An Electrophysiological Study of Vagal Reflex Pathways Activated by Upper Gastrointestinal Stimuli

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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(Ms. Elita Partosoedarso)

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General Abstract

1. This study investigates the complexity of the vagal reflexes arising from the upper gastrointestinal tract (GIT) by recording single unit vagal afferents and efferents in the ferret. The potential involvement of various neurotransmitters in mediating and modulating GIT inputs was also explored.

2. The discharge rate of single vagal efferent fibres were modulated by oesophageal, gastric and colonic distension, oesophageal acid and capsaicin infusion, and close intraarterial cholecystokinin, bradykinin and capsaicin. Responses to distension were rapidly evoked, and maintained only for the duration of the stimuli. Responses to oesophageal infusion had a longer latency and were maintained until washout of the oesophageal lumen. Responses to chemical injections had a variable latency and duration. Not all fibres responded to all peripheral stimuli tested. The intensity and direction of response varied with different stimuli and in different fibres. Oesophageal afferent inputs onto these central neurones were vagal in origin. A non-vagal, possibly spinal, gastrointestinal input onto these neurones also exists.

3. The involvement of several different neurotransmitters in gastrointestinal vagal reflexes was investigated. Antagonism of the NK-1, NMDA, and M2 muscarinic receptors with CP96345 and CP99994, CGS19755 and methoctramine respectively, did not affect most vagal efferent responses to any of the peripheral stimuli listed above. Antagonism of the M1 cholinoceptor with pirenzepine selectively reduced the efferent responses to gastric distension in 75% of fibres tested. Antagonism of the non-NMDA and CGRP receptor with CNQX and hCGRP8-37 respectively, reduced or blocked, and in 1 study reversed the direction of response, the responses of some fibres to some of the stimuli. However, no one receptor antagonist was able to block all responses to a particular stimuli.

4. GABAb receptor influences on vagal gastro-oesophageal tension and mucosal receptors and vagal efferents were studied. GABAb receptor agonism with baclofen selectively attenuated the responses of some tension receptors with endings in the corpus, but not

those with endings in the oesophagus or antrum, to distension. Baclofen also potentiated mucosal receptor responses to cholecystokinin, although this effect was probably indirect through modulation of somatostatin levels. With vagal efferent studies, systemic and central baclofen inhibited most efferent responses to gastric and colonic distension. The baclofen effects on gastric distension, but not on colonic distension, were always reversible with the GABAb receptor antagonist CGP35348. Our data indicate that the overriding effect is probably central.

4. The acute chemical sensitivity of vagal gastro-oesophageal tension and mucosal receptors were also studied. Capsaicin, but not acid, directly stimulated these afferents and affected the tension receptor responses to distension. Inflammation of the gastric mucosa attenuated responses to some stimuli while potentiating others.

5. Great heterogeneity exists within vagal reflex pathways. Vagal efferents receive a wide range of inputs from mechano- and chemo-sensitive receptors located within the gastrointestinal tract. The degree of influence of these inputs which converge onto vagal efferents are not uniformally proportional, such that individual efferents may respond with greater intensity to one stimulus than to others. Although there is a great diversity in the central connections of the vagal reflex pathways in terms of the neurotransmitters and the neuronmodulators present, there does seem to be a loose chemical specificity present within the ferret brainstem.

Abbreviations

Name	Function/Definition
ACID	Oesophageal acid infusion (150mM HCl)
AD	Antral distension (4ml saline)
AP	Area Postrema
ATP	Adenosine triphosphate
Baclofen	GABAb receptor agonist (β–ρ-chlorophenyl-GABA)
BK	Bradykinin
Cap	Capsaicin (8-methyl-N- vanillyl-6-nonenamide)
CCK	Cholecystokinin octapeptide, sulphated
CD	Corpus distension (5-20ml saline)
CGP35348	GABAb receptor antagonist (3-amino-propyl(diethoxymethyl) phosphinic acid)
CGRP	calcitonin gene-related peptide
CGS19755	NMDA receptor antagonist (cis-4-(phosphonomethyl)piperidine-2-carboxylic acid)
CNQX	non-NMDA receptor antagonist (kainate/AMPA) (6-cyano-7nitroquinoxaline-2,3-(1H,4H)-dione)
ColD	Colonic distension (3ml saline)
CP96345	NK-1 receptor antagonist ((2S,3S)-cis-2-diphenylmethyl-N-(2-methoxyphenyl)-methyl-1-azabicyclo octanamine)
CP99994	NK-1 receptor antagonist ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine)
DMVN	Dorsal motor nucleus of the vagus
DVC	Dorsal vagal complex
GABA	γ-amino butyric acid
GD	Gastric distension (40-60ml saline)
hCGRP8-37	CGRP receptor antagonist
5-HT	5-hydroxytryptamine
IR	Immunoreactivity
Methoctramine	M2 muscarinic receptor antagonist (N,N-bis[6-[[(2methoxyphenyl)methyl]amino]hexyl]-1,8-octane diamine tetracholoride)
NA	Nucleus ambiguus

Name	Function/Definition
NK	Neurokinin
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
nRO	Nucleus raphe obscurus
NTS	Nucleus of the solitary tract
OBD	Oesophageal balloon distension (0.5-3.0ml air)
Pirenzepine	M1 muscarinic receptor antagonist (5,11-dihydro-11-[(4-methyl-1-piperazinyl)acetyl]-6H-pyridol[2,3-b][1,4]benzodiazepin-6-one dihydrochlroride)
QUIS	Quisqualic acid
TLOSR	Transient lower oesophageal sphincter relxations
TRH	Thyrotrophin releasing hormone
VAD	Vagal afferent discharge
VED	Vagal efferent discharge
VIP	Vasoactive intestinal peptide

Introduction

1 THE VAGUS NERVE

The vagus nerve is the main source of parasympathetic innervation, and is involved in a variety of different functions, including cardiovascular responses, respiration, osmoregulation, endocrine effects, arousal, food and water intake, taste preference, vomiting and the coordination of gastrointestinal secretion and motility. Its extensive interconnections between the cardiovascular, respiratory and the gastrointestinal systems allows it to play a pivotal role in the central integration of these functions in the whole animal. However, only the vagal functions connected to the digestive tract will be elucidated below, as any discussion of the vagal control of other systems is beyond the scope of this thesis.

2 ELUCIDATION OF THE VAGAL REFLEX PATHWAY

Any description of vagal reflexes is apt to be bulky and complex. I will attempt to streamline the following by sketching a picture based on the reflex pathway taken by the vagus nerve. This will begin with an anatomical description of the vagal afferent endings, both in the periphery and within brain stem nuclei and be continued by that of the central and peripheral terminations of vagal efferent fibres. A brief description of the neuroanatomical connections of spinal afferents will be given, as activation of these can lead to stimulation of vagal central neurones. It will be followed by a summary of the electrophysiological responses of vagal and spinal afferents and vagal efferents when activated by peripheral stimuli. It will then be finished by a brief overview of the various gastrointestinal functions modulated by the vagus.

3 NEUROANATOMY

The vagus is a sensorimotor nerve which innervates the oesophagus, lower oesophageal sphincter, stomach, pylorus sphincter, duodenum, jejunum, ileum, proximal colon, liver, pancreas, biliary tree, gall bladder and adrenal glands. The peripheral endings

of vagal afferents are thought to be bare, unspecialised nerve endings and are classified by their location within the gut wall as mucosal, muscular or serosal afferents. These afferents course within the main vagal trunks and have their cell bodies contained within the nodose ganglion. Upon reaching the brainstem, the main site of termination is within the nucleus tractus solitarius (NTS) where connections are made, either directly or via interneurones, with preganglionic motor neurones in the nucleus ambiguus (NA) or the dorsal motor vagal nucleus (DMVN). Efferent fibres from the NA and the DMVN then project to the oesophageal body and the rest of the upper GI tract. These motoneurones exert their functions by synapsing extensively with the intramural ganglia such as the myenteric ganglia. Post ganglionic neurones within these plexuses then provide the final pathway for both vagal and enteric reflexes. Each step of the neuroanatomical pathway will be detailed below. It should also be emphasised that the brainstem nuclei that are involved in the control of gastrointestinal function are also involved in the control of all viscera. Therefore it may often be difficult to ascertain whether these structures are relevant to GI function.

3.1 Peripheral endings of vagal afferents

Although the vagus was originally viewed as only a motor nerve, the proportion of sensory afferents projecting from the periphery has been found to be much greater than the number of motor neurones arising from the brainstem. This often held perception arose as the sensory information from the GI tract rarely reaches the levels of consciousness under normal circumstances. The afferent fibres provides the CNS with information regarding the physical and chemical changes occuring in the peripheral environment. As there are a large number of afferents, this information can be very detailed and accurate as the size of the receptive fields of these fibres is small (<10mm).

An electron microscopy study by Asala and Bower[18] found that, of the 27,000 axons within both ferret abdominal vagal trunks, approximately 90% of these are afferent. These afferents were identified as such by performing a supranodose vagotomy which

allows efferent fibres to degenerate before the number of afferent axons are counted. This ratio is also true at the cervical level, where the total number of axons is doubled. Of these, 13% of the total number of axons are myelinated, although the degree of myelination between the cervical and abdominal vagal trunks differs, with myelinated fibres consisting of less than 15% and 1% of the cervical and abdominal axons respectively. In this study, no attempt was undertaken to determine either the site of central or peripheral terminations of the vagal fibres. It simply sought to determine the number of fibres present at the cervical and the subdiaphragmatic levels. The proportion of myelinated:unmyelinated fibres and of afferent:efferent fibres are in agreement with those performed on other species including the cat, rabbit, and man[104, 178, 190].

Vagal afferent endings have been identified histologically within the mucosal, muscular and serosal layers of the gut wall. These peripheral endings are not differentiated, and so assignment of functional specificity of these endings is often based on the site of the termination within the gut wall. Electrophysiologically, vagal afferents have been classified as serosal, muscular, and mucosal receptors based on their responses to peripheral stimuli designed to selectively activate endings located in the each layer of the gut wall.

Vagal afferents in the mucosal layer of the rat, cat, rabbit and monkey upper gastrointestinal tract have been identified[27, 28, 30, 198, 222, 224, 225, 233]. These peripheral nerve endings pass through the submucosal plexus to the mucosa and were identified in the epithelium and subepithelium throughout the oesophagus[225], stomach and duodenum[30, 233]. In the oesophagus, the density of labelling of these nerve terminals is high in the proximal oesophagus and decreases aborally down the oesophageal body[198]. Electron microscopy studies of these oesophageal endings at the ultrastructural level in the cat and monkey have found them to be bare and unspecialised[222]. In the rat stomach, vagal fibres were found to course within the submucosa, often being associated with blood vessels[30]. Terminations were also seen to enter the mucosa, innervating the muscularis

mucosa and lamina propria. The terminal arborizations occur mainly between the crypts and the villous lamina propria, coming into contact with the basal lamina[28]. In the lamina propria, they come into close contact with fibrocyte-like cells which may be interstitial cells of Cajal and with granulocytes. The degree of vagal mucosal innervation was variable, with some areas showing no evidence of vagal innervation while other villi possessed dense networks of arborizing terminal fibres.

Vagal afferent endings in the muscular layer have been traced in the rat, rabbit. cat and monkey[27, 29, 30, 198, 223, 225, 233]. The density of labelled vagal terminals and fibres was highest in the myenteric plexus of the stomach and proximal duodenum, medium in the rest of the small intestine, caecum and ascending colon, and very low in the descending colon of the rat. Labelled axons were found along the length of the oesophagus, and for a short distance into the cardia of the stomach, between the muscularis externa and interna, and within the myenteric ganglia. These nerve fibres run along the entire depth of both the longitudinal and circular muscle layers, and were mostly oriented parallel to the muscle bundles[30]. They form a highly arborizing network of fibres which form a complex lattice, running closely with the interstitial cells within the connective tissue matrix that is oriented parallel to the smooth muscle layer. Vagal nerve fibres were also seen to terminate within myenteric ganglia as intraganglionic laminar endings (IGLEs)[29, 198, 223]. These IGLEs are peripheral afferent endings which possess flattened branches that are in contact with the inner surface of the connective tissue capsule which envelopes the myenteric ganglia. They are found in all regions of the gut which receive vagal innervation[29] and are thought to form the structural basis for the tension receptor which has been characterised electrophysiologically[30].

Vagal fibres innervating the serosal layer are found in the subserous plexus between the serosa and the longitudinal muscle layer in the gastroduodenal wall of the rabbit[233]. These were supplied with IGLEs at the cardia of the rat[199]. The ventral vagal trunk enters the stomach wall at the level of the cardia and ramified, forming the subserous plexus.

Histochemistry of vagal afferents

Cell bodies of vagal afferent fibres are located within the nodose and jugular ganglia. Axons from these cell bodies project peripherally to different layers of various target organs and centrally to the nucleus of the solitary tract (NTS). These ganglions are also the site of protein synthesis, with neuropeptides and receptor proteins being produced within the cell bodies. These neurotransmitters and receptors are then transported to the central and peripheral axonal terminations where they are able to effect a physiological function. There are several ways of identifying neurotransmitters which are present within vagal afferents: 1) the nodose ganglion may be removed and the change in transmitter content in the NTS or the periphery determined immunohistochemically, 2) injection of a tracer into the nodose ganglion which would label afferent fibres anterogradely in the NTS or retrogradely in the periphery and 3) activating a specific group of neurones and visualising them using c-fos and immunohistochemical techniques. This knowledge of the neurotransmitter content provides important information about the involvement of neurotransmitters in vagal reflexes and inputs to the CNS. The histological data has also been largely supported by data from available functional studies.

Generally, the nodose ganglion contains acetylcholine, γ-amino butyric acid (GABA), glutamate, aspartate, 5-hydroxytryptamine (5-HT), substance P, calcitonin gene-related peptide (CGRP), cholecystokinin, vasointestinal peptide, and somatostatin[19, 20, 63, 88, 97, 116, 136, 164, 175, 195, 251, 255]. These compounds are transported along axons projecting both peripherally to the target organ and centrally to the NTS[195]. However, these transmitters and modulators may not be evenly distributed. This is seen with substance P where over 95% was found to be peripherally transported along the vagus[58]. Receptors that are found within the nodose ganglion include those for acetylcholine (including muscarinic and nicotinic receptors), histamine, bradykinin, GABA (including GABAa receptors), 5-HT (including 5-HT_3 receptors), cholecystokinin (including CCKb receptors), opiate, angiotensin II, neurotensin and peptide YY[139, 194, 267].

Specifically, glutamate and substance P, which are found in the central terminations of vagal afferent fibres, have been implicated in cardiovascular responses[137, 164]. This is confirmed by physiological studies where NK-1 receptor antagonism within the NTS in an *in vitro* preparation reduced excitatory post-synaptic potentials evoked by vagal nerve stimulation and chemoreceptor input from left ventricular receptors to the NTS[211]. Also, cardiovascular afferents which demonstrate substance P-immunoreactivity (IR) make contact with preganglionic neurones which are located in the region of origin of cardiovascular motor neurones within the nucleus ambiguus (NA)[182]. Likewise, glutamate is implicated in the CNS control of respiration and cardiovascular responses[56, 269, 276]. These functional studies show that glutamate is involved in the production of the Breuer-Hering reflex, the integration of baroreceptor afferent inputs in the NTS and can mimic the shallow breathing evoked by phenylbiguanide, a sensory C fibre stimulant which activates vagal cardiopulmonary C fibre endings supplied by pulmonary circulation. However, similar data regarding neurotransmitter involvement in vagal gastrointestinal reflexes is lacking. In this thesis, I will investigate the contribution of some putative neurotransmitters which may be involved in control of gastrointestinal function.

Specifically wtihin the gastrointestinal tract, CGRP and substance P are contained in vagal afferent fibres which end in the oesophagus[225] and the stomach[122]. However, in the rat stomach, only 5% and 10% of vagal fibres show substance P- and CGRP-IR respectively[122]. This is in contrast with the 50% and 85% of gastric spinal afferents which are immunoreactive for the same peptides. The CGRP found in the stomach and oesophagus is mainly extrinsic in origin and may be released during inflammation to provide gastric mucosal protection via an increase in blood flow[173].

3.2 Central connections of the vagus

Vagal afferents and efferents possess extensive interconnections within brainstem nuclei, the cerebellum and the forebrain. The portion below will concentrate mainly on the dorsal vagal complex (DVC) and the nucleus ambiguus (NA), the connections between

different nuclei contained within it and the connections with other parts of the brain. The DVC includes the nucleus of the solitary tract (NTS), area postrema (AP) and dorsal motor vagal nucleus (DMVN). The NTS and AP are the main site of vagal afferent terminations from the gastrointestinal region and the NA and DMVN that of vagal efferent somata. There is substantial overlap within the DVC in the termination of fibres coming from and projecting to the thoracic and abdominal viscera. The information received by the DVC is processed and communicated with other regions of the brain via reciprocal connections.

3.2.1 The nucleus of the solitary tract

The nucleus of the solitary tract (NTS) is located on either side of the midline in a rostrocaudal direction at the level of the obex. It is the site of termination of the majority of vagal afferents from the cervical, thoracic and abdominal viscera. Vagal afferent fibres enter the dorsolateral aspect of the medulla oblongata and proceed in a dorsomedial direction towards the ipsilateral solitary tract and terminate mainly within the ipsilateral NTS. Afferents from the gastrointestinal tract terminate mainly within the medial and dorsal subnuclei of the NTS.

In the ferret, vagal afferents terminate mainly in the ipsilateral NTS[203], where labelled fibres were found along its entire length. Minor projections to the contralateral NTS, the AP and the DMVN were also identified, indicating that a proportion of vagal afferents terminate directly within these regions. No labelling was found in this study in the NA, the medial and lateral external cuneate nuclei, the dorsal horn of the first and second cervical segments of the spinal cord, any part of the trigeminal nuclei and the reticular formation, in contrast to findings in other species, where terminations in the nucleus of the spinal tract of the trigeminal nerve have been identified in the rat[168]. This discrepancy may have arisen due to the different methods used: tritiated leucine, used as the marker in the ferret study, is known to only label cell bodies whereas horseradish peroxidase (HRP), used in the rat, labels axons both retrogradely and anterogradely.

A rough viscerotopic organisation exists within the NTS, with the majority of gastric vagal afferents terminating in the subnucleus gelatinosus of the dorsomedial NTS at the level of the obex as well as in the medial NTS and the commissural NTS[113, 221, 243], most oesophageal vagal afferents terminating in the medial portion of the NTS designated as the central subnucleus[221], cardiac afferents terminating in the commissural NTS, baroreceptor and chemoreceptor inputs terminating in the dorsal and dorsolateral subnuclei and respiratory afferents terminating within the intermadiate, ventral and ventrolateral subnuclei. Even within the subnuclei, further topographic organisation exist, with afferents from the more rostral part of the oesophagus projecting to the more rostral aspects of the central subnucleus[2].

The NTS also receives inputs from other loci within the brainstem and from the cerebellum and forebrain which may serve as part of a feedback mechanism regulating input from the forebrain to the hindbrain as well as providing viscerosensory information necessary for the control of endocrine or autonomic nervous functions. These projections include those to 1) the parabrachial nucleus which serves as a relay centre for both ascending and descending visceral fibre systems including those that mediate taste. This nucleus is also in communication with forebrain regions and is implicated in the complex integration of gastrointestinal, cardiovascular and respiratory activity in autonomic reflexes such as vomiting[249]; 2) the paraventricular nucleus of the hypothalamus[202, 220], the site of vasopressin and oxytocin synthesis. Release of vasopressin has been implicated in the emetic reflex and is released as a result of nausea and vagal stimulation. However, in the ferret, injections of vasopressin are unable to evoke an emetic response. DiI injections in the rat show that there are numerous connections from this into the DMVN[27]; 3) the central nucleus of the amygdala which is part of the limbic system[202, 220] and is indicated to be concerned with emotion and emotional responses. Again, the emetic reflex may evoke strong emotional responses which are probably important in the future avoidance of foods which may led to nausea and vomiting. There are projections in the rat from this, the posterolateral hypothalamus, and the bed nucleus of the stria terminalis into the ventral

NTS[27]. As such, the NTS functions as a central sorting station for the large quantities of information that it receives from the periphery as well as from other brain centres.

Histochemistry of the NTS

Synapses immunoreactive for glutamate and GABA, the main excitatory and inhibitory neurotransmitters in the CNS, have been abundantly identified throughout the NTS[228, 250]. At least half of the glutamate and GABA-immunoreactive terminals are central projections of primary vagal afferents as vagotomy led to a decrease in the content of both neurotransmitters[97]. Terminals immunoreactive for GABA would often synapse onto the same dendritic profile as glutamate-IR terminals. This may form the anatomical basis for GABA mediated inhibition of glutamatergic excitatory inputs to the NTS, ie it may be the mechanism through which excitatory afferent responses are altered into the inhibitory responses seen in electrophysiological recordings of vagal efferent neurones.

Other neurotransmitters were found to be associated with specific subnuclei within the NTS, ie these neurotransmitters are not ubiquitously distributed within the NTS. For example, substance P- and CGRP-IR terminals were found in the medial subnuclei of the NTS which receive inputs from gastrointestinal afferents[151, 228, 250] while those subnuclei which receive inputs from respiratory afferents show immunoreactivity for somatostatin and, to a lesser extent, for substance P and enkephalin[151]. Enkephalin-IR levels are high in regions of the NTS which receive input from cardiovascular afferents[166].

Other neurotransmitters that were found within the NTS include aspartate, acetylcholine (inferred from the presence of the synthetic and degradative enzymes for this compound), cholecystokinin, noradrenaline and dopamine[97, 119, 166, 229]. Receptors which have been shown to be present within the NTS include those for glutamate, opiate, histamine, insulin, GABA, substance P, thyrotropin-releasing hormone (TRH) and 5-HT (including the 5-HT_{1A} and 5-HT_{1B} receptor subtypes)[180, 244].

3.2.2 The area postrema

The area postrema (AP) is located dorsal to the NTS. At the level of the obex, it is bound on one side by the fourth ventricle and on another by the subnucleus gelatinosus and the medial subnucleus of the NTS. This circumventricular organ with its dense vascular supply and the lack of a blood-brain barrier can encourage the direct influence of both blood- and cerebrospinal fluid- borne humoral elements[170], such as peripherally circulating peptide hormones. Due to its proximity to both the fourth ventricle and the NTS, its location is ideal for the processing of information from blood, CSF, and the periphery. The AP is thought to be mainly involved in the generation and coordination of emesis and conditioned taste aversion.

In the ferret, reciprocal connections to and from the AP exist only with various subnuclei of the NTS, especially to the medial and gelatinosus subnuclei[249]. In other species, vagal afferents can project directly to the AP and connections to the DMVN, NA, spinal trigeminal tract and parabrachial nucleus exist[167, 168, 242]. However, regardless of whether the AP has connections exclusively with the NTS or not, the majority of projections do arise from the NTS. Due to this and its proximity with the fourth ventricle, it has been considered a highly vascularized part of the NTS.

Histochemistry of the AP

Some vagal afferents which project to the AP are immunoreactive for 5-HT and dopamine, which is consistent with the involvement of 5-HT3 and dopamine receptors in the emetic reflex[250]. Other neurotransmitters that are found within the AP include noradrenaline, adrenaline, enkephalin, CCK, GABA, substance P, and glutamate[97, 166]. Receptors which have been shown to be present within the NTS include those for dopamine, CCK and 5-HT[166]. Data on the neurotransmitter content of the AP is scarce as most effort is concentrated on the DMVN and the NTS.

3.2.3 The dorsal motor nucleus of the vagus

The dorsal motor nucleus of the vagus (DMVN) and the nucleus ambiguus (NA) are sources of vagal preganglionic parasympathetic fibres which innervate the abdominal and thoracic viscera[168, 270]. The DMVN supplies vagal preganglionic motor neurones to the corpus, antrum, jejunum, duodenum, ileum, caecum and colon[26, 78].

The DMVN is located dorsolateral to the hypoglossal nucleus which extends along the entire length of the medulla. It forms a cell column which extends further rostrocaudally than the hypoglossal nucleus. At the rostral end, it is positioned adjacent to the AP. At the caudal end, the DMVN approaches the midline immediately dorsolateral to the central canal. It consists of several morphologically distinct subnuclei arranged longitudinally throughout the dorsal medulla oblongata. A rough structure-function relationship has been suggested as region specific differences were observed[113]. A study by Fox and Powley[112] suggests that the 4 major columns within the DMVN are organised into 4 of 5 major subdiaphragmatic vagal branches. These columns form 2 bilaterally symmetrical longitudinal clusters on each side of the medulla and contain the somata of the cells running along the gastric and coeliac branches of the vagus. Vagal fibres running along the paired gastric branches arise from the 2 columns on either side of the medulla which run the rostrocaudal length of the DMVN. Some gastric fibres also arise from the compact group in the rostral quarter of the NA[168]. Vagal fibres which follow the coeliac branches of the subdiaphragmatic vagus arise mainly from the 2 columns on the lateral portions on either side of the DMVN. The fibres forming the hepatic branch arise from a fifth column of cells which is scattered thoughout the longitudinal length of the DMVN cell column that projects to the anterior gastric branch.

The main source of input to both the DMVN and the NA comes from the vagal afferent fibres from the NTS and the AP. A 'typical' DMVN neurone in the rat would contain several dendrites which have extensive arborizations mainly in the lateral plane. Although most of these dendritic ramifications were contained within their column of origin, a proportion of them projected into other subnuclei of the DMVN, on both the

ipsilateral side and across the central canal to contralateral subnuclei[201]. Dendritic ramifications have also been found to extend to subnuclei of the NTS that receive gastrointestinal inputs. Projections to the central canal, fourth ventricle and the AP also exist. These contacts allow communication between preganglionic efferent neurones and afferents, with humoral input from the CSF and/or peripheral plasma[113, 243].

Another source of input to the DMVN is the nucleus raphe obscurus (nRO) which is also located within the dorsal medulla. The nRO has been implicated in the vagal control of gastric motility as it has direct connections with the DVC which includes the DMVN and the NTS[184].

The central connections of the DMVN and the NA are similar in that they receive inputs from regions that are in communication with vagal sensory nuclei. For example, reciprocal connections with the parabrachial nucleus exist, as do monosynaptic connections with the hypothalamic, limbic and cortical areas, which are likely to be involved in integrating autonomic, endocrine, protective and defensive functions.

Histochemistry of the DMVN

Both TRH and the TRH receptor are found within the DMVN[180]. TRH is thought to be released within the DMVN when the nRO is activated[143]. The activation of DMVN neurones by TRH would then lead to an increase in vagal efferent activity and induce the release of acetylcholine from peripheral efferent terminals. This cholinergic activation of the stomach or enteric system would lead to an increase in gastric motility and gastric tone. Other neurotransmitters that are found within the DMVN include GABA, glutamate and enkephalin[97, 166]. Other receptors which have been shown to be present within the DMVN include those for glutamate (including NMDA receptors), GABA (including GABAa receptors), substance P, histamine and acetylcholine (including muscarininc receptors)[62, 244].

3.2.4 The nucleus ambiguus

The nucleus ambiguus (NA) is composed of 2 longitudinal columns located in the ventrolateral medulla in areas of the brain stem that are coextensive with the DMVN. The dorsal column is divided into 3 sections: the rostral compact formation (NA_C) is the source of motor neurones which project to the entire oesophageal body, from the cervical and thoracic region, to the subdiaphragmatic portion, the intermediate subcompact formation (NA_{SC}) provides the motor innervation to the palatopharyngeal portion of the alimentary tract and the cricothyroid muscle and the caudal loose formation supplies motor innervation to the larynx. The ventral column contains parasympathetic preganglioinic neurones which innervate supradiaphragmatic structures such as the heart and trachea and contains bulbospinal respiratory motoneurones. The NA_C and NA_{SC} possess extensive dendritic network which might be involved in the generation and coordination of complex motor events such as swallowing and the subsequent peristaltic movement of a liquid or solid bolus aborally through the oesphageal body[3]. In the rat, NA neurones which project to the stomach innervate mainly the forestomach[113].

Histochemistry of the NA

The neuropeptide CGRP was found in the NA_C[250] which is consistent with other data that have identified motor endplates within the muscle layers of the oesophageal body which are also CGRP-IR[225]. Other neurotransmitters that are found within the NA include substance P, somatostatin, enkephalin, galanin, brain natriuretic peptide, N-acetylaspartylglutamate and acetylcholine[87, 182]. Receptors which have been shown to be present within the NA include those for acetylcholine (including muscarinic receptors)[166].

3.3 Peripheral endings of vagal efferents

Vagal efferent fibres originate from the DMVN, the NA, and the reticular formation between the two[144, 156, 159, 270]. These efferents follow the 2 main vagal trunks, projecting to the oesophagus, stomach and the rest of the gut[8, 9, 144, 199]. At the

subdiaphragmatic level, the 2 nerve trunks divide into 3 main divisions, the paired gastric, paired coeliac and unpaired hepatic branches. The gastric branches have the stomach as their main, and almost exclusive, target[26]. The hepatic branches obviously supply the liver as well as the duodenum. The coeliac branches initially descend along the left gastric artery toward the coeliac plexus, after which it was named, and innervate the jejunum, ileum, caecum and entire colon. Although a degree of specificity exists, there is some overlap between the 3 subdivision of the vagus, with all 3 branches innervating the proximal duodenum, the gastric branch also sending projections to the distal duodenum and caecum, the coeliac branches to the corpus and antrum, and the hepatic branch to the distal antrum and the intestines[26].

Vagal fibres arising from the NA enter the oesophagus in thick bundles and are divided into branches[199]. These fibres could then be traced to motor endplates which were oriented in various directions, reflecting the direction of muscle fibres in the longitudinal and circular muscle layers. The endplates arising from each axon were always aligned parallel to each other, indicating that these efferent neurones are positioned to parallel one set of agonist muscle fibres within one muscle layer.

Once the vagal efferent fibres reaches the target organ, they may terminate within myenteric ganglia located throughout the gut[26, 80, 156]. These terminations were more numerous in the stomach than in the small intestine and generally became more sparse distally, although a few were found in the ileo-colic junction. It is known that the parafascicular ganglia, which are located between the myenteric and the subserosal plexuses, are also associated with vagal nerve fascicles. This ganglia receive convergent inputs from the vagus and may therefore act as peripheral centres for the reception and distribution of central inputs[155].

Histochemistry of the peripheral vagal efferent terminals

Neurotransmitters that are found within the peripheral terminals of vagal efferents include galanin, CGRP and substance P[156, 224, 225].

3.4 The enteric nervous system

The enteric (or intrinsic) nervous system is confined within the walls of the gastrointestinal tract, from the lower oesophagus to the anus. It consists of 2 layers of nerve 'nets': one of these, the myenteric plexus, is located between the longitudinal and circular muscle layers whereas the second, or submucosal plexus, is positioned between the circular muscle and the submucosal layers. Electrophysiological studies in the intestines have shown that the complex network of sensory neurones, interneurones and motor neurones belonging to these intrinsic nerve plexus form a nervous sytem that can function independently of the extrinsic nervous sytems. Some of these neurones do function as relay stations for parasympathetic outflow- this is also seen in the disruption of motility patterns immediately after vagotomy, although normal functioning is regained several weeks after sectioning of the thoracic vagus. The overall function of the enteric nervous system is the regulation of digestion.

Enteric motor neurones to the circular and longitudinal muscle layers have been identified as either orally directed excitatory motor neurones or anally projecting inhibitory motor neurones[80]. These motor neurones are typically Dogiel type I neurones with multiple short dendrites and a long axonal process and form approximately half the total number of myenteric neurones. Another third of myenteric neurones are likely to have a sensory function. These are morphologically Dogiel type II neurones with a smoother cell body and several long processes and may account for ganglionic transmission in enteric reflexes. Another population of enteric neurones act as interneurones: these can either be annally directed or orally projecting. In this way, the enteric nervous system can receive adequate information to enable its functioning independently of extrinsic neural control.

Histochemistry of the enteric nervous system

Acetylcholine is a major neurotransmitter released by excitatory motor neurones and most probably act via muscarinic receptors[80]. Together with substance P or another tachykinin, these excitatory motor neurones supply both the longitudinal and circular muscle layers. Acetylcholine also acts on other enteric neurones via nicotinic receptors (which mediate fast synaptic transmission) and muscarinic receptors (which is responsible for slow synaptic potentials). Both receptor subtypes are involved with enteric reflexes responsible for peristalsis. Transmitters which are involved in relaxation mediated by enteric inhibitory neurones include vasointestinal intestinal polypeptide (VIP) (a slow acting transmitter) and NO. VIP is also proposed to be involved in secretomotor functions mediated by those neurones located in the submucus ganglia.

Other neurotransmitters that are found within the myenteric ganglia include GABA, glutamate, galanin, noradrenaline, histamine, ATP, CGRP, somatostatin, CCK, 5-HT, neuropeptide Y, peptide histidine isoleucine (PHI), gastrin releasing peptide (GRP) and enkephalin[100, 118, 156, 157]. Receptors which have been shown to be present within these ganglia include those for glutamate (including NMDA, AMPA and and kainate receptors)[157].

3.5 Spinal afferents

The neuroanatomy of afferents following the sympathetic pathway will be described as inputs from non-vagal afferents have been shown to converge onto vagal central neurons (see later). There is also histological evidence proving that reciprocal connections exist between neurones within the NTS and DMVN and those projecting from the spinal cord[103, 196, 218]. Therefore, a brief description of the peripheral endings of these afferents, the pathway they follow and the terminations within the spinal cord will be made here.

The majority of non-vagal afferents innervating the proximal gastrointestinal tract follow the splanchnic nerves. Like the peripheral endings of vagal afferents, those of spinal afferents are also bare unspecialised endings terminating at different levels within the gut wall[128]. In the cat gastro-oesophageal region, terminations were observed in all layers of the gut wall, with the largest concentrations occuring within the myenteric area and mucosa[74]. Work done in our laboratory has found spinal afferents following the inferior mesenteric nerves in the mucosa of the rat colon *in vitro*[176]. The fibres with endings in the myenteric area may be the structural correlate of the tension receptor while those in the mucosa may be involved in nociceptive responses.

These afferents have their cell bodies in the dorsal root ganglion (DRG) at the cervical, thoracic and lumbar levels and project mainly to the laminae I and V of the spinal cord[67, 77]. The dorsal root ganglion cells form the route through which information is transmitted from the periphery to the CNS. The primary afferents terminate mainly in the spinal cord dorsal horn and the dorsal column nuclei. The splanchnic afferents arising from the gut consist of only 7% of the total number of cells within the DRG. However, they converge extensively onto somatosensory pathways through the spino-thalamic and spino-reticular pathways.

Histochemistry of dorsal root ganglion cells

In the spinal cord, immunohistochemistry and pharmacological evidence indicates that glutamate is involved in neurotransmission between most, if not all, primary afferent fibres and dorsal horn neurones[59]. This means that information transfer from different modalities is partially, or completely, dependent on the release of this excitatory amino acid. ATP can also function as a fast neurotransmitter and may be involved in antinociception. ATP and glutamate may also be coreleased from primary afferent terminals. GABA-IR neurones are also found throughout the dorsal horn, with the major concentration being in laminae I-III. GABAb-positive boutons are present in axodendritic and axosomatic synapses as well as axoaxonic ones, which provides a basis for both post-

and presynaptic GABAergic inhibition[59]. The presynaptic axoaxonic contacts appear to be limited to Aδ-high threshold mechanoreceptors and are absent in the terminals of C-fibres. This may be an indication that presynaptic GABA modulation, presumable through GABAb receptors, occurs in transmission from myelinated primary afferent fibres to dorsal horn neurones.

A wide variety of peptides are also present within the spinal cord. These peptides seem to be associated with small to medium sized primary afferent fibres, and thus would be confined to C- and Aδ-fibres. Substance P and CGRP are two peptides that are frequently colocalised in dorsal root ganglion cells and are distributed evenly along the length of the spinal cord[118]. These peptides, together with bombesin, CCK, gastrin releasing peptide and VIP, are most probably released by primary afferent fibres. Other peptides like somatostatin, on the other hand, are thought to be found only in intraspinal neurones or neurones with descending projections to the spinal cord.

These peptides are likely to play a modulatory role by attenuating or potentiating synaptic transmission through both pre- and post-synaptic mechanisms. For example, CGRP may act presynaptically to potentiate the release of substance P but may prolong the action of substance P by inhibiting its extracellular enzymatic degradation. Substance P may alter the excitability of primary afferent axons by acting presynaptically. Substance P and CGRP are also involved in nociception and are released after noxious stimulation.

With regards to the gut, substance P is found in 50% and CGRP in 85% of spinal afferents projecting to the stomach[122]. In fact, most of the gastric CGRP content is thought to be associated with spinal neurones as coeliac ganglionectomy, splanchnectomy anor capsaicin treatment virtually abolished the CGRP-IR in the stomach. On the other hand, somatostatin, which was localised in cell bodies of the DRG, was not found to be associated with gastric spinal afferents. It should be noted again that afferents projecting from the gut make up only a small proportion of the total number of spinal afferents. As such, it would be difficult to assign neurochemical specificity for these afferents.

4 ELECTROPHYSIOLOGY OF GASTROINTESTINAL FIBRES

From the neuroanatomical section of this introduction, it is obvious that the vagus has reciprocal connections with many different parts of the CNS. In this section, electrophysiological studies exploring the behaviour of both vagal and non-vagal afferents and of vagal efferents in respond to peripherally administered mechanical, pharmacological and chemical stimuli will be discussed.

These afferents and efferents modulate their discharge frequency (aka firing rate) when activated by a particular stimulus. Only recordings from single units will be discussed as limited information can be derived from multiunit or whole nerve trunk recordings. Recordings from single afferent units allow the location of the peripheral ending of the fibre to be identified by the application of a discrete stimulus to a particular region of the gut designed to activate the peripheral endings of the afferent. The fibre can also be classified according to its conduction velocity. Efferent recordings require single unit recordings to be interpretable as individually, these fibres receive extensive convergent inputs from different afferent populations. Multiunit recordings are harder to interpret as any response generated by a peripheral stimulus may be complicated by fluctuations in cardio-respiratory parameters due to changes in firing rhythms from afferents arising within the cardiovascular and respiratory systems. Furthermore, the stimulus administered may also affect more than one type of gastrointestinal afferent, albeit sometimes transiently, which means that the changes in firing rate in multiunit recordings could be due to the activation of more than one afferent population. The information derived from single unit studies can be interpreted fairly easily in that a cause-effect relationship is in most cases easy to establish.

Single unit recordings are made using either one of 2 basic techniques. The first technique involves teasing fine nerve filaments from a main nerve trunk and recording from the peripheral end of the filament, in the case of afferents, or from the central end, in the case of efferents. The second technique involves extracellular recordings made from inserting microelectrodes adjacent to cell bodies either in the nodose ganglion, in the case

of afferents, in the DMVN, in the case of efferents, or in the NTS, in the case of interneurones or second order afferent neurones. Recordings from NTS neurones are often from second or third order neurones which have their cell bodies in the DVC and may show a convergence of inputs from primary afferents. Recordings from DMVN neurones require collision for identification of neurones[8, 9].

4.1 Vagal mucosal afferents

Afferents with nerve endings in or near the mucosal epithelium are able to monitor the constantly changing physical and chemical composition of gut luminal contents. These fibres are thought to act as pre- or peri-absorptive receptors which transmit information to the central nervous system to aid in the coordination of the digestive process. These are mainly C-fibres with conduction velocities ranging from 0.4-1.7m/sec and a receptive field of up to 5.0mm^2[37, 72, 90, 191]. Their peripheral endings are thought to be located near the epithelium as topical application of local anaesthetic led to a cessation of activity and abolished responses to previously effective peripheral stimuli[191].

Although mucosal afferents have been studied in different organs of various species, including the rat oesophagus[5], antrum and small intestine[72], cat oesophagus[90] and small intestine[148, 189, 192], sheep duodenum[83] and ferret oesophagus, corpus, antrum and duodenum[35, 37, 38, 207] the majority of these studies have concentrated on the mechano- and chemo-sensitivity of gastric mucosal afferents.

4.1.1 Mechanosensitivity of vagal mucosal afferents

Electrophysiological recordings have shown that mucosal receptors are generally silent in the absence of any stimulus[148, 189], although mucosal units with spontaneous activity have been described in the sheep proximal duodenum[83]. Spontaneous activity may also develop in response to repeated chemical stimulation[37, 83, 189].

Mucosal receptors are primarily identified by their sensitivity to light mucosal stroking and are therefore probably capable of fine tactile discrimination. Responses consist of rapidly evoked, intense, and short lasting bursts of activity as the stimulus passes over the receptive field of the afferent. These can be generated by light stroking of the mucosa with a piece of moist cotton wool or tissue paper[5, 72, 83], a brush or a blunt probe[72, 191]. The threshold force needed to evoke a response has been tested with calibrated probes or von Frey hairs, and range from 130mg in the *in vivo* sheep duodenum[83], 300mg in the *in vivo* cat stomach and duodenum[90], and 10mg in the *in vitro* ferret oesophagus[207].

Mucosal receptors are relatively non-responsive to distension or contraction except where it results in excessive stretching of the mucosa[72]. In cases where a response to balloon distension has been observed, the brief burst of activity coincident with the start and end of the distension period may be due to the friction generated between the mucosal surface and the balloon during the dynamic phase of balloon inflation and deflation [72, 90].

An exception to the non-responsiveness to distension or contraction was found in our laboratory by Page and Blackshaw[207] using an *in vitro* preparation. A proportion of fibres identified in the ferret oesophagus which were initially classified as mucosal afferents based on their sensitivity to mucosal stroking were also activated by circular stretch. The circular stretch-evoked responses were similar to those evoked by muscular fibres in the same preparation and led to the classification of these 'dual response' fibres as tension/mucosal (T/M) fibres. Preliminary evidence from another *in vitro* preparation in our laboratory indicates that a similar population of fibres may exist in the rat colon (personal communication with P.A. Lynn). These T/M fibres are thought to have their peripheral endings located within the muscularis mucosa as the resting activity of the fibres were often rhythmic and concomitant with the peak contractions of the muscularis mucosa.

4.1.2 Chemosensitivity and thermosensitivity of vagal mucosal afferents

Mucosal receptors are known to respond to a number of chemical stimuli, such as hyper- and hypo-tonic saline, acid, glucose and amino acids, and to changes in temperature. Reports vary as to whether these receptors are unimodal, ie they respond to a single specified stimulus to the exclusion of all other stimuli, or polymodal, ie they respond to a variety of stimuli.

Unimodal mucosal afferents

Unimodal mucosal afferents which are insensitive to mechanical stimuli and preferentially activated only by a distinct subgroup of chemicals have been identified in the duodenum and proximal jejunum of the cat[148, 189, 192]. These spontaneously silent, C-fibres were not mechanosensitve to stimuli commonly used to activate mucosal afferents, ie mucosal stroking, digital compression of the gut segment, gross distension of the bowel, rapid air infusion and rapid emptying of perfusion. However, the mechanosensitivity of these afferents was not always tested (see below). Chemicals were perfused intraluminally into a closed segment of gut. When activated by chemical perfusion, responses were rapidly evoked and slowly adapting. These mucosal afferents were categorised as 1) 'glucoreceptors' which responded to glucose and other absorbable and non-absorbable carbohydrates, 2) 'amino acid receptors' which responded to solutions of single or multiple amino acids in a seemingly concentration dependent manner, although desensitization generally occured with repeated perfusions, either with the same or different infusion mixtures and concentrations and 3) 'lipid receptors' which were subdivided into those that responded to glycerol and short chain lipids and those that were activated by long-chain lipids. None of these afferents responded to perfusion of KCl or NaCl which were iso-osmolar with other solutions used, to perfusion of acidic or alkaline solutions, or to intravenously administered glucose.

These receptors are thought to be quality specific sensors, able to determine the nutrient content within the small intestine. They were proposed to be involved with

nutrient absorption, regulating the amount of nutrients being delivered into the small intestine by affecting the rate of gastric emptying. However, the location of the receptive fields of these afferents were not identified. They were assumed to be within the portion of the gut that was being perfused as they responded to the perfusate. Where mechanical stimulation was used to attempt to identify the receptive field, the stimuli included strong balloon distension or digital compression of the perfused portion of gut, ie they tended to be more suitable for the identification of muscular, and not mucosal, receptors. In studies where the mucosa was stroked with a soft tipped probe, no responses were observed. This may be due to the probe being too coarse a stimulus or the receptive field being located in another segment of the gut other than the region being stimulated. Also, the range of chemical stimuli tested within each study was not extensive. This means that it was unknown whether these afferents would also respond additionally to stimuli such as close intraarterial cholecystokinin, bradykinin, α–β-methylene ATP, and adrenaline which have been shown in other studies to stimulate mucosal afferents[35, 37, 38, 72]. Thus, it cannot be totally ascertained that these afferents were truly unimodal or that their endings were located in the mucosal layers as the receptive fields were not always identified and the range of chemical stimuli used was restricted.

Chemically unimodal afferents which are mechanosensitive, ie they respond to gentle stroking of the mucosa, have been described by Davison[90] in the cat gastric and duodenal mucosa. These mucosal units respond to either alkaline (sodium hydroxide, pH>8.0) or acidic (acetic or hydrochloric acid, pH<3.0) solutions but not to both. The responses were concentration dependent and remained consistent and specific throughout the duration of the study. None of these afferents were activated by hypertonic solutions, bile, or glucose. These afferents were thought to function as pH detectors, involved with the control of motility as duodenal acidification inhibits gastric peristalsis.

There also exist mucosal afferents which are mechanosensitive but do not exhibit any chemosensitivity[37, 38]. When chemicals were administered either intraluminally or intraarterially, no activation was seen in these fibres. This contrasts greatly with other

afferents identified using the same preparation which responded to close intraarterial cholecystokinin (CCK) and 5-hydroxytryptamine (5-HT) and to intraluminal acid and hypertonic saline. As these 'pure mechanosensitive mucosal afferents' are activated by light stroking, they are highly sensitive to physical changes in the local environment of the receptive field and may be involved in sensing the 'texture' of boli present in the gut.

Polymodal mucosal afferents

Polymodal mucosal afferents have also been found in the gastroduodenal region[35, 37, 38, 72, 83, 125, 191, 200]. These act as rapidly adapting mechanoreceptors in response to light mucosal stroking and often respond to more than one chemical stimuli. Chemical stimuli were applied as perfusions through an open or closed loop of a gut segment, topically on the mucosal surface, close intraarterially into coeliac axis via the abdominal aorta, or intravenously through the jugular vein. The stimuli used included luminal organic and inorganic acids, tap water and distilled water, alcohol, warm isotonic saline (39-55°C), hypertonic saline, sodium hydroxide, copper sulphate, casein hydrosylate, mustard powder, cayenne pepper, fatty acids, olive oil, CCK, 5HT, prostaglandin $F_{2\alpha}$, prostaglandin E_2, papaverine, phenylbiguanide, insulin, acetylcholine, close intraarterial prostaglandin $F_{2\alpha}$, prostaglandin E_2, papaverine, phenylbiguanide, insulin, acetylcholine, CCK, 5HT, bradykinin, adrenaline and α–β-methylene ATP. Responses to intraluminal stimuli were slowly adapting and were often weakly activated. Where large increases in activity was elicited by intraluminal stimuli, eg by copper sulphate and sulphuric acid, the afferent was often rendered non-responsive to subsequent chemical stimulation[200]. Responses to close intraarterial stimuli were often powerfully evoked and were not correlated with changes in motility patterns of the region where the receptive field was located. Responses to close intraarterial 5HT and CCK were still observable after smooth muscle activity was reduced with atropine and hexamethonium, indicating that activation was likely to be direct and not secondary to motor responses. These afferents were proposed to function primarily as chemoreceptors as they would be sensitive to changes in the chemical environment of the gut[37, 38]. The sensitivity may be via a non-specific mechanism through mechanical

deformation of the receptor site as hypo- and hyper-osmolar solutions have been shown to cause histological changes in the mucosa, epithelium and villi.

While the chemosensitivity of gastrointestinal organs distal to the oesophagus have been extensively characterised, few studies have looked at the sensitivity of oesophageal afferents to chemical stimuli. Previously, only 2 reports[76, 131] have investigated the properties of oesophageal mucosal fibres *in vivo*. Recently, *in vitro* studies on ferret oesophageal mucosal receptors have been performed in our laboratory[207, 208] and found that only a few mucosal fibres are chemosensitive.

The first preliminary report[131] showed that a proportion of oesophageal mucosal afferents responded directly to acid (100mM HCl), hypertonic saline (>345mM) and to sodium hydroxide (50mM). These afferents responded with sharp bursts of activity during oesophageal balloon inflation and deflation. The bursts of activity were coincident with friction generated between the balloon and the mucosal surface during the dynamic phases of inflation and deflation. The second study[76] provided evidence that some mucosal afferents in the lower oesophageal sphincter were stimulated by perfusion of warm saline (50-52 C) and acid (HCl, pH2), but not by glucose perfusion. These mucosal afferents were not activated by moderate balloon distension but were found to be stimulated by strong and rapid balloon distension, mucosal stroking, or digital compression of the sphincteric region. In our laboratory, *in vitro* recordings made from the ferret oesophagus found mucosal afferents to be responsive to mucosal stroking and forces as small as 10mg. However, very few were activated by topical application of chemical stimuli, such as capsaicin, 5-HT, prostaglandin E_2, acid (HCl) and bradykinin[208].

Thermosensitivity of vagal mucosal afferents

One uncorroborated study has explored the presence of specific thermoreceptors[101]. These have been identified in the cat lower oesophagus and stomach

by continuous infusions of saline at specific temperatures and were classified into 3 groups; cold receptors discharged at 10-36°C, warm receptors were activated at 39-40°C, and mixed receptors were stimulated at 10-35° and 40-50°C. These afferents were neither chemo- nor mechano-sensitive and were proposed to be involved in thermoregulation and the coordination of motility as the rate of evoked oesophageal contractions can be altered by warm and cold stimulations. As the receptive field of these afferents were not identified, it is hard to assign a definitive role to them.

4.2 Vagal muscular afferents

Afferents with peripheral endings in the muscular layer of the gastrointestinal tract are often referred to as mechanoreceptors as they are mechanically sensitive and respond to distension, contraction, and compression of the region where the receptive field is located. In some cases, they are also referred to as tension receptors as the degree of activation is often related to the intraluminal pressure generated during distension which is proportional to changes in intramural tension. Muscular afferents have been suggested to serve different functions depending of their location within the gastrointestinal tract. For example, receptors located in the lower oesophageal sphincter were suggested to act as sensors of sphincter opening and closing[76]. Muscular afferents located in the corpus and fundus, on the other hand, have been proposed to signal the degree of distension whereas those in the antrum may provide information of the amplitude, rate and duration of antral contractions[12].

4.2.1 Mechanosensitivity of vagal muscular afferents

Oesophageal muscular afferents

Vagal oesophageal tension receptors have been identified both *in vitro* and *in vivo* in the ferret, opossum, dog, cat and sheep[76, 107, 207, 210, 232, 239, 240]. They are classified as a mixture of C-fibres and Aδ-fibres based on their conduction velocities (<2.5m/sec for C-fibres). These posses a spontaneous irregular discharge in the absence of

any stimulus which can be modified by lung inflation or exhibit a rhythm synchronous with the arterial pulse[232]. Oesophageal tension receptors are usually identified by distension of an oesophageal segment either with a balloon or with fluid in the case of *in vivo* preparations or with circular stretch in the case of *in vitro* preparations.

The responses to distension follow a general triphasic pattern; the initial phase consisted of a sharp rise in discharge levels during the dynamic period of distension. In the second phase, the discharge adapted to a lower level but remained above resting level throughout the static portion of the distension period. The third phase occurred when pressure was rapidly withdrawn, resulting in a fall in discharge levels below the resting level. The discharge of some fibres during the third period ceased for 2-4 seconds (silent period) before prestimulus levels of discharge were regained. Large increases in discharge could be obtained with small pressure changes; these increases in discharge were linear over a narrow pressure range and were saturated within normal physiological ranges. Fibres with a higher conduction velocity had a higher rate of firing in response to oesophageal balloon distension[239, 240].

Oesophageal muscular fibres can also be activated by oesophageal contractions elicited by electrical stimulation of the vagus[239]. Oesophageal contractions produced a strong response in these receptors which was followed by a silent period before spontaneous activity was resumed. Muscular afferents located with the lower oesophageal sphincter modulated their discharge in response to stretching of the sphincteric region. Reinforcement of sphincter closure elicited by vagal electrical stimulation led to an increase in discharge[76].

These oesophageal muscular fibres were classified as being in series with either the longitudinal or the circular muscle based on their response to swallow induced oesophageal peristalsis[239]. The fibres that responded during peristalsis with a short burst of activity which occurred concurrently with the peristaltic contractions were classified as being in series with circular muscle layers as this layer is known to contract for a similar duration

during peristalsis. The second group of afferents were activated prior to the onset of the contraction and lasted for several seconds after the contraction. These afferents responded additionally to longitudinal muscle stretch and were thought to be in series with the longitudinal muscle layers as these are known to contract for a longer period during peristalsis. Regardless of their orientation within the muscular layers, these oesophageal muscular receptors are thought to be involved in the normal coordination of oesophageal peristalsis as their response to distension is linear over a physiological range.

Some of these oesophageal tension senstive fibres send projections, not only to centrally via the cervial vagal branch, but also peripherally along the gastric vagal branches[266]. These 'centrifugally' directed strands were seen to have a typical tension receptor response to oesophageal distension and were thought to represent the functional correlates of those axon collateral reflexes which mediate lower oesophageal sphincter relaxation when the oesophagus is distended.

Gastric muscular afferents

The behaviour of gastric muscular afferents differs according to their location within the stomach[12, 45, 91]. The corpus functions as a reservoir and can accommodate large increases in volume with a relatively small increase in intraluminal pressure. On the other hand, the antrum functions primarily as a mechanical pump designed to pulverise and break down food in order to deliver it at a regulated pace into the duodenum. In the ferret, distension of the intact stomach with saline resulted in 80% of fluid being disproportionately accommodated in the fundus and corpus and the remainder entering the antrum[12]. Thus, there are variations in the pattern of spontaneous discharge of vagal afferents from the corpus and the antrum and their response to motor activity and intraluminal pressure.

Afferent fibres from the corpus possess a low level, spontaneous discharge in the absence of any stimulus[12, 45]. Recordings from antral tension receptors can show resting

activity which was modulated in phase with spontaneously occurring antral contractions[12]. Distension of the ferret corpus resulted in a response similar to that evoked in oesophageal muscular afferents by distension[12, 36, 38]. A rapid increase in vagal afferent discharge was immediately apparent when fluid was instilled into the corpus. The slowly adapting response was maintained until the corpus was deflated and were sometimes further modulated by contractile activity of the corpus, often resulting in phasic oscillations of discharge. Upon deflation of the corpus, the discharge quickly returned to predistension levels, often after a period of reduced activity. The degree of activation produced by distension was proportional to the volume of fluid instilled into the corpus. Distension of the corpus under near-isotonic conditions led to small changes in gastric pressure and vagal activity, even with a large change in volume. On the other hand, contractions and relaxations of the region produced under isovolumetric conditions led to oscillations in discharge that were closely related with motor activity. This suggests that corpus muscular receptors function as tension receptors as the activity of these afferents is modulated by changes in intraluminal pressure rather than length or volume.

Distension of the ferret antrum, on the other hand, resulted in an increase in afferent discharge in phase with the increase in antral motility. Unlike corpus tension receptors, antral mechanoreceptors do not necessarily respond in a volume dependent manner to fluid distension. Instead, they exhibit a phasic discharge pattern which is closely correlated to the contractile activity of the antrum. Thus, the discharge pattern is often closely related to the rate, amplitude, and duration of each wave of contraction.

In the rat, muscular receptors in the fundus, corpus, and antrum also respond to distension in the a similar manner to that of ferret corpus muscular fibres, ie the response was triphasic[91, 183, 234, 236]. Distension resulted in a sharp increase in discharge levels during the dynamic phase of inflation which adapted over a period of several seconds whereby discharge was maintained at a lower static level until removal of the gastric contents. Deflation was often accompanied by a drop in discharge below basal levels, often resulting in a distinct 'silent period' before discharge returned to resting levels. A

similar triphasic response could also be obtained by longitudinal stretching of the gastric wall. The degree of afferent excitation was roughly proportional to the degree of distension, although there is a great variability in the threshold volume, and therefore intraluminal pressure, required to elicit a response.

In the distal portion of the gut, muscular afferents in the small intestine and colon respond to distension and contractions[82, 84, 85, 188, 235], to generate continual information on the passage of food through the digestive tract and the level of contractile activity in the various areas of the gut.

4.2.2 Chemosensitivity of vagal muscular afferents

There is an ongoing debate as whether muscular afferents respond to chemical stimuli directly or secondarily though changes in muscle tension. This issue is made even more controversial as in some studies, the motility patterns of the organ being studied were not properly controlled. In other studies, however, the increase in discharge was seen to coincide with contractile activity of the muscle.

One such study was performed in the opossum oesophagus[240] where intraarterial administration of bradykinin (2-300μg/kg) evoked longitudinal muscle contractions which in turn activated a proportion of vagal muscular afferents. The same was also true of muscular afferent responses to 5-HT, also administered close intraarterially[36]. Here, the responses of vagal muscular afferents to 5-HT was seen to be directly dependent on the changes in motor activity, which were either contraction or relaxation depending on the region of the gut. Tension receptor responses which are secondary to changes in muscle length, tension or intraluminal pressure have also been evoked by drugs such as cholecystokinin, noradrenaline, acetylcholine, pentagastrin, prostaglandins and bradykinin[36-38, 81, 84, 109, 124] and perfusions of chemicals including KCl, HCl, glucose, peptone, acetic acid, butanoic acid, sodium hydroxide and sodium bicarbonate[85, 235].

Other studies have proposed that mechanoreceptors are directly sensitive to chemical stimuli[91, 234, 236]. Here, vagal afferents which were shown to have a typical tension receptor response to increased intraluminal pressure caused by distension were activated by both cholecystokinin (CCK) and gastrin releasing peptide (GRP). However, CCK inhibited antral (or whole gastric) pressure while GRP increased the contractile activity of the antrum. Thus, the activation by cholecystokinin was thought to be direct through activation of CCK receptors present in the peripheral endings of the afferents[195] whereas the GRP response was presumed to be secondary to changes in motility. This is obviously in direct contrast to those studies in which mechanoreceptors were proposed to be indirectly activated by chemical stimuli. The discrepancy might be due to the dose of cholecystokinin used as well as the method for recording intraluminal pressure. Firstly, in studies where CCK was thought to have a direct action on muscular receptors, the octapeptide was administered at doses which may have been above the physiological range. Secondly, in these studies, intraluminal pressure may not have been accurately recorded. Antral mechanoreceptors can be briefly stimulated by CCK secondary to contractions[38]. However, if whole gastric pressure was being recorded, the overall effect may be one of relaxation due to proximal gastric relaxation. It may not be a true reflection of the localised antral contractions which may have occurred to give rise to an increase in mechanoreceptor activity.

In other studies, changing the chemical composition of the perfusate did not alter the responses of muscular afferents to distension[183]. In this study, vagal afferents which responded to gastric distension with physiological saline were also similarly affected by distension with glucose, peptone or hypertonic saline. The authors concluded that these tension sensitive afferents responded to changes in volume and not to the actual chemical composition of the gastric contents, ie they were pure mechanoreceptors which did not exhibit any chemosensitivity.

4.3 Vagal serosal afferents

Afferents with peripheral endings below the serosal surface are generally thought to be involved in mediating visceral pain, and as such traditionally associated with splanchnic and other sympathetic nerves[75]. They can have multiple receptive fields and have been classified as C-fibres by their conduction velocity. Although few in number when compared to mucosal and muscular afferents, vagal fibres with endings in the serosal layer have been identified electrophysiologically in the sheep and rabbit duodenum and in the cat oesophagus[76, 82, 85]. These are thought to be activated in circumstances where strong stretching and distension occur, such as vomiting.

4.3.1 Mechanosensitivity of vagal serosal afferents

Vagal serosal receptors cannot be stimulated by gentle probing of the mucosal surface, mucosal stroking or contraction of the muscular layer[76, 82, 85]. Rapidly adapting responses were generated by touching or probing the serous membrane, tangential and longitudinal stretch of the serosa, strong balloon distension, application of punctate pressure on the serous membrane and compression of the serosal membrane. Maximum frequencies were generated during the initial second of the response, after which it quickly decreased to disappear completely 10 seconds after the commencement of the stimulus. Application of a local anaesthetic to the serosa diminished responses to even the most potent stimuli.

4.3.2 Chemosensitivity of vagal serosal afferents

The chemosensitivity of these afferents has only been evaluated in one study[85]. Serosal units responded to topical serosal applications of veratrine, CCK, KCl, atropine and hexamethonium and to intraarterial 5-HT. As changes in wall tension were not monitored regularly in this study, it was not possible to ascertain whether the afferents responded directly to the chemicals or secondarily to changes in wall tension.

4.4 Functional roles of vagal afferent

Vagal afferents have been implicated in nausea and vomiting, satiety and hunger, heartburn and possibly pain.

For example, CCK has been shown to strongly stimulate vagal gastroduodenal mucosal afferents[38, 39]. Endogenous CCK released in the duodenum by the presence of nutrients may have a paracrine action on afferents which inhibits gastric emptying via vagal reflexes and in parallel directly influences food intake by causing satiety. In ferrets, electrical stimulation of the unique supradiaphragmatic vagal communicating branch or intraduodenal injections of hypertonic saline, both of which have been shown to induce vomiting, leads to activation of neurones within the DVC[47]. Vagal mucosal afferents are implicated in the emetic response as vagotomy and granisetron, a 5-HT3 antagonist, abolished the emetic response triggered by a number of emetic agents[6].

The involvement of vagal afferents in pain is seen when gastric distension at a level which is considered to be noxious (80mm Hg) leads to expression of c-fos activity in the NTS and DMVN[258]. This is mainly vagally mediated as bilateral section of the nerve results in a marked reduction in c-fos content. In fact, the induction of c-fos in the NTS and DMVN by noxious gastric ditension was greater than that in the thoracic spinal cord, which may indicate that vagal afferents are involved in pain perception.

4.5 Spinal afferents

The electrophysiology and functional roles of afferents following non-vagal (spinal) pathways will be discussed as inputs following pathways other than the vagus have been recognised to be important in influencing vagal outflow to the gut[126].

Non-vagal mechanosensitive afferents with endings in the cat, dog and opossum have been described. The location of the afferent endings were found within the oesophagus, gastric fundus, corpus and antrum, duodenum, pancreas, spleen, small

intestine, and colon[75, 110, 135, 146, 238]. These afferents may have multiple receptive fields, between 1-8 punctate locations, distributed along the course of the nerves. The receptive fields were found in the serosa, the muscular layer, mucosa, mesentery or periptoneal ligament attached to the gut. The density of spinal innervation varied between organs. Within the oesophageal body, there were significantly more vagal than spinal afferents. However, the proportion of spinal afferents increases distally along the gastrointestinal tract.

Spinal afferents were identified as C- and Aδ fibres by their conduction velocities. The majority of spinal afferents from the proximal gastrointestinal tract follow the splanchnic, lumbar colonic, or pelvic nerves although many oesophageal afferents follow the sympathetic chain[241].

4.5.1 Mechanosensitivity of spinal afferents

Mechanoreceptors with endings in the muscular layers were mainly spontaneously active[75, 174]. However, this basal discharge was often changed after administration of several stimuli. Those located in the oesophageal body were activated by contraction of the LOS (elicited by vagal or splanchnic stimulation), local balloon distension, digital compression, and longitudinal stretching of the lower oesophagus. These responses were slowly adapting and had a low threshold of excitation. Muscular receptors located elsewhere within the gastrointestinal tract were activated by gentle probing, or placement of a saline soaked pledget. Application of a local anaesthetic on the serosal or the mucosal surface did not affect either spontaneous or evoked discharge.

Mechanoreceptors with endings in the serosal layers, on the other hand, were silent in the absence of any stimulus[75, 110, 174]. They did not respond to mucosal stroking or contraction of the muscular layers. Serosal mechanoreceptors in the oesophagus were activated by strong distension, serosal stroking and longitudinal stretching. The responses elicited were rapidly adapting and required a high threshold for activation. Serosal

mechanoreceptors in other gastrointestinal regions were tonically activated by maintained pressure administered by probing the serosal surface with a blunt probe or to stretching of the gut wall[110]. They could also be activated by air blown lightly on the serosal surface. Like the responses of vagal serosal fibres, the responses of splanchnic serosal fibres were abolished with serosal application of a local anaesthetic[32, 76, 82, 85].

In some studies, no attempt was made to investigate the location of the peripheral afferent endings within the gut wall. These were identified as mechanoreceptors by their response to distension and have been identified in the colon and oesophagus[46, 135, 146, 147, 238, 240, 241]. Spinal afferents from the opossum oesophagus were categorised into 2 groups based on their threshold of response to balloon distension[240, 241]. Wide dynamic range mechano-nociceptors (WDR-MNs) have a low threshold pressure of response and were activated by increases in pressure that were both innocuous and noxious whereas high threshold mechanonociceptors (HT-MNs) were activated only at higher increases in pressures which were in the noxious range. The responses to balloon distension of both types of afferents were non-adapting and reproducible with repeated periods of distension. WDR-MNs responded additionally to oesophageal contractions evoked by vagal stimulation and to longitudinal stretch of the oesophageal body. HT-MNs did not respond to the contractile activity of the oesophagus but responded to intense longitudinal muscle stretching. As WDR-MNs can be activated by pressures which range from the normal physiological range to the noxious range, these afferents may be involved in both normal coordination of oesophageal peristalsis as well as involved in mediating painful sensation. On the other hand, HT-MNs may well be recruited (activated) only when nociceptive events occur.

In the colon[46, 135, 146, 147, 238], the activity of afferent fibres were recorded from the inferior splanchnic nerves or the sacral dorsal roots (pelvic nerves). These afferents were initally identified by pelvic nerve stimulation, in the case of recordings made from the dorsal rootlets, or lumbar colonic nerve stimulation, in the case of recordings made from the inferior splanchnic nerves. Only a proportion of afferents had

identified peripheral endings in the wall of the colon or in the mesentery. Also, not all afferents were mechanosenstiive, ie some did not respond to any of the mechanical stimuli applied, ie colon distension, probing with a glass rod or contractile activity. When a response was obtained to distension, these were mostly rapidly evoked, slowly adapting and lasted only for the duration of the stimulus. The responses were proportional to the increase in intraluminal pressure. These distension-sensitive afferents also responded to contractions. The threshold pressure needed to activate afferents was variable and may play a part in differentiating between those afferents that are activated by normal physiological increases in pressure and those responding only to increases in intraluminal pressure that are within the noxious range.

4.5.2 Chemosensitivity of spinal afferents

The 2 most common chemicals used in examining the chemosensitivity of spinal afferents are bradykinin which is released at the site of tissue injury and produces an acute inflammatory response, causing pain, oedema and increased blood flow at the site of injury[262] and capsaicin, which is known to activate small diameter unmyelinated nerve fibres. In most cases, the responses to the chemicals were similar in latency, duration, and amplitude regardless of whether they were administered either topically or close intraarterially[109, 135, 174].

Responses to bradykinin were either thought to be direct on the afferent endings or secondary to mechanical activity. Bradykinin led to the direct activation of mechanosensitive afferents in the oesophagus and colon via the B_2 receptor subtype[238, 240]. In the oesophagus, the increase evoked by HT-MNs was almost five-fold the intensity of the response generated by WDR-MNs which is consistent with the possible function of HT-MNs as primarily nociceptive fibres. Responses to bradykinin were considered to be direct as 1) partial tachyphylaxis was seen in the afferent response with repeated administration even though tachyphylaxis was not observed with muscle

contractions, 2) the intensity of response was well in excess of the response generated by balloon distension, and 3) antagonism of fibre responses was seen with BK analogues without changes in muscle contracility.

Those which were thought to respond indirectly include hypogastric afferents which were sensitive to bladder distension and contraction and splanchnic afferents from the colon, small intestine, pancreas and ileocaecal junction[109]. These responses are secondary to the contractile activity of the bladder as abolition of mechanical activity by transection or cooling of the spinal cord abolished the afferent responses to BK.

Other afferents were thought to have both direct and indirect components of their response to bradykinin. This was the case with splanchnic afferents innervating the cat colon[135]. The increases in discharge were originally thought to be indirect through contractions of the colon. However, a direct component is thought to exist as in some fibres, a response was elicited in the absence of any contractile activity of the colon. The bradykinin response was still present even when contractile was abolished with loperamide, an opiate agonist, which paralysed the colonic smooth muscle.

In other studies, the changes in motility evoked by bradykinin and capsaicin were not properly observed[174]. The effects of bradykinin and capsaicin were presumed to be indirect in fibres which were also mechanically sensitive (in the case of A fibres) and direct in those that were insensitive to mechanical stimuli (in the case of C fibres).

Spinal afferents[135] were also activated by ischaemia, intraarterial injections of KCl (330mM) and hypertonic saline (1.2M). Both solutions evoked short latency, rapidly adapting responses. Ischaemia of the colon produced increases in resting activity which was partially, but not exclusively, due to the increased contractility of the colon and also enhanced afferent responses to distension.

4.5.3 Functional roles of spinal afferents

Spinal afferents are involved in various roles including the mediation of pain and sensation and spino-spinal and spino-vagal reflexes[66, 67].

Spinal afferents are involved in coordinating gastrointestinal motility. It is known that intestinal and colonic distension can lead to an inhibition of gastric motility and tone[129]. This is considered to have a sympathetic component to the reflex, with the inhibition due to catecholamines occurring at 3 levels, ie presynaptically onto vagal preganglionic fibres, onto myenteric neurones and directly onto smooth muscle. The tonic inhibitory sympathetic activity is uncovered by splanchnectomy and spinal section, whereby the decrease in gastric motility and tone evoked by colonic distension is reduced.

Generally, spinal afferents are presumed to be involved in painful sensations whereas the vagus is thought to mediate general sensations[123]. This is seen in studies by Sengupta et al[239-241] where, with the same experimental model, recordings were made from both vagal and non-vagal (spinal) afferents. Vagal mechanoreceptors responded only to intraluminal pressures within normal physiological ranges whereas spinal afferents responded to distension pressures above the normal physiological range, including those considered noxious. This would imply that while vagal afferents are primarily involved in monitoring responses within the normal physiological range, those following sympathetic pathways are mainly involved with sensing responses in the nociceptive range. However, the presence of the WDR-MNs which respond to both innocuous and noxious pressures may mean that there is some overlap in sensitivity, with spinal afferents able to contribute to normal gastrointestinal function as well as non-painful sensations.

Although less than 10% of afferents at the level of the spinal cord are gastrointestinal in origin, they can converge with inputs from somatosensory afferents and project to higher sensory areas[67]. These afferents thus possess extensive actions on neurones within the spino-thalamic and spino-reticular tracts. Activation of visceral spinal afferents would therefore result in the stimulation of somatic sensory pathways. It may be

possible that this diffuse organisation of afferent input is similar to a trip wire alarm system which does not necessarily discriminate inputs from different regions but has the potential to trigger a great effector response in the form of diffuse pain, visceral reflexes and muscle spasms. This would lead to a sensation that would be referred to the origin of the sensory drive and may form the basis for referred visceral pain.

4.6 Processing of afferent inputs in the brainstem

As stated previously in the neuroanatomy section of this introduction, the majority of procesing of gastrointestinal inputs to vagal central neurones occurs within the dorsal vagal complex (DVC) in the dorsal medulla. Processing of inputs can be examined at various stages along the reflex pathway. Recordings from single unit vagal central neurones have been made either within the DVC or from the cervical vagus. Extracellular recordings from the DVC are either from second-order or interneurones within the NTS or from neurones within the DMVN, which is the site of most cell bodies of vagal efferent fibres. Those made in the cervical vagus are from the peripherally projecting axons of these cell bodies. Obviously, the location that the recording is made may influence the degree of convergence observed.

4.6.1 Convergence of gastrointestinal inputs onto vagal central neurones

Vagal efferents have been shown to receive convergent inputs from different populations of afferents, ie from mechano- and chemo-sensitive afferents, from mucosal, muscular and serosal afferents, from afferents with peripheral endings located in different organs and from those following different pathways.

Recordings made from the NTS and DMVN have shown that these central neurones receive inputs from gastric and duodenal mechanoreceptors, 'volume receptors' in the hepatic circulation, hepatic glucoreceptors, cholecystokinin-, substance P- and duodenal acid- sensitive afferents[16, 21-24, 71, 185, 219, 277]. Other recordings made further down

the reflex pathway, ie from single unit vagal efferent fibres at the level of the cervical vagus, have investigated the reflex modulation of efferents by activation of mechanoreceptors in the oesophagus, stomach, duodenum, jejenum and colon by distending that particular portion of the gut and of mucosal receptors sensitive to intraluminally administered acid, hypertonic saline, serotonin and sodium hydroxide[16, 33, 41, 43, 92, 93, 105, 126].

Activation of mechanoreceptors via distension led to either an increase or a decrease, either partial or complete, in vagal efferent discharge. The excitatory responses were similar in profile to the responses obtained by afferent fibres to the same stimuli, ie they were rapidly evoked, showed a dynamic and static component, and returned rapidly to predistension levels upon deflation after a short period of inhibition. The inhibitory responses were mirror images of the afferent responses to distension. The intensity of the response was proportional to the level of inflation once the threshold volume had been reached. In the rat, a few fibres were inhibited at low levels of gastric distension and excited at high levels of distension, or vice versa[93]. In some fibres, the change in discharge generated by distension was also modulated by motility patterns such that an increase in discharge was seen whenever contractions occurred. The modulation of discharge patterns by gastric motility may be related to the peripheral location of the afferent inputs, ie those located along the greater curvature of the antrum may be subject to a higher degree of contractions.

Efferent responses to chemical stimuli were more variable in their latencies. This ranged from 5-120 seconds, ie from a gradual change in efferent discharge to a sharp, well defined response, and may be influenced by diffusion through the mucosal barrier. Responses would be either inhibitory or excitatory, with some fibres showing an increase in discharge to one stimulus and a decrease to others. Responses to chemical stimuli were generally smaller in amplitude than those to mechanical stimuli, implying that inputs from chemosensitive afferents were minor compared with those from mechanoreceptors.

In these recordings of vagal central neurones either from the DVC or from the cervical vagal region, most studies have discovered that these neurones receive convergent inputs from mechanoreceptors in different regions of the gut and from chemosensitive afferents, ie they receive sensory information from more than one type of afferent.

4.6.2 Convergence of spinal and vagal inputs onto vagal central neurones

The involvement of spinal afferents in vagal reflexes is seen in electrophysiological studies[40, 71, 126, 129, 219]. The non-vagal component of the afferent inputs onto vagal central neurones is revealed when vagal efferent activity, both basal and in response to certain stimuli, is maintained after bilateral vagotomy. Vagotomy led to the loss of over half the responses to gastric distension whereas those to duodenal and colonic distension were relatively unaffected. Responses to intestinal distension were elicited with both high and low pressure distension and were demonstrated to be spinal in origin as temporary spinal cooling, after vagotomy, reversibly abolished the distension response. The input from splanchnic afferents is further confirmed in studies where stimulation of the greater splanchnic nerve reflexly activated responses in vagal efferent fibres[40]. This data has several implications; 1) the major afferent pathway from gastric mechanoreceptors is vagal whereas those from duodenal and colonic mechanoreceptors are mainly non-vagal and 2) both vagal and non-vagal afferents converge onto vagal central neurones and 3) these ascending spinal pathways convey both innocuous and noxious information to the DMVN.

4.6.3 Pharmacology of afferent inputs to spinal cord and brainstem

The neurotransmitters involved in mediating afferent inputs to the spinal cord and brainstem has been investigated extensively in the control of respiration and cardiovascular responses. For example, an *in vitro* heart-brainstem slice preparation developed in neonatal mice, NK1 receptors in the NTS were established to be involved in mediating inputs from left ventricular vagal receptors[211]. Non-NMDA receptors are thought to be present in the area of the NTS which is involved in the Breuer-Hering reflex[56]. Both

NMDA and non-NMDA receptors in the NTS are also involved in the integration of baroreceptor afferent inputs in the NTS[276] as well as in mediating inputs from vagal cardiopulmonary fibres[261, 269].

While direct evidence exists for the involvement of various neurotransmitters in cardio-respiratory reflexes, most of the data regarding the involvement of neurotransmitters in afferent pathways arising from the gastrointestinal tract can only be indirectly inferred from behavioural studies. In one such study[215], sensitisation of visceral afferent pathways was inferred by the increased number of abdominal contractions evoked by colorectal balloon distension after intracolonic administration of acetic acid. The occurence of abdominal contractions was taken to be a measure of visceral pain. The involvement of visceral afferents was implied as hypersensitivity was not seen in anmals which were previously treated with systemic capsaicin. CGRP was thought to be involved as intravenous administration of CGRP mimicked the effects of acetic acid and the hypersensitivity caused by either CGRP or acetic acid was abolished by intrathecal administration of the CGRP receptor antagonist, hCGRP8-37. In a second similar study, the involvement of the neurokinin 1 (NK1) and 2 (NK2) receptors[150] was investigated. Here, the effects of NK1 and NK2 receptor agonists and antagonists on abdominal contractions and inhibition of colonic motility evoked by rectal balloon distension were reported. NK1 receptor antagonists, injected intracerebroventricularly or intraperitoneally, abolished the colonic inhibition but not the abdominal response induced by rectal distension. On the other hand, either central or peripheral administration of the NK2 receptor antagonist reduced the abdominal response while leaving the colonic inhibitory response unaffected. With these two studies, it can be seen that both CGRP and NK2 receptors appear to be involved in mediating visceral pain and that there is a central component in this pathway.

There is one study which investigated the neurotransmitter involvement of gastric inputs to the NTS[275]. However, in this *in vitro* neonatal gastric-brainstem preparation, no attempt was made to duplicate the physiological stimulus which could activate these vagal

afferents. Afferents were characterised as being of gastric origin when an action potential was generated with electrical stimulation of the gastric branch of the subdiaphragmatic vagus. In addition, only a small proportion of afferents reduced their evoked discharge when GABAa and GABAb receptor agonists were applied to the medial NTS region.

Thus, it can be seen that there is a lack of information regarding the pharmacology of central processing within the spinal cord and brainstem as it relates to information arising from the gastrointestinal tract. The evidence that we have tends to be circumstantial at best, or incomplete.

5 VAGAL CONTROL OF GASTROINTESTINAL MOTILITY

The extensive convergence of afferent input onto vagal efferent fibres reflects the wide range of gut reflexes mediated by the vagus. These range from the mechanical passage of food through the digestive tract to the secretion of hormones and other substances to aid in the breakdown and ingestion of nutrients. Each process would require information from a variety of afferent inputs. For example, the rate of gastric emptying is dependent on the extent of duodenal distension, the amount of CCK release in response to the nutrient load, and can also be controlled by regulating the amount of food that enters the antrum from the main body of the stomach. The CNS would therefore require information from gastric and duodenal tension receptors and from mucosal afferents so that it would properly coordinate the gastric emptying process. Those efferent fibres which are involved in the control of peristalsis would require information from tension receptors oral and aboral to the position of the bolus. Thus fibres mediating different functions would have inputs from several distinct populations of afferents.

It may be conceptually difficult to assign a definitive role for the vagus in the control of gastrointestinal function as extensive innervation by the enteric nervous system is undeniably capable of modulating changes in motility and secretion independently of any central or spinal connections. However, despite the ability of this intrinsic system in

providing sensory and motor functions in the local control of gastrointestinal function, the vagus is still required to integrate and coordinate reflex events which occur in response to the passage of food along the length of the gastrointestinal tract.

The vagus is able to coordinate between different regions of the gut during the digestive process through feedback and feedforward mechanisms, eg the stimulation of pancreatic juice secretion caused by food in the stomach, and the slowing of gastric emptying by duodenal distension. It can also faciliate rapid communication with other organ systems, eg vomiting evoked by activation of vagal afferents leads to reflex changes involving synchronisation of cardiovascular, respiratory and gastrointestinal systems. Its extensive connections with the higher brain centres ensures that integration of neuroendocrine and behavioural functions occurs in response to external environmental factors.

The vagus is involved in the modulation of a wide range of gastrointestinal functions including coordination of motility throughout the length of the entire gut, tonic control of the lower oesophageal and ileocaecal sphincters, and release of somatostatin, gastrin and other mediators.

Its role can be elucidated by studying changes in motility and secretion patterns following acute section of the nerve or temporary blockade of activity by cooling of the vagal trunks. The former technique also allows study of the efferent function of the vagus through stimulation of the peripheral end of the severed nerve [14, 127, 132]. With the latter technique, observations can be made after vagal drive is restored.

5.1 Vagal control of motility

In the stomach, the vagus is heavily involved in controlling motility during distension. Tonic control of intragastric pressure is mediated via vagal cholinergic excitatory fibres which can be reflexly activated by distension to cause an increase in wall

tension, and therefore a rise in gastric pressure. This elevated pressure response is blocked in the ferret by atropine. Vagal non-cholinergic, non-adrenergic inhibitory and splanchnic fibres also play a role in regulating the gastric pressure response, although the effects of the latter are suppressed in the vagally intact animal[10].

The passage of food along the digestive tract is coordinated by the vagus. For example, swallowing, sham feeding and oesophageal distension induces relaxation of the lower oesophageal sphincter via a vagal inhibitory non-cholinergic, non-adrenergic transmitter mechanism. Upon entering the stomach, the majority of fluid is accomodated in the corpus[12]. This triggers a receptive relaxation such that large quantities of food can be accomodated in the main reservoir of the stomach without a large increase in pressure. The mechanisms involved in mediating the corpus relaxation include inhibition of vagal cholinergic excitatory pathways and the stimulation of myenteric neurones that release inhibitory neurotransmitters such as vasoactive intestinal peptide (VIP) and nitric oxide (NO)[98]. Corpus distension also triggers peristaltic contractions in the antrum, aiding in the mechanical pulverising of the food as well the control of gastric emptying. The antral contractile activity is severely curtailed by bilateral cervical vagotomy and enhanced by splanchnectomy, indicating the presence of an inhibitory tonic sympathetic pathway and an excitatory vagal reflex pathway[11].

The vagus is involved with normal feedback regulation in different portions of the gut. For example, there exists a splanchno-vagal reflex which inhibits gastric motility and tone with distension of either the duodenum or the colon[129]. As this inhibition is elicited with low levels of duodenal distension, this reflex may play a part in inhibiting antral contractions, which would slow gastric emptying and limit the quantity of food-chyme passing through to the intestines and hence prevent overfilling.

The vagus is also involved in the control of secretion as both gastric distension and electrical stimulation of the peripheral end of the cut vagus in the ferret leads to secretion of gastric acid and pepsin, and pancreatic amylase[11, 127, 132]. This gastric acid secretion

was found to be independent of gastrin release. Vagal stimulation also leads to intestinal and gastric contractions regardless of the stimulation frequency used[14, 132].

5.2 Transient lower oesophageal sphincter relaxations

Transient lower oesophageal sphincter relaxations (TLOSRs) are considered to be the main mechanism by which the pathophysiological gastro-oesophageal reflux disease develops. TLOSRs are triggered spontaneously and allow for gas and acid reflux from the stomach that occurs with belching and gastro-oesophageal reflux. They are not associated with the normal LOS relaxations that occur during a normal peristaltic event, eg one triggered with swallowing. They occur mainly postprandially when the stomach is distended. In the laboratory setting, TLOSRs can be induced by gastric air insufflation in the conscious dog[181]. This phenomenon is at least partially vagally mediated as cooling of both vagal trunks abolished their occurence. Vagal blockade also abolished common cavities and stomal gas venting, two consequences of TLOSRs which are caused by gaseous distension of the stomach.

As TLOSRs can only be triggered in conscious animals, the pharmacology of the neurotransmitter mechanisms was studied in our laboratory by evoking LOS relaxations either with oesophageal infusions of acid or with vagal nerve stimulation in the anaesthetised animal[34, 152]. Oesophageal acidification led to LOS relaxations which increased in depth with repeated infusions. This inhibition of LOS pressure is mediated via the release of substance P from vagal or spinal axon collaterals and acts via the neurokinin-1 (NK-1) receptor[34]. LOS relaxations produced by stimulation of the central cut end of the cervical vagus nerve, on the other hand, involves α-adrenergic input to the myenteric plexus which may cause the release of nitric oxide from inhibitory myenteric motor neurones[34]. Thus the control of lower oesopahgeal sphincter pressure is still being investigated in order to better our understanding of TLOSRs and reflux disease.

6. AIMS OF THE STUDY

This study seeks to further characterise vagal afferent and efferent fibres using an established electrophysiological technique. Although extensive research has been performed on the mechanical and chemical sensitivity of vagal afferent fibres, the chemosensitivity of vagal oesophageal mucosal afferents has only been investigated in 2 studies. Also, the presence of receptors on the peripheral terminals of the mucosal and muscular vagal afferents in the upper gastrointestinal tract will be investigated. In this study, more work was done to determine the extent of convergent inputs received by vagal efferent fibres. I investigated the role of various neurotransmitters in mediating these inputs onto vagal central neurones. Although there is a large number of potential candidates involved in the transmission of information from the gut, I have selected those most strongly indicated by anatomical, physiological and pharmacological literature to be involved in these reflexes. These include acetylcholine via the muscarinic receptors, substance P via the neurokinin-1 (NK-1) receptor, calcitonin gene-related peptide (CGRP) via the CGRP1 receptor, and glutamate via both N-methyl-D-aspartate (NMDA) and non-NMDA receptors. The involvement of the neuromodulator γ-amino-butyric acid (GABA) via the GABAb receptor was also investigated.

Methods

1 GENERAL

Experiments were performed on 117 adult ferrets (Mustela putorius furo L.) of either sex weighing between 0.5-1.4kg. They were fed a standard carnivore diet with free access to water but were deprived of food for approximately 18 hours prior to experimentation. No residual food was found in the stomach upon cannulation following laporatomy. All animals were in good general health and did not display any signs or symptoms of illness. Female ferrets were routinely checked for signs of pregnancy prior to the administration of anaesthetic to avoid using pregnant ferrets for experimentation. Care was taken when handling the animals to prevent undue stress.

2 ANAESTHESIA

Ferrets were initially anaesthetised with 1.25g/kg Urethane intraperitoneally. The abolition of the hindlimb-withdrawal reflex upon pinching the limb was taken to indicate that a sufficient depth of anaesthesia had been achieved. Animals were given additional 0.2ml bolus injections of Urethane (0.5g/ml ip) as necessary to reach the required level of anaesthesia prior to commencing the surgical procedures. The hindlimb-pinch test was routinely performed throughout the experiment to monitor the depth of anaesthesia with additional anaesthetic administered as a 0.25g/ml solution intravenously as required.

3 SURGERY

The surgical preparation of the animals varied slightly from study to study as vagal efferents are known to receive input from a wide variety of afferents. Flexibility in the preparation of individual animals thus ensured that the vagal efferent responses to a range of afferent inputs could be examined in detail (Figure 1). Surgical preparation for the vagal afferent studies was essentially similar to that for the efferent studies.

A 3-4cm midline incision was made in the neck. An endotracheal cannula was inserted orally or via a tracheostomy. This enabled the animal to breath freely and facilitated the removal of accumulated mucus from the respiratory tract. A multi lumen assembly was positioned within the oesophageal body perorally (see later). The external right jugular vein was cannulated for administration of intravenous drugs and further anaesthetic. The vagal trunks were separated from their adjacent carotid arteries. The right vagal trunk was used for electrophysiological recordings. The left carotid artery was cannulated so that blood pressure could be monitored using a pressure transducer (Transpac IV, Abbott Critical Care Systems). Mean arterial pressure of at least 100 mmHg was maintained throughout the experiments. The animal was kept warm at approximately 38$\pm$5°C on a warming pad. Temperature was monitored in several studies with a rectally inserted bulb thermometer.

A 5-6cm midline laparotomy was performed. A triple lumen polyethylene (total OD 1.2mm) cannula was introduced via the aorta at the iliac bifurcation and positioned so that the tip of the catheter lay at the coeliac axis. This allowed close intraarterial drug administration to the upper gastrointestinal tract region. The position of the tip was confirmed post mortem. Dye injections in previous studies have confirmed that these injections predominantly reach the arterial supply of the stomach and upper small intestine[36].

The stomach, either as an intact organ (n=69) or as corpus and antrum separately (n=29), and colon (n=2) were cannulated to enable fluid distension and the measurement of intraluminal pressure. For whole gastric distension, a cannula was inserted into the stomach via the pylorus. For studies where the effects of antral and corpus distension were examined separately, the stomach was divided into its corpus and antral regions by a silk ligature tied tightly around the circumference of the stomach at the level of the incisura angularis. The corpus was cannulated immediately proximal to the ligature, while the antrum was cannulated 1mm proximal to the pylorus for distension and drainage in addition to intraluminal pressure recordings. The antrum and duodenum were separated at the level of the pylorus by a ligature. The duodenum was cannulated in an oral direction distal to the

ligament of Treitz to allow free drainage of bile from within the duodenum. A cannula was inserted into the colon via the anal sphincter and secured with a ligature at the rectum for distension and intraluminal pressure recordings. A second ligature was tied at the level of the splenic flexure. In 14 studies where oesophageal afferents were investigated, a large bore cannula (OD 2cm) was positioned in the antrum such that the oesophageal stimulating assembly could be inserted through the cannula into the oesophageal body via the lower oesophageal sphincter.

Pressure transducers (Transpac IV, Abbott Critical Care Systems) and the syringes used for distension and deflation were connected to the gastric and colonic cannulae via three way taps which allowed for simultaneous intraluminal pressure recordings and inflation and deflation of the stomach. Gastric pressure output and blood pressure signals were then amplified (Polygraf Synectics, Sweden), digitised (NBMIO16 A-D card, National Instruments) and stored on hard disk via an Apple Macintosh IIci computer. Data acquisition and off-line analysis was performed using MAD software (Royal Adelaide Hospital/Charles Malbert) based on LabView (National Instruments).

3.1 Insertion of intracerebroventricular cannula

This was performed in 20 ferrets in order to study the effects of receptor activation and blockade selectively within the central nervous system on vagal efferent responses to upper gastro-intestinal mechanical and chemical stimuli. This was achieved by delivering drugs into the fourth ventricle because in the ferret, the dorsal vagal complex (the main site of terminations of vagal fibres) is situated close to the fourth ventricle[159, 249].

A 3-4cm midline skin incision was made at the dorsal base of the skull. The skin and muscle were reflected until the atlanto-occipital membrane was reached. Bleeding was stopped by irrigation of the area with adrenaline (100µg/ml) and calcium chloride (300mM). A diathermy was also used to contain prolonged bleeding (System 500, Davol Electro Medical Systems, Colorado, USA). A small diameter polyethylene cannula (0.5 mm OD)

was introduced via a minute hole in the atlanto-occipital membrane and secured with cyanoacrylate adhesive so that the tip lay in the fourth ventricle at the level of the obex. Care was taken to avoid damage to the underlying neural tissue and to seal the preparation with cyanoacrylate to prevent leakage of cerebrospinal fluid. This was used for central application of normal saline (control), the non-NMDA receptor antagonist CNQX (77-155nmol icv, n=15), the calcitonin gene-related peptide antagonist $hCGRP_{8-37}$ (3.2-6.4nmol icv, n=13), the $GABA_b$ receptor agonist baclofen (3-6nmol/kg icv, n=7) and the $GABA_b$ receptor antagonist CGP35348 (100nmol/kg icv, n=7). Catheter position was confirmed with the aspiration of a small amount of cerebrospinal fluid into a 100µl Hamilton syringe. Blood pressure responses were observed closely during catheter insertion into the fourth ventricle and subsequent drug administration.

3.2 Bilateral vagotomy and splanchnectomy

Bilateral cervical vagotomy and splanchnectomy were performed in order to study the relative contribution of vagal and splanchnic afferents respectively activated by peripheral stimuli onto vagal central neurones. Vagotomy was accomplished as the penultimate treatment prior to splanchnectomy in 1 atudy and as the last treatment in 15 studies after prior administration of at least one receptor agonist/antagonist. Splanchnectomy, only performed in 1 study, was done subsequent to vagotomy: this was the last treatment of that study. At least 2 minutes were allowed before the responses to any stimulus were evaluated in order to determine the effects of nerve section on basal discharge frequency.

The left vagal trunk was sectioned completely in the neck at the level of the recording site. A fine silk ligature was positioned around the right vagal trunk approximately 10mm caudal to the recording electrodes. The right trunk was severed almost completely such that only a small portion of perineural connective tissue remained. The fine ligature was then tightened around this tissue to ensure any remaining nerve fibres would be crushed and damaged. The ligature also served to maintain tension along the length of the nerve in order to provide the stability required for maintenance of nerve recordings.

The greater splanchnic nerves were isolated at the crus of the diaphragm and severed with a pair of fine scissors.

4 OESOPHAGEAL STIMULATING ASSEMBLY

The oesophaeal assemblies used consisted of multi lumen tubing, or extrusions, containing up to 9 channels, or lumen, and with a maximum outer diameter of 3.5mm (Figure 2). Balloons were assembled by stretching a cylindrical piece of silicon rubber membrane around the extrusion and sealing the membrane at both ends with a fine silk ligature. The balloons were distended via multiple holes trefined within a specified channel of the extrusion. Balloon distension was used to stimulate oesophageal afferents mechanically. Other channels were used to infuse fluid into and drain fluid from the distal oesophagus. The ports for these were constructed by drilling outlets in the specified channels within the assembly. Oesophageal afferents could be chemically activated by solutions infused through these multiple infusion ports.

The lumina used for balloon distension, infusion and drainage were exteriorised using PVC tubing attached to female leur locks. A three way tap was attached to the luer lock so that pressure could be measured via a pressure transducer and infusion and distension could be performed with a syringe. Ring stimulating electrodes were used to calculate the conduction velocity of the afferent fibre being studied. These were either fed into a sidehole and run along a channel or positioned along the outside of the assembly. The platinum wires were soldered onto insulated wires and connected to a stimulator via 2mm stackable plugs (see section 5 Stimulating electrodes). The catheter used in the efferent studies was similar to the second one used in the afferent studies, and was used interchangeably.

1. Afferent studies #1 (Figure 2A): This 4 lumen polyethylene assembly (OD 3mm) was introduced orally and positioned just above the lower oesophageal sphincter. A fine brush made from paint bristles was mounted 1cm above the tip. A 2cm long balloon was mounted

2.5cm above the tip. The assembly also contained 2 infusion ports and a drainage port positioned 5.0cm above the tip. An additional separate tube was often required to facilitate drainage. 2 ring stimulating electrodes were positioned on either end of the infusion and drainage ports. The electrode wires were adhered to the exterior of the assembly. This assembly was flexible but stiff enough to move up and down easily within the oesophageal body. Light stroking of the oesophageal mucosa was performed by moving the catheter in a circular motion as well as longitudinally along the oesophageal body.

2. Afferent studies #2 (Figure 2B): This 9 lumen silicon rubber assembly (OD 3.5mm) was inserted through a large bore antral cannula. Two 2 cm long balloons were positioned 1cm and 6.5cm from the tip. These balloons were distended through separate sideholes with a maximum volume of 3ml of air. Four infusion ports and a larger drainage port were positioned 5cm from the tip. Two ring stimulating electrodes were positioned 0.5cm above and below the infusion ports. The electrode wires were then inserted through a sidehole and fed into that channel in order to streamline the exterior of the assembly.

3. Efferent studies (Figure 2C): This 9 lumen silicon rubber assembly (OD 3.5mm) was introduced orally and positioned just above the lower oesophageal sphincter. The assembly contained four infusion ports positioned 2.5cm above the tip. A 1cm long balloon was mounted 3.5cm from the tip and was distended with air through a sidehole with a maximum volume of 2ml. A drainage channel positioned 6cm above the tip allowed drainage of infused fluid. An additional, separate drainage tube (OD 1.0mm) was often required to facilitate drainage. Nickel titanium wire (not shown in Figure 2C) was inserted into an extra channel to stiffen the assembly and therefore allow easy movement of the assembly within the oesophagus.

5 STIMULATING ELECTRODES

Stimulating electrodes were also used in afferent studies to measure conduction velocities of afferent fibres. In the case of gastric afferents, these were extraluminal hand

held electrodes. In the case of oesophageal afferents, they were mounted as ring electrodes on the oesophageal assembly.

Exteriorised electrode wires were soldered onto insulated wires and connected to an isolated stimulator (ST-1, JRAK, Melbourne, Australia) via 2mm stackable plugs. The stimulator was driven by a period generator (PG-1, JRAK). Electrical stimulation was delivered via square pulses of 20V, 0.5ms pulse width, 1Hz, for 50 seconds.

6 ELECTROPHYSIOLOGICAL RECORDINGS

A pool was made in the neck by suturing the skin and muscle around the incision to a round metal ring (diameter 2.5cm). This was filled with paraffin. The right cervical vagal trunk was placed on a black perspex platform submerged within the paraffin pool. With the aid of a microscope (SZ60, Olympus), the vagal sheath was then opened longitudinally to a length of 5mm using a sharp blade. Silver or platinum wire hook electrodes were positioned approximately 5mm above the nerve trunk. With a pair of fine forceps, fine nerve filaments were dissected in a rostral or caudal direction and placed on the recording electrode. A piece of connective tissue of similar dimensions was placed on the reference electrode. For efferent studies, recordings were made from the central end of the cut vagal strand. For afferent studies, the peripheral end of the nerve filament was placed on the recording electrode. The signal was amplified and filtered (SA-1, BA-1, JRAK, Melbourne, Australia) and displayed on an oscilloscope (Tektronix 5111). The amplified signal was stored on DAT audio tapes using a digital audio recorder (PCM-2300, Sony).

The discharge frequency of the unit was calculated in 3 ways: 1) The action potential was gated with the aid of a window discriminator (WD-1, JRAK) which discriminated the width, depth and amplitude of either the rising or falling portion of the action potential wave form. The number of action potentials, or pulses, was then counted in bins of 1-10 seconds by a pulse counter (NPC-1, JRAK) to generate an integrated output which was entered into an Apple Macintosh IIci computer via a NBMIO16 A-D card (National Instruments),

displayed on screen, stored on hard disk and analysed off-line using MAD software (Royal Adelaide Hospital/Charles Malbert) based on LabView (National Instruments); 2) the action potential was gated in the same manner described above and the pulses fed directly into the computer via the A-D card where an internal counter generated an integrated output. The integrated frequency of discharge, together with measurements of gastric and blood pressure, was displayed on screen, acquired, stored on hard disk and analysed off-line using MAD software; 3) the filtered and amplified signal was entered into a Power Mac where it was displayed on screen and stored on hard disk. Individual action potentials were then discriminated using a template recognition software (Spike2, Cambridge Electronic Design) and analysed off-line.

7 PERIPHERAL STIMULI

The following stimuli were applied in similar ways in both afferent and efferent studies in order to facilitate interpretation of the data obtained from both types of studies.

7.1 Mechanical stimuli

Distension of the distal oesophagus, stomach and colon was used to activate oesophageal, gastric and colonic tension receptors respectively.

Oesophageal distension in efferent studies was performed by rapid inflation of an assembly mounted silicon rubber balloon with 1-2ml air. This was maintained for a period of 30secondsbefore being deflated. A volume of 2ml was used as it has been found to evoke a response from vagal mechanoreceptors in a previous study in the cat oesophagus[131]. The catheter was positioned such that the balloon was within the distal 5cm of the oesophageal body. In afferent studies, oesophageal balloon distension of a 2cm long silastic balloon was performed in 0.5ml step increments every 30seconds to a maximum of 3mls after the receptive field of the oesophageal receptor had been identified. Step distension in afferent studies were used to determine the volume required to generate a maximal increase in discharge.

In 69 studies, the intact stomach was distended. This was accomplished by the rapid (<5seconds) infusion of 40-60ml isotonic saline which was maintained for 60 seconds before aspiration by syringe was performed. 40-60 ml was chosen as the volume used for whole stomach distension as ferrets were found to drink well in excess of that amount when they had free access to milk after an overnight fast[12]. The distension volume used varied as the size of the ferrets ranged from 500 to 1500g. Thus, 40 ml was used in ferrets weighing between 500-700g, 50ml for ferrets between 700-1000g and 60ml for ferrets above 1000g.

In 29 studies, the stomach was divided into its separate corpus and antral compartments. This was performed mainly during afferent studies where the effects of GABAb receptor ligands on mechanoreceptors and mucosal receptors were investigated. Distension of the antrum was performed in the same manner as whole gastric distension with 10ml isotonic saline or in 1ml incrrements up to a maximum of 5mls. Corpus distension was performed with 20ml saline either as a bolus or in 5ml increments over 2 minutes.

In 2 studies, colonic distension was performed with 3ml saline for 1minute.

7.2 Intra-oesophageal chemical stimuli

Intra-oesophageal acid and capsaicin infusion was performed to stimulate chemosensitive afferent endings. Each solution was infused through a separate channel in the catheter to avoid errors due to flushing the dead space when switching over from one solution to another. The sidehole of each channel was located at the same distance from the assembly tip so that fluid would bathe the same area of the oesophagus.

For efferent studies, chemical and saline solutions were infused slowly as a 2ml bolus over approximately 15 seconds. For afferent studies, chemical solutions were infused in 0.5ml boli every 30 seconds for a maximum of 5 minutes. In these studies, saline was infused in 2ml boli every minute for a maximum of 7 minutes. The protocol followed in

afferent studies ensured that the oesophageal lumen was continuously bathed in solution and was a refinement of the protocol used for the earlier efferent studies.

Isotonic saline (2ml) was first infused slowly into the oesophagus to determine the fibre response to the mechanical flow of an innocous fluid across the receptive field. This also served to clear any blockages in the drainage channel. Hydrochloric acid (150mM) was administered intra-oesophageally, during which free drainage was allowed in order to avoid undue distension of the oesophageal body. The oesophageal lumen was then washed out with a minimum of 2 infusions of isotonic saline 2 minutes after initiating the acid infusion. At least five minutes were allowed between the last saline infusion and the next acid or capsaicin infusion. The concentration of acid used in these experiments has been previously shown to excite vagal mucosal afferents in the ferret duodenum or jejenum and cause modulation of vagal afferent discharge[37, 43].

Oesophageal capsaicin infusion (3.2-6.5mM) was applied in the same manner as the acid infusion, although more isotonic saline infusions were often required over a longer time period in order to bring the discharge rate to pre-stimulus levels. The vehicle, ethanol/Tween 80/saline (10%/10%/80% v/v), was administered in the same manner prior to capsaicin infusion and was not associated with any fibre response.

7.3 Close intraarterial chemical and pharmacological stimuli

Close intraarterial chemical and pharmacological stimuli were applied to stimulate chemosensitive endings in the upper gastrointestinal tract. These were applied as close intraarterial boli via the coeliac axis. Dye injections in previous studies have confirmed that the doses administered predominantly supply the stomach and upper small intestine[36]. Each drug solution was administered in 0.1ml boli via a separate channel of the triple lumen cannula in order to avoid cross contamination between solutions. The gastric pressure and blood pressure responses to each drug were also observed.

Cholecystokinin (sulphated octapeptide, 100pmol) was used to activate gastric and duodenal mucosal and mechanoreceptors[38, 39]. Bradykinin (18nmol) was used to stimulate all vagal and spinal afferent pathways[174, 240]. A similar dose was used intraarterially to stimulate splanchnic afferents in the cat colon and mucosal and muscular vagal afferents in the ferret stomach and duodenum[35, 135]. In efferent and early afferent studies, capsaicin (65nmol) was used to selectively activate all unmyelinated C-fibres in the original belief that all populations would be sensitive[141]. However, this generalisation was brought into question by later experiments (see Chapter 2). 5-hydroxytryptamine (130nmol) was administered in one study of an antral mucosal receptor in order to selectively stimulate it. No dose response curves were obtained for any of the above chemicals as the distribution of the injection bolus is variable from receptor site to receptor site in the same animal and from animal to animal. However, the duration and intensity of response to each chemical within each experiment were consistent within each treatment group.

8 EXPERIMENTAL PROTOCOL

At least 30 minutes were allowed after surgery before characterisation of responses. An equilibration period of 1-2 minutes following each stimulus and of 5 minutes following each treatment was allowed in order to achieve a steady basal level of discharge prior to the next test.

8.1 Afferent studies

Upon confirmation that a single afferent unit was being recorded, the spontaneous activity of the unit was evaluated. The majority of afferent strands examined were discarded as they possessed fibres that were cardiorespiratory in origin, as evidenced by rhythmic firing patterns associated with cardiac and/or respiratory cycles. Strands with 3 or more active units were not studied due to the difficulties of distinguishing the shape and waveform of the action potential of each individual unit. Only single units which were clearly distinguishable from any other units present on the same strand throughout the recording period were included for data analysis.

Various search strategies were used to classify different classes of afferent fibres. Oesophageal tension and mucosal receptors were located by circular and longitudinal movement of the oesophageal stimulating assembly or a stiff rod (OD 0.5mm) with a ball (OD 0.65mm) at the tip within the distal 5cm of the oesophagus. Only units which responded with a ≥50% increase of activity when the assembly or rod passed over the receptive field of the unit were studied further. The assembly was then positioned such that the balloon or sideholes were located at the level of the receptive field of the unit for balloon distension or infusion respectively. Oesophageal balloon distension was performed in 0.5ml increments of air every 30 seconds to a maximum of 3mls. In 11 studies, responses to oesophageal infusions of isotonic saline, acid (HCl, 150mM) and capsaicin (3.2-6.4mM) solution were then observed. The number and order of infusions varied between studies. In 6 studies, the response of oesophageal tension receptors to oesophageal balloon distension was also ascertained after chemical infusion. In 3 studies, the effects of the GABAb receptor agonist baclofen (14μmol/kg iv) on the response of oesophageal tension receptors to oesophageal balloon distension were obtained.

Corpus and antral tension receptors (n=8) were identified by distension of the corpus (5-20ml in 5ml increments every 30 seconds) or antrum (1-5ml in 1ml increments every 30 seconds) respectively. Only units which responded with a ≥50% sustained increase in discharge from predistension levels were studied further. The effects of the GABAb receptor agonist baclofen (14μmol/kg iv) and subsequent addition of the GABAb receptor antagonist CGP35348 or CGP36742 (100μmol/kg iv) on vagal afferent discharge and gastric pressure in response to distension were obtained in 8 studies.

The receptive fields of corpus and antral mucosal receptors were located by probing the gastric serosal surface with a blunt probe. This generated a shear force as folds of mucosa rubbed against each other and produced a sharp burst of activity when the receptive field was probed. Units which responded with a ≥50% increase in discharge were studied further. The response of 1 corpus mucosal receptor to close intraarterial cholecystokinin (100pmol), bradykinin (18nmol), and capsaicin (65nmol) was evaluated under control

conditions and to CCK after treatment with the GABAb receptor agonist baclofen (14μmol/kg iv). Another presumably mucosal receptor with an unidentified receptive field was activated when close intraarterial cholecystokinin resulted in a burst of discharge. This unit was on the same strand as a corpus tension receptor being studied (see Chapter 4, Figure 4-6). The effect of the GABAb receptor agonist baclofen (14μmol/kg iv) on the response to cholecystokinin was evaluated.

In one study of an antral mucosal receptor, the stomach was opened along the greater curvature. The responses of this unit to close intraarterial 5-HT and CCK and to various chemical solutions applied topically to the area surrounding the receptive field were obtained. The area was irrigated with isotonic saline before the next chemical solution was applied. Response to saturated glucose, hypertonic saline (500mM and 1M) and acid (150mM HCl) were determined. Inflammation was then induced by placing a piece of cotton wool over the receptive field which was soaked with an acidified aspirin solution (made by dissolving soluble aspirin in 150mM HCl solution) for 5 minutes[158]. The area was then washed out with isotonic saline before the responses to the four solutions listed above was tested again. Due to the difficulties encountered in isolating gastric mucosal afferents, this line of investigation was not continued.

8.2 Efferent studies

The minimum criteria used to study efferents were the same as those used to evaluate the validity of afferents, ie that efferents fibres did not have spontaneous activities which correlated with the cardiovascular and/or respiratory rhythms of the animal, that the strand had less than 3 active units on it, and that the shape and waveform of the action potential being studied could be easily distinguished.

The 2 common search stimuli used were gastric distension (40-60ml saline, 1 minute) and oesophageal balloon distension (1.5-2.0ml air, 30 seconds). Efferent units which were either silent in the absence of any stimulus or possessed an irregular

spontaneous discharge and responded to gastric and/or oesophageal balloon distension with a ≥50% change from resting discharge frequency were included for analysis.

The responses to oesophageal infusion of acid (HCl, 150mM) and capsaicin (3.2-6.4mM) were evaluated. At least 3 successive infusions of acid were performed prior to the initial capsaicin infusion. Responses to repeated capsaicin infusions were also tested in some studies. Units were also tested with close intraarterial capsaicin (65nmol), bradykinin (18nmol), and cholecystokinin (100pmol). The whole procedure was repeated after treatment with each drug agonist and/or antagonist and after bilateral vagotomy.

9 DATA ANALYSIS

Basal discharge was assessed for 60 seconds upon commencing each experiment and after the administration of each agonist or antagonist. Resting discharge was also calculated for 45 seconds immediately prior to each stimulus to average any fluctuations due to gastric contractile rhythms. Responses to distension are expressed as the change in mean discharge rate during the slowly adapting portion of the response. Responses to chemical stimulation are expressed as the change in discharge rate during the nadir or peak of the response.

Intragastric pressure and blood pressure were monitored continuously throughout most studies. Intragastric pressure was measured during distension and used to calculate the change in discharge/change in pressure. A paired t-test or ANOVA was used to assess effects of treatments on gastric pressure and blood pressure.

Data are expressed as mean$\pm$sem (standard error of the mean), unless stated otherwise. The number of observations=n. The Wilcoxon's signed rank paired test was used to examine the statistical significance of different treatments on neuronal activity as this data was non-parametric.

10 Drugs

Drugs and chemicals used.

Compound	Dose	MW	Source
Adrenaline	100µg/ml irrigation		Astra
Baclofen	7-21µmol/kg intravenous (iv) 3-6nmol/kg intracerebro-ventricular (icv)	214	Sigma
Bradykinin	18nmol close intraarterial (ia)	1060	Auspep
Capsaicin	65nmol close ia 3.2-6.4mM intra-oesophageal	305	Sigma
Calcium chloride	300mM irrigation		BDH
CGS19755	13µmol/kg iv	223	gift from Astra Hässle
CGP35348	100µmol/kg iv 100nmol/kg icv	225	gift from Astra Hässle
CGP36742	100µmol/kg iv	179	gift from Astra Hässle
Cholecystokinin-8 sulphated	100pmol close ia	1144	Auspep
CNQX	77-155nmol icv	232	RBI
CP96345, as HCl salt	8µmol/kg iv	485	gift from Pfizer
CP99994, as HCl salt	12µmol/kg iv	333	gift from Astra Hässle
hCGRP8-37	3.2-6.4nmol/kg icv	3124	Auspep
Heparinised Saline			Astra
Hydrochloric Acid	150mM intra-oesophageal		Sigma
5-hydroxytryptamine, creatinine sulphate complex	130nmol close ia	387	Sigma
Methoctramine	7-14µmol/kg iv	729	RBI
Pirenzepine	2.5-5.0µmol/kg iv	424	RBI
Urethane (ethyl carbamate)	1.25g/kg intraperitoneal (ip)		Sigma

Bradykinin and cholecystokinin were initially dissolved in isotonic saline with 0.1% bovine serum albumin. Capsaicin was dissolved initially in 10/10/80v/v ethanol/Tween80/saline and subsequently in isotonic saline. All other drugs were dissolved in isotonic saline.

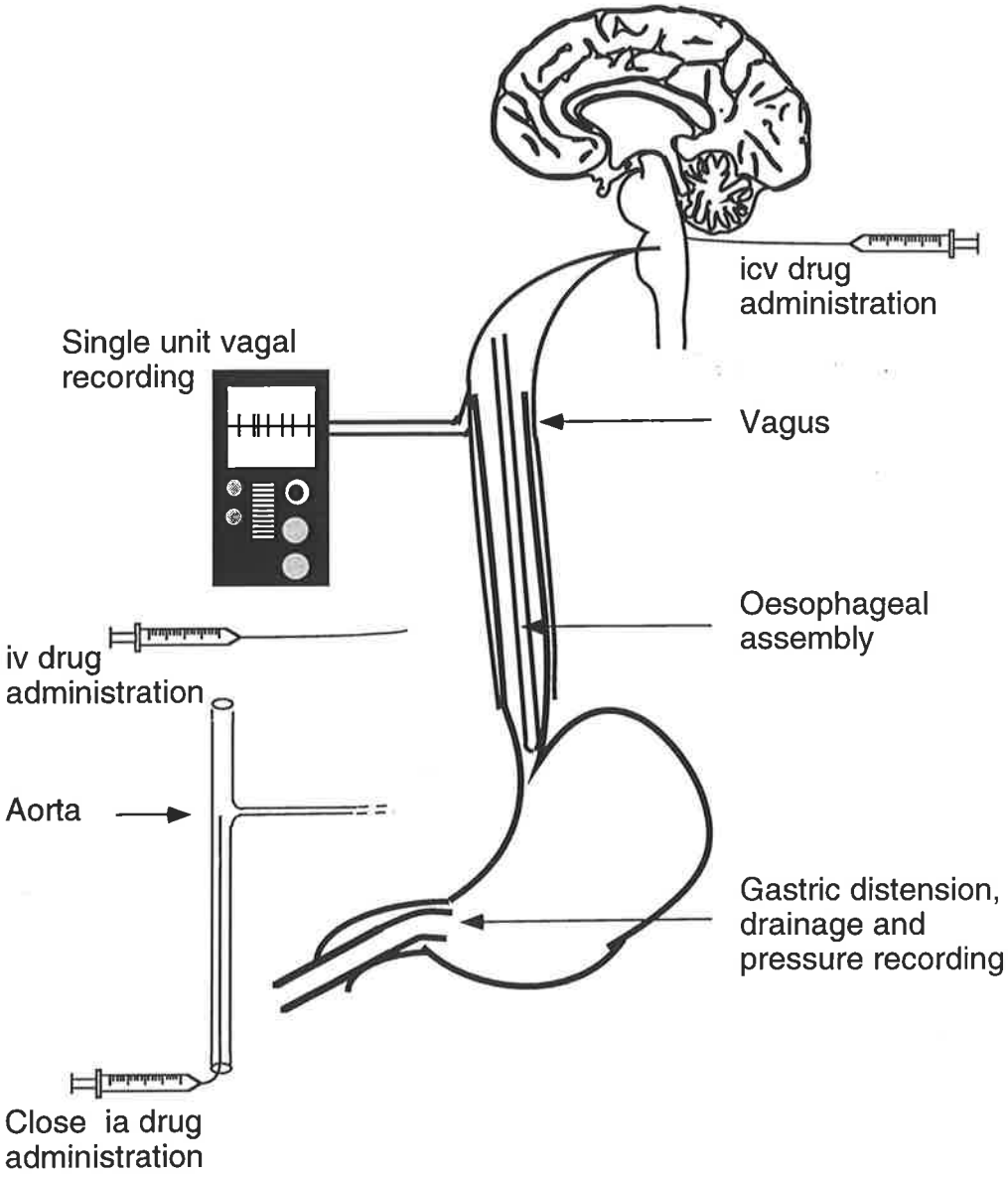


Figure 1. Schematic representation of the preparation used for single unit vagal recordings.
Single unit recordings were obtained from the right cervical vagus. Compounds and drugs could be administered via the right external jugular vein (iv), the abdominal aorta at the level of the coeliac axis (close ia) or the fourth ventricle (icv). The stomach was cannulated for fluid distension and intraluminal pressure recordings. The duodenum was cannulated to allow free drainage of bile (not shown). The oesophageal assembly was inserted either orally or via a wide bore gastric cannula in order to distend the distal oesophagus via a balloon and perfuse the lumen via sideholes. The assembly used for vagal afferent recordings also had ring stimulating electrodes for use in the measurement of conduction velocities (see details in Figure 2).

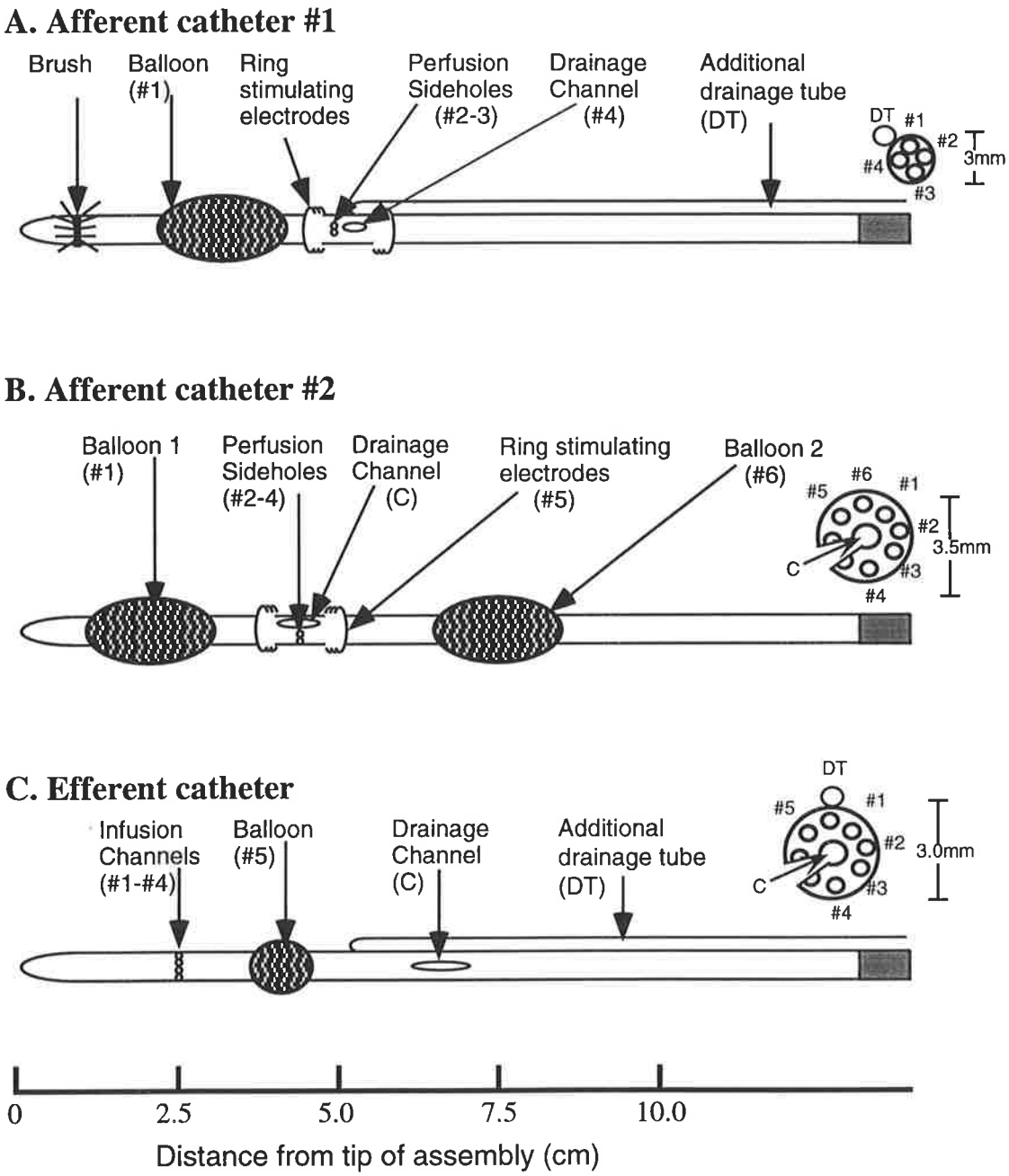


Figure 2. Schematic representation of the assembly used to evoke single unit responses to oesophageal chemical and mechanical stimuli.
A-B. Both assemblies had stimulating ring electrodes, balloon(s) and infusion and drainage sideholes for measuring conduction velocity, oesophageal distension, infusion and drainage respectively. Afferent catheter #1 was inserted orally, and had a brush to stimulate mucosal afferents. Afferent catheter #2 was inserted into the oesophagus in an oral direction via a wide bore gastric cannula.

C. This orally-inserted catheter contained 4 infusion channels, a drainage channel, a silastic balloon for intraoesophageal infusion, drainage, and distension respectively.

CHAPTER 1.
Acute mechanical & chemical sensitivity of gastro-oesophageal vagal afferents

1 SUMMARY

1. Single unit afferent activity was recorded from the ferret cervical vagus to study the properties of gastro-oesophageal afferents in the ferret.

2. Recordings were made from 13 oesophageal tension receptors and 3 mucosal receptors (n=1 oesophagus, n=1 corpus, n=1 antral). These were classified according to their responses to oesophageal balloon distension in the case of tension receptors or to probing in the case of mucosal receptors.

3. Oesophageal balloon distension (OBD), performed in cumulative stepwise increments, elicited a slowly adapting response in oesophageal tension receptors. 2/10 of these receptors responded directly to capsaicin solution (3.2-6.4mM) infused intraluminally at the level of the identified receptive field. Infusions of acid solution (HCl, 150mM) in the same manner failed to evoke a direct response in 10/10 fibres tested.

4. The responses to OBD of 6/6 oesophageal tension receptors tested after capsaicin infusion were significantly attenuated. This was independent of the direct response of the tension receptors to capsaicin. No change in the responses to OBD of 4/4 oesophageal tension receptors tested were evoked after acid infusion.

5. The mucosal afferents responded to probing with sharp bursts of discharge. The oesophageal mucosal receptor responded additionally to capsaicin and acid solutions infused intraluminally at the level of the identified receptive field. The corpus mucosal receptor was also activated by close intraarterial administration of cholecystokinin (100pmol), bradykinin (18nmol), and capsaicin (65nmol). Topical mucosal application of acid (HCl, 150mM), saturated glucose and NaCl (500mM and 1M) on the receptive field of the antral mucosal afferent evoked increases in discharge. Inflammation produced by local perfusion of an acidified aspirin solution led to the abolition of the response to glucose, an enhanced response to 500mM NaCl, and unchanged responses to the other 2 solutions.

6. In conclusion, this study confirms previous observations and provides evidence that oesophageal tension receptors can respond directly to intraluminal capsaicin. Capsaicin infusion also reduces the mechanosensitivity of these tension receptors regardless of their direct response. My study on gastro-oesophageal mucosal receptors show them to be mechanosensitive and polymodal in their response to chemical stimuli.

2 INTRODUCTION

Diseases involving oesophageal pain are quite prevalent. However, the aetiology of oesophageal pain is often unknown, probably due to the poor localisation of the sensation in patients. Some patients undergo coronary angiography to determine whether the cause of the pain is cardiovascular in origin. Oesophageal acid perfusion test conducted on these patients often reveal that they suffer from gastro-oesophageal reflux disease (GORD). However, the symptoms reported by patients with non-cardiac chest pain (NCCP) are often different from those patients who suffer from heartburn. Also, there is a poor correlation between the severity of symptoms perceived in those with NCCP and the degree of GORD that they suffer. In addition, there is controversy as to whether GORD patients undergo oesophageal sensitisation to repeated acid exposure. Although the mechanism by which GORD is developed is largely unknown, it is believed to involve a defective functioning of the lower oesophageal sphincter (LOS) which provides a barrier to gastric acid from refluxing into the normally non-acidic environment of the oesophageal body. Afferent inputs to vagal motor neurones involved in the control of the LOS may originate from the oesophagus, stomach or LOS itself. This is demonstrated in studies[181, 216] where stimuli applied to these areas evoke a reflex effect on the LOS. As these inputs may follow either vagal or spinal pathways, one of the aims of this chapter is to investigate the effects of intraluminal acidification on the activity of vagal afferents arising from the oesophagus and stomach.

Electrophysiological studies have investigated the chemosensitivity of mucosal afferents within the gastrointestinal tract. These gastrointestinal mucosal afferents have been

found to respond to a whole range of chemicals including acid and alkaline and to pharmacological stimuli such as bradykinin, cholecystokinin and 5-hydroxytryptamine [35, 37, 38, 72]. Studies on the vagal afferent innervation of the oesophagus, on the other hand, have concentrated on tension receptor sensitivity to distension, with only 2 studies reporting on the effects of chemical stimuli on oesophageal afferents[76, 131]. In these studies, only those mucosal afferents located between the cervical region and the LOS were acid-sensitive, with a few responding additionally to hypertonic saline and sodium hydroxide.

The need for more information to be obtained regarding the chemosensitivity of vagal afferents located in the distal oesophagus was prompted by 1) the paucity of available literature, 2) findings in our laboratory that acidification of the distal oesophageal lumen led to relaxation of the LOS[34] and 3) other findings also in our laboratory that the same process of acidification activated vagal efferent fibres (see Chapted 2 and [209]). Both LOS and afferent inputs to vagal efferents were sensitised with repeated episodes of acidification. As a significant portion of my thesis is concerned with vagal efferent responses to peripheral inputs from the oesophagus, the nature of these inputs is investigated in this section by using a protocol similar to that employed to investigate vagal efferent responses to the same stimuli (see Methods and Chapter 2).

3 METHOD AND PROTOCOL

3.1 Method and protocol

The methods used in this series of experiments are outlined in detail in the Methods section of this thesis. The various protocols used are detailed in section 8.1 in the Methods section of this thesis.

3.2 Methodological considerations

Two different oesophageal assemblies were used to locate and activate oesophageal afferents (see Methods, Figure 2A&B). Both contained infusion and drainage ports for the

infusion of different chemical solutions, a balloon to distend the oesophagus, and ring stimulating electrodes, positioned on either side of the infusion ports, to measure the conduction velocity of the afferent unit being studied. The first assembly which was inserted orally also had a brush mounted 1cm from the tip. The second assembly was inserted through a wide bore gastric cannula into the oesophagus and had an additional balloon located 6.5cm from the tip.

No recordings from oesophageal afferents were obtained using the first assembly. Movement of this assembly within the distal oesophagus frequently led to bleeding and mucus accumulation caused by the abrasive action of the brush on the oesophageal mucosal surface. Also, when a current was passed through the ring stimulating electrodes mounted on the assembly in order to measure the conduction velocity of the afferent unit, the stimulus interfered with the recording as the proximity of the ring stimulating electrode wires to the recording electrodes at the level of the recording site was less than 15mm. This exaggerated the size of the stimulus artefact on the oscilloscope and prevented detection of the action potential of any orthodromically triggered afferent. Thus, no oesophageal afferents were identified using this assembly due to difficulties in stimulating the receptive fields of afferents mechanically and electrically.

The second assembly was designed to minimise problems inherent with the initial catheter design. This was achieved by inserting the assembly via the stomach, ensuring an adequate distance between the stimulating electrode wires and the recording electrodes and thus reducing the artefact size. Electrode wires were fed through a sidehole and run within the catheter in order to streamline the catheter and minimise friction of the wires against the mucosal surface. Oesophageal afferents were located by inflating either of the 2 balloons mounted 3cm apart or by movement of a separate stiff rod with a ball attached to its tip. Both tension and mucosal receptors were identified and characterised using this catheter.

4 RESULTS

Recordings were made from 13 oesophageal tension receptors, and 3 mucosal receptors with endings in the oesophagus (n=1), corpus (n=1) and antrum (n=1). The mechano- and chemo-sensitivity of these afferents are discussed in detail.

4.1 Blood pressure responses to intra-oesophageal chemical infusion

The effects of intra-oesophageal infusions of isotonic saline, acid (HCl, 150mM) and capsaicin (3.2-6.4mM) solutions on blood pressure were monitored. Changes in blood pressure would indicate that the oesophageal infusions were effective in crossing the mucosal epithelium and were administered in sufficient doses to have systemic effects.

Oesophageal infusions of capsaicin solution led to a significant increase in blood pressure ($p<0.01$, Figure 1-1). This increase occurred even when the capsaicin solution was infused as the first chemical infusion, prior to any acid infusions, as seen in 3 studies. Blood pressure was unaffected by infusions of either saline or acid solution into the distal oesophagus, regardless of whether prior infusions of the capsaicin solution had occurred.

4.2 Oesophageal tension receptors

The responses of 13 oesophageal tension receptors were recorded. The catheter used for these studies fitted snugly within the oesophageal lumen. Tension receptors were identified when moving the oesophageal assembly or a stiff rod up and down the distal oesophagus. A sustained increase in discharge was evoked when the catheter or ball was moved within the receptive site. Respiratory modulation was seen occasionally; however, this influence was not evident when a response to mechanical or chemical stimulus was observed.

The conduction velocities of 3/13 afferents were determined. The conduction velocity of 3/3 fibres fell within the C fibre range (<1.8m/sec).

4.2.1 Oesophageal tension receptor responses to mechanical stimuli

Oesophageal balloon distension (OBD) was performed in 0.5ml increments every 30 seconds, up to a total of 3ml. The vagal oesophageal tension receptor response can be divided into 3 parts which are depicted in the afferent response in Figure 1-2. In this fibre, Phase 1 is seen only at higher levels of distension (1.5-3.0ml air), when the dynamic phase of distension produced a sharp increase in vagal afferent discharge (VAD). At lower levels of balloon distension (0.5-1.0ml air), Phase 1 was not obvious. Phase 2 consisted of a tonic increase in discharge during the static period of distension that was lower than the initial increase seen but was still greater than basal discharge levels. This persisted until either the next increment of inflation or balloon deflation. Phase 3 was seen with rapid balloon deflation which led to an abrupt decrease of afferent discharge below resting discharge for <5seconds before returning to its basal, pre-distension levels. In this fibre, a complete inhibition was seen in this third phase.

Vagal afferent discharge increased in response to each increment of oesophageal balloon (Figure 1-3). This increase was statistically significant even at the lowest level of distension ($p<0.01$ vs Control). Vagal tension receptor responses to OBD in general reached a plateau at a volume of 1.0ml, although some fibres reached their maximal firing rate after 0.5ml air while the discharge frequency of other fibres were linear up to a volume of 3mls.

4.2.2 Direct responses of oesophageal tension receptors to chemical infusion

The direct effects of tension receptor activity to oesophageal acid and capsaicin infusion were measured in 10 studies. In 7/10 studies, at least one acid infusion was administered prior to the initial capsaicin infusion. However, in the other 3/10 studies, capsaicin was infused prior to any oesophageal acid infusions.

The direct responses of 10 fibres to oesophageal capsaicin infusions were observed. 2/10 fibres tested showed a sustained increase in VAD with capsaicin infusions which lasted

for >10minutes, returning to basal levels only after multiple saline washouts (see example in Figure 1-4). In 1 of 2 fibres which responded to the initial capsaicin infusion, a second series of capsaicin infusions was performed: no desensitization was not seen in the fibre response with the second capsaicin infusion. The other 8/10 fibres tested did not show any prolonged responses to this chemical. Infusions of vehicle solution was also ineffective in eliciting changes in afferent discharge. Although oesophageal pressure was not recorded in these studies, other studies performed in the laboratory have shown that capsaicin infusion did not evoke oesophageal contractions or changes in muscle length[34, 210].

No prolonged direct changes in afferent activity in response to oesophageal acid infusion were observed in 10/10 fibres tested, regardless of whether any prior acid or capsaicin infusions were performed. A brief increase in discharge was often elicited in response to acid infusion (see example in Figure 1-4). This was similar in amplitude and duration to the increase in discharge is seen in response to saline infusions and hence is likely to be due to the mechanical effect of fluid passing over the receptive field.

4.2.3 Effect of chemical infusion on the mechanosensitivity of oesophageal tension receptors

The effects of oesophageal acid and capsaicin infusion on oesophageal tension receptor responses to OBD were evaluated in 6 studies. Under control conditions, graded OBD in the distal oesophagus led to a progressive increase in VAD (Figure 1-3). Graded OBD was also performed after at least 1 acid infusion in 4 studies and after at least 1 capsaicin infusion in 6 studies.

After oesophageal capsaicin infusion, there was a reduction in the tension receptor response to OBD which was especially evident at higher distension volumes (Figure 1-5). This was statistically significant, when the combined responses to OBD at 1.0-2.0ml before and after capsaicin infusion were compared ($p<0.05$). This reduction in mechanosensitivity was independent of any direct response of the oesophageal tension receptor to capsaicin

infusion, as only 2 of the 10 receptors tested with capsaicin infusion responded with an increase in discharge during the infusion period. This reduction was also acute as a similar reduction was not seen in response to acid or saline infusions, even when these were infused after capsaicin. There was no discernible difference in the oesophageal tension receptor response to OBD before and after acid infusion (Figure 1-5).

4.3 Mucosal receptor responses

The responses of 3 mucosal afferents were recorded. One had a receptive field in the distal oesophagus, the second and third were located within the corpus and antrum respectively. In the absence of any stimulus, the oesophageal afferent had a low frequency irregular firing pattern and the 2 gastric mucosal receptors were silent.

4.3.1 Mucosal receptor responses to mechanical stimuli

3/3 mucosal receptors responded to probing of the mucosal surface with brief bursts of activity (Figure 1-6). These had a short latency and were rapidly adapting, lasting only for the duration of the brief contact of the probe with the mucosa.

The oesophageal mucosal afferent was initially classified as a tension receptor as it appeared to respond to oesophageal balloon distension (Figure 1-7). However, repetition of this stimulus did not lead to a 'typical' tension receptor response, ie the discharge did not increase in a linear manner with further increments of distension. Instead, a random pattern of discharge was elicited. Movement of the oesophageal assembly within the oesophageal body led to short bursts of activity characteristic of mucosal receptors. This pattern of discharge was reproducible with repeated movement of the assembly.

Similarly, the antral receptor was originally classified as a tension receptor as there were intermittent bursts of activity in the absence of any applied stimulus which was characteristic of the spontaneous rhythm of antral tension receptors. When the contractile

activity of the antrum was observed, the brief burst of activity occurred whenever the antrum contracted during distension. When the antrum was opened along the greater curvature, subsequent examination showed a food particle lodged in the mucosal folds adjacent to the cannula. The brief bursts were seen to be caused by friction generated by the force of the particle against the gastric mucosa (Figure 1-8). When the food particle was removed, brief bursts of activity were evoked from the otherwise silent unit by mucosal stimulation with a blunt probe (Figure 1-6).

The receptive field of the corpus mucosal receptor was located by probing the gastric serosal surface with a blunt glass rod. Each downward movement of the rod generated a shear force as folds of the gastric mucosa rubbed against each other. Probing an area $0.5mm^2$ around the incisura angularis led to sharp bursts of activity, indicating the receptive field of the unit had been located.

4.3.2 Mucosal receptor responses to intraoesophageal chemical infusion

The direct response of the oesophageal mucosal receptor to infusions of saline, acid and capsaicin solution was determined (Figure 1-9). The flow of fluid over the receptive field, be it saline, acid or capsaicin, led to a brief burst followed by a short period of inhibition. Discharge levels either then returned to basal levels, in the case of saline infusions, or developed a sustained increase in activity in the case of acid and capsaicin infusions.

With this fibre, multiple series of oesophageal acid and capsaicin infusions were administered. A series of acid infusions were administered initially, followed by 2 series of capsaicin infusions. This was followed by a second series of acid infusions and then a third series of capsaicin infusions. The responses shown in Figure 1-9 are of the second acid infusion and the third capsaicin infusion series.

Oesophageal acidification led to a >50% sustained increase in discharge which was maintained until washout of the oesophageal lumen with isotonic saline. Subsequent capsaicin infusion evoked a similar response in the fibre. The response to capsaicin infusion was reproducible throughout the study whereas the response to acid infusion was only discernible with the second series of acid infusions. The prolonged increase in receptor discharge was presumed to be a direct response to the chemical infusion and not secondary to changes in muscle length or tension as shown in previous studies[34, 210].

4.3.3 Mucosal receptor responses to close intraarterial drug administration

Changes in corpus mucosal receptor discharge, intracorpus pressure and blood pressure were measured in response to close intraarterial capsaicin (Cap, 65nmol), bradykinin (BK, 18nmol) and cholecystokinin (CCK, 100pmol).

Capsaicin caused a rapid increase in discharge which lasted for approximately 4 minutes (Figure 1-10). Blood pressure was also increased for a similar period. The brief increase in gastric pressure occurred simultaneously with a sharp intake of breath.

Bradykinin evoked biphasic increase in discharge (Figure 1-10), with the initial part of the response reaching a maximum at the same instant as the peak of the corpus contraction. The secondary response which lasted for >2minutes was not correlated with any further changes in corpus or blood pressure. Saline injected through the same port led to an afferent response similar to that evoked by bradykinin, due to the bradykinin solution left in the dead space of the catheter. Bradykinin caused a brief decrease in blood pressure and evoked an increase in gastric pressure <1minute which was accompanied by a large intake of breath.

Cholecystokinin was administered after the corpus was slightly distended with 5ml saline (Figure 1-10). Afferent discharge was increased in response to the octapeptide. Cholecystokinin also caused an increase in blood pressure together with a concomitant

decrease in intragastric pressure. The duration of response for all three parameters to cholecystokinin was >7 minutes.

The response of the antral mucosal receptor to close intraarterial 5-hydroxytryptamine (130nmol) and capsaicin (65nmol) was also tested under control conditions. The discharge of this afferent was unchanged by either compound (not shown).

4.3.4 Effect of inflammation on responses to topical application of chemicals

Responses of the antral mucosal receptor to topical application of hypertonic saline (500mM and 1M NaCl) and saturated glucose were determined under control conditions and after mucosal inflammation with acidified aspirin. Mucosal irrigation with all 4 solutions yielded excitatory responses, with maximum responses generated by glucose and 1M hypertonic saline (Figure 1-11).

Inflammation was induced by irrigating the mucosal surface containing the receptive field with an acidified aspirin solution. Inflammation was characterised by a marked increase in gastric mucosal secretion, the sloughing of the mucosal layer, and profuse gastric bleeding. When the responses to the 4 stimuli were again tested 7 minutes after inflammation was induced, responses to acid and 1M saline were unchanged, that to saturated glucose was lost and that to 500mM NaCl potentiated.

5 DISCUSSION

The present study provides evidence for the direct activation of vagal oesophageal tension and mucosal receptors by acute intraoesophageal administration of capsaicin. This study also shows that capsaicin can cause a decrease in the mechanosensitivity of the tension receptors in the absence of activation of the peripheral endings. My data on the vagal gastric and oesophageal mucosal receptors, though few in number, also confirms previous findings on the presence of mechanosensitive polymodal mucosal receptors. A portion of the data from this study has been presented at the International Society of Autonomic Neuroscience[210] in conjunction with results from *in vitro* work performed by Dr. Amanda Page and *in vivo* work performed by Dr. Ashley Blackshaw.

5.1 Oesophageal acid and capsaicin infusions

This project investigating the effect of oesophageal acid and capsaicin infusions on oesophageal vagal afferents *in vivo* is part of the wider aims of our research laboratory and is related to determining the effects of oesophageal acidification on LOS pressure as it pertained to the aetiology of gastro-oesophageal reflux disease. In concurrent studies using a similar protocol in the same animal model, we have found firstly, that oesophageal acid infusions led to a decrease in lower oesophageal sphincter (LOS) pressure which returned to basal levels when the oesophageal lumen was bathed with isotonic saline[34]. This decrease in LOS pressure became more pronounced with successive acid infusions and with infusions of capsaicin solution. Secondly, a similar sensitisation is seen in vagal efferent responses to oesophageal acidification (Chapter 2 and [209]). In fact, the vagal efferent responses occured concomitantly with the LOS pressure responses in combined manometry and electrophysiological studies. Lastly, direct responses to acute administration of oesophageal acid and capsaicin were evoked in a few recordings of *in vitro* oesophageal mucosal and tension receptors[210].

The effect of oesophageal capsaicin infusion was investigated as protons have been proposed as the endogenous activators of the capsaicin, or vanilloid, receptor (VR1)[31, 162,

[212]. The vanilloid receptor has been identified in the dorsal horn of the spinal cord and dorsal root ganglion in man, rat and pig[253]. As yet, the receptor has not been identified in the vagus, which may be due to a different subtype being present in the vagus. However, they have been found to be functionally expressed on the peripheral endings of vagal C-fibres in the guinea pig trachea[111]. In this study, acid, braykinin and capsaicin applied directly on the epithelial surface of the guinea pig trachea *in vitro* activated single vagal afferent fibres. When the selective capsaicin antagonist capsazepine was perfused onto the receptive fields, the responses to low pH and capsaicin were abolished, but the response to bradykinin was maintained. Removal of the epithelium did not affect the responses to any of the chemical stimuli or the effects of capsazepine. This implies that protons and capsaicin act via the same mechanism and that their effects are probably neurally mediated.

5.2 Oesophageal tension receptors

5.2.1 Mechanosensitivity of oesophageal tension receptors

The oesophageal tension receptors recorded in this study appear to be highly sensitive to changes in intraluminal pressure, as evident by their response to balloon distension even at the lowest level of distension. This suggests that they are well positioned to detect the passage of food as the bolus progresses along the oesophageal body. The proportional increase in receptor activity with increasing volumes of distension may also be important in providing information on the size of the bolus. In these aspects, these tension receptors resemble the low threshold vagal mechanoreceptors (LTMs) described in the opposum[239, 240] as these fibres only required a slight increase in intraluminal pressure to evoke an increase in discharge. Also, the maximal firing rate obtained by the LTMs were similar to that evoked by the present ferret vagal tension receptors. As intraballoon, and therefore intraoesophageal, pressure was not routinely measured, the saturation pressure of the ferret tension receptors could not be ascertained. However, most ferret oesophageal tension receptors reached their maximal firing rate before the highest level of distension was performed.Like the opossum LTMs, vagal tension receptors in the ferret are likely to bve involved in detecting increases in pressure within the normal physiological range.

The pattern of response of the oesophageal tension receptors to oesophageal distension is similar to that described previously[239, 240] where balloon distension led to a tri-phasic response in vagal tension-sensitive receptors. These LTMs were present in the smooth muscle portion of the opossum oesophagus and thought to be connected in series to either circular or longitudinal muscle fibres, where they can be activated by passive distension or active muscle contraction. Although the precise function of these fibres have yet to be elucidated, it is certain that these relay information regarding the tension in both circular and longitudinal muscle layers during both oesophageal distension and oesophageal peristalsis[239]. These would probably terminate within the myenteric ganglia via intraganglionic laminar endings (IGLEs)[29, 198, 223].

5.2.2 Chemosensitivity of oesophageal tension receptors

Both acid and capsaicin solutions were infused into the oesophageal lumen. Acid was infused prior to capsaicin in most studies in order to make the oesophageal epithelium more permeable[257]. This 'priming' of the oesophageal lumen with previous infusions of acid was later found to be unnecessary as capsaicin infusions increased mean arterial pressure even without prior oesophageal acid infusions. The significant increase in mean arterial pressure evoked by capsaicin infusions indicate that it effectively diffused across the mucosal epithelium of the oesophagus and therefore would have reached the peripheral endings of the fibres being recorded.

The non-responsiveness of my tension receptors to oesophageal acid was consistent with results from an earlier study by Harding and Titchen[131] in the cat. Generally, vagal tension receptors in the oesophagus[240] and stomach[37, 38, 84, 109] respond to chemicals indirectly, ie secondary to changes in muscle length or tension. The direct activation of oesophageal tension receptors by chemicals applied peripherally either topically or systemically is only found in one report by Sengupta[240] in which recordings from the vagus and the thoracic sympathetic nerves of the oppossum were made. Bradykinin was applied

systemically to stimulate both vagal and sympathetic oesophageal afferents. Only a proportion of vagal receptors responded to bradykinin: these responses were found to be secondary to longitudinal muscle contraction. On the other hand, all sympathetic afferents tested were activated directly by bradykinin.

In contrast to the results obtained with oesophageal acidification, a proportion of my vagal tension receptors were found to respond directly to capsaicin. Repeated capsaicin infusion did not lead to desensitisation. Responses were not due to changes in muscle length or oesophageal contractions as these parameters have been monitored in previous studies and found to be unaffected by oesophageal infusion of capsaicin in other studies performed in our laboratory[34, 230]. This may be the first instance where a chemical stimulus was able to activate vagal tension-sensitive endings in the absence of motor activity.

Regardless of the direct oesophageal tension receptor response to oesophageal infusions of acid and capsaicin, the afferent response to oesophageal balloon distension was significantly attenuated after intraluminal infusion of capsaicin. These results are consistent with *in vitro* data from our laboratory which show that a proportion of oesophageal tension receptors are desensitised to further mechanical stimulation after topical capsaicin application[210]. Unmyelinated afferents in the cat's cornea which were directly activated by capsaicin became unresponsive to mechanical (von Frey hairs) and chemical (hypertonic saline, acetic acid) stimuli after exposure to capsaicin solution[115]. Taken together, acute capsaicin administration can cause cross-desensitization even when no direct changes in afferent activity is discernible.

This may be a novel finding as capsaicin is traditionally thought to cause initial activation of afferents which is then followed by desensitisation if the exposure to capsaicin is sufficiently large[141]. In this present study, capsaicin is shown to cause a decrease in the mechanosensitivity of oesophageal tension receptors without any noticeable prior direct activation of the afferent endings.

5.3 Oesophageal mucosal receptors

5.3.1 Identification of oesophageal mucosal receptors

The extent of difficulties encountered when attempting to identify and characterise oesophageal mucosal receptors *in vivo* was unexpected. As the oesophagus is a hollow tubular structure within the thoracic cavity, the minute receptive fields of mucosal fibres are virtually impossible to locate with any precision *in vivo* without removal of the diaphragm and artificial ventilation. Of the 14 oesophageal afferents recorded, 13 were classified as tension receptors. Tension receptors were easier to identify as they usually had an irregular, low frequency discharge which was modulated by movement of the stimulating assembly within the oesophageal body. The respiratory activity of the animal also modulated the discharge of tension receptors although this input was overridden when an optimal level of response was generated by distension or chemical stimuli. Respiratory modulation in the resting discharge pattern of some fibres was also observed by Clarke and Davision[73].

Only one out of the 14 oesophageal afferent fibres that were recorded had a receptive field in the mucosa. This particular unit was initially mistakenly classified as a tension receptor due to its initial response to oesophageal balloon distension which was similar in profile to that generated by oesophageal tension receptors. Parallel *in vitro* studies of ferret oesophageal mucosal fibres in our laboratory[207] have shown these afferents are sensitive to stroking with von Frey hairs with forces as small as 10mg and have receptive fields of approximately 0.5mm^2. It was difficult to have an analogous stimulus to apply to the intact oesophagus *in vivo*. *In vitro*, the tissue can be pinned out flat, thus facilitating identification of the receptive field. Indeed, equal numbers of tension and mucosal fibres have been identified *in vitro* where fine tactile stimulation can be applied to a given area with great control. Responses to chemical stimuli have been obtained by isolating the receptive field and applying solutions locally onto the area.

The proportion of mucosal and muscular fibres observed in the present study is in contrast to that seen in the literature. Only two other *in vivo* studies are reported whereby the chemosensitivity of oesophageal vagal afferents to acid have been examined. The first

was an initial report by Harding and Titchen[131] which was based on recordings made from 18 tension receptors and 14 mucosal receptors in the cat. The second study was performed by Clerc and Mei[76], again using the cat. In this study, recordings were made from 31 tension receptors, 29 mucosal receptors, and 2 serosal receptors. The difference between these 2 studies and the present one may be due to a number of factors. Firstly, my experiments were conducted on ferrets weighing an average of 800g and ranging in size from 500-1500g. The other 2 studies were conducted on cats weighing between 2.5-5.0 kg. In my study, manipulation of a specific portion of the oesophagus was difficult as both the rod and the assembly used fitted snugly within the oesophageal lumen. With a larger animal model such as the cat, oesophageal manipulation could presumably be performed with a cylindrical object which was smaller in diameter than the oesophageal lumen, thus ensuring an isolated stimulation of a particular portion of the oesophageal body. Secondly, the diaphragm around the gastro-oesophageal junction was removed in the study by Clerc and Mei[76] and the animal artificially ventilated. This means that the receptive fields of afferents located within the lower oesophagus would be easier to identify and isolate with stimulus such as digital compression.

5.3.2 Chemosensitivity of oesophageal mucosal receptors

The single mucosal oesophageal afferent that was obtained for this study was responsive to both acid (HCl 150mM) and capsaicin (3.2mM) infusion. The receptive field of this vagal unit was located within the distal 5 cm of the oesophageal body. This unit was more chemosensitive than the oesophageal tension receptors recorded in this study. Firstly, the mucosal afferent responded directly to acid infusion which none of the oesophageal tension receptors did. Secondly, the concentration of capsaicin solution used in oesophageal infusions of the mucosal receptor study was 50% that used in most of the tension receptor studies.

The responsiveness of these oesophageal mucosal afferents to infusion of acid and other chemicals may be at least partially dictated by the location of the receptive field along

the oesophagus, ie all mucosal afferents located in the proximal oesophagus were shown to be acid-insensitive, those located within the middle and distal oesophagus were activated by acid and other chemicals, whereas only a proportion of those located in the region of the lower oesophageal sphincter were acid-sensitive[76, 131]. The peripheral terminations of mucosal afferents are also located closer to the lumen and those of muscular, or tension, receptors. Chemicals infused intraluminally would therefore have a shorter distance to travel to reach mucosal afferents. These would indicate that mucosal receptors within the mid to distal section of the oesophageal body may be primarily responsible for the detection of the presence of acid and other substances refluxed from the stomach.

The response of my oesophageal mucosal receptor to both acid and capsaicin infusion may either be due to the vagal afferent being a non-specific, or polymodal mucosal unit, similar to those identified elsewhere in the GI tract[35, 37, 38, 72] or that both acid and capsaicin act via the same mechanism, and probably through the same receptors. In other studies where the effects of protons and capsaicin on the vanilloid receptor VR1 were compared, it was found that protons potentiated the effects of capsaicin if the latter were applied at a submaximal dose[65].

5.4 Gastric mucosal receptors

Gastric mucosal afferents in my study responded to light mucosal stroking and a variety of chemical stimuli. One corpus mucosal receptor responded to close intraarterial injections of cholecystokinin, bradykinin, and capsaicin. The other gastric mucosal receptor responded to topical application of hypertonic saline, acid, and saturated glucose, but not to close intraarterial injections of 5-hydroxy tryptamine and capsaicin.

The activation of these receptors by more than one chemical classifies them as polymodal receptors. Polymodal, or non-specific, receptors have been identified in the ferret and rat gastro-duodenal region[35, 37, 38, 72]. These receptors are sensitive to light mucosal stroking and respond directly to intraluminal administration of acid, alcohol,

hypertonic saline, copper sulphate, and to close intraarterial administration of cholecystokinin, bradykinin, adrenaline, and 5-hydroxytryptamine. They are qualitatively different to unimodal afferents which have been described in the cat stomach and duodenal and are only activated by one chemical to the exclusion of all others[148, 189, 192].

With the antral mucosal receptor, inflammation induced by irrigating the mucosal surface containing the receptive field with an acidified aspirin solution affected the responses to topical administration of chemicals in different ways. After the induction of inflammation, the response to the lower concentration of NaCl (500mM) was potentiated while that to the higher concentration (1M) was unchanged. There may be several reasons for this. Firstly, 500mM NaCl was the first solution tested under control conditions. The response of the mucosal receptor may not have been maximal in the same way that the oesophageal mucosal receptor in this study also did not respond to the first acid infusion. Secondly, the threshold required to elicit maximal responses may have been lowered by the inflammation induced. Thus, the response to the lower concentration of NaCl was very similar in intensity to the ones generated by the higher concentration of NaCl under either condition. It may well be a combination of the two reasons listed above.

5.5 Conclusion

This study may be the first to document the novel finding of capsaicin induced desensitization without prior direct activation of afferent endings. It also shows that tension receptors within the oesophagus are able to respond directly to a chemical infusion, albeit a strong and potentially noxious one. The mucosal fibres that I recorded from are polymodal in nature, each responding to more than 1 chemical.

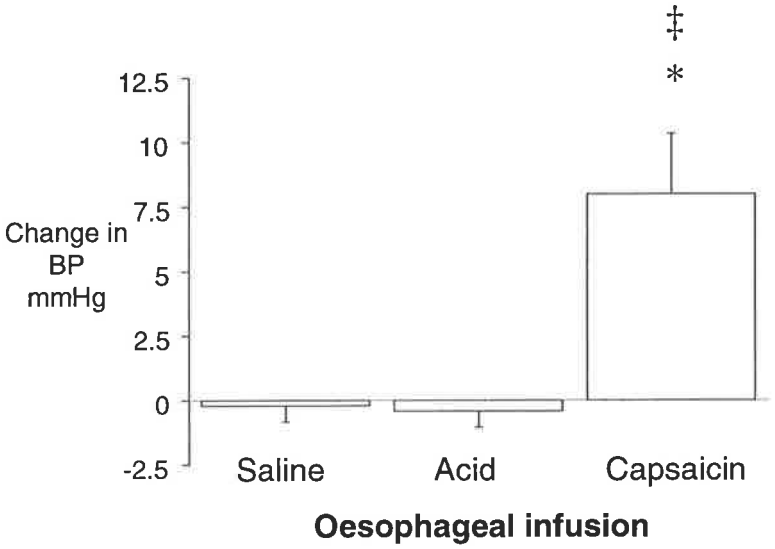


Figure 1-1. Group data representing effects of oesophageal acid and capsaicin infusion on mean arterial pressure.

Infusions of saline (isotonic), acid (HCl, 150mM), and capsaicin (3.2-6.4mM) solutions were administered into the distal oesophageal lumen in random order. Blood pressure was calculated before and after each oesophageal infusion.

* $p<0.01$ vs Saline, and
‡ $p<0.01$ vs Acid using paired t-test
$n\geq10$

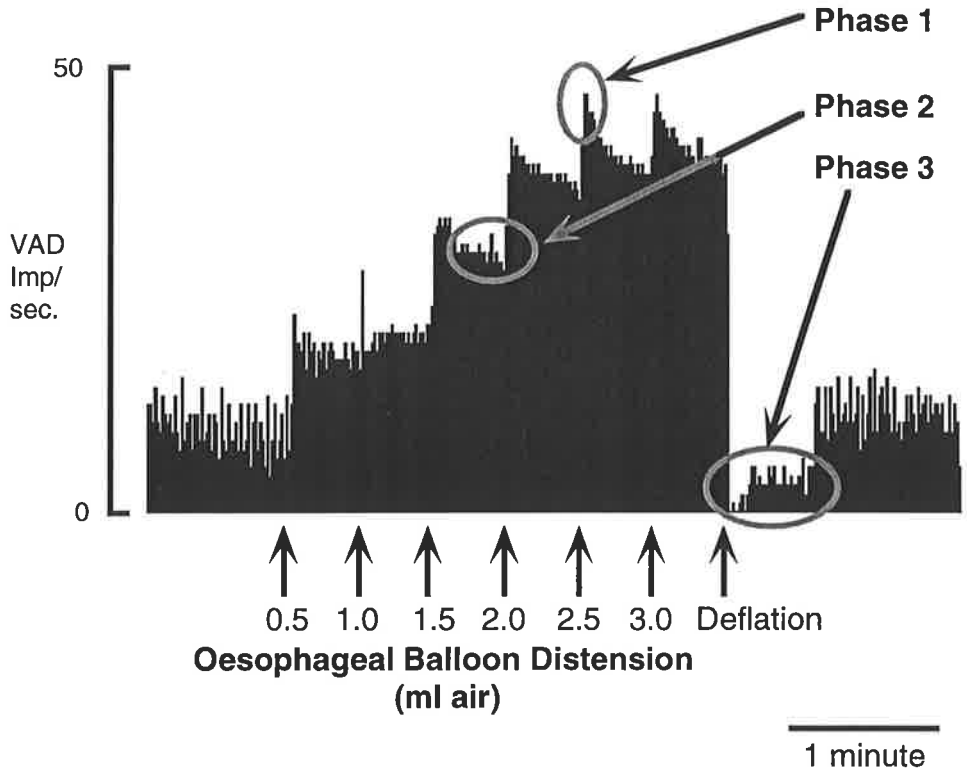


Figure 1-2. Integrated record of the vagal afferent discharge (VAD) of an oesophageal tension receptor in response to incremental oesophageal balloon distension.

The silicone rubber balloon was positioned within the distal 5 cm of the ferret oesophagus and was inflated in 0.5ml increments to a total of 3ml. The response can be divided into 3 phases: in Phase 1, onset of distension evoked a sharp increase in discharge. The discharge level in Phase 2 is less than that during Phase 1, although it remained above basal levels. Phase 3 commenced with balloon deflation, and led to a complete cessation of activity before discharge slowly returned to prestimulus levels.

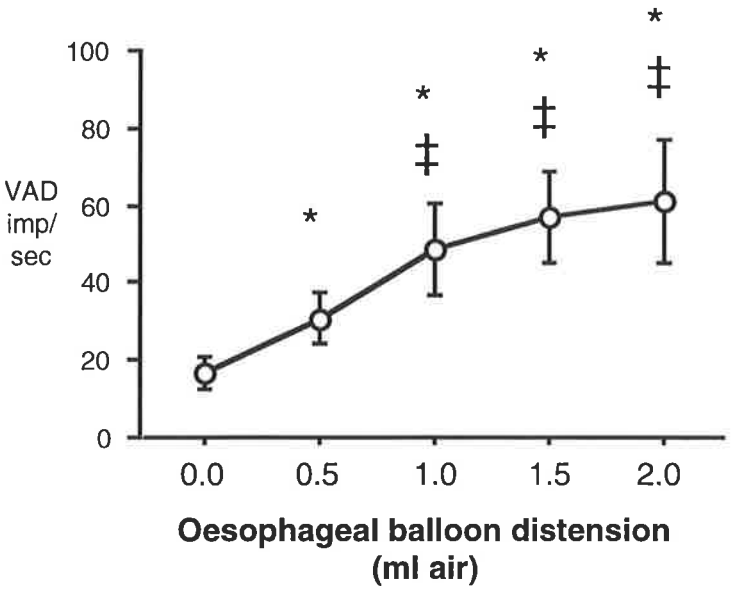


Figure 1-3. Group data representing discharge rate (VAD) of oesophageal tension receptors (n=13) in response to oesophageal balloon distension.

The discharge rate of oesophageal tension receptors was calculated during Phase II, ie the slowly adapting period, of the response at each level of distension. Overall, the increase in discharge was proportional to the degree of distension and was significant up to a volume of 1ml.

* $p<0.01$ vs Control (ie, OBD 0.0 ml),

‡ $p<0.05$ vs. OBD 0.5 ml
using paired t-test

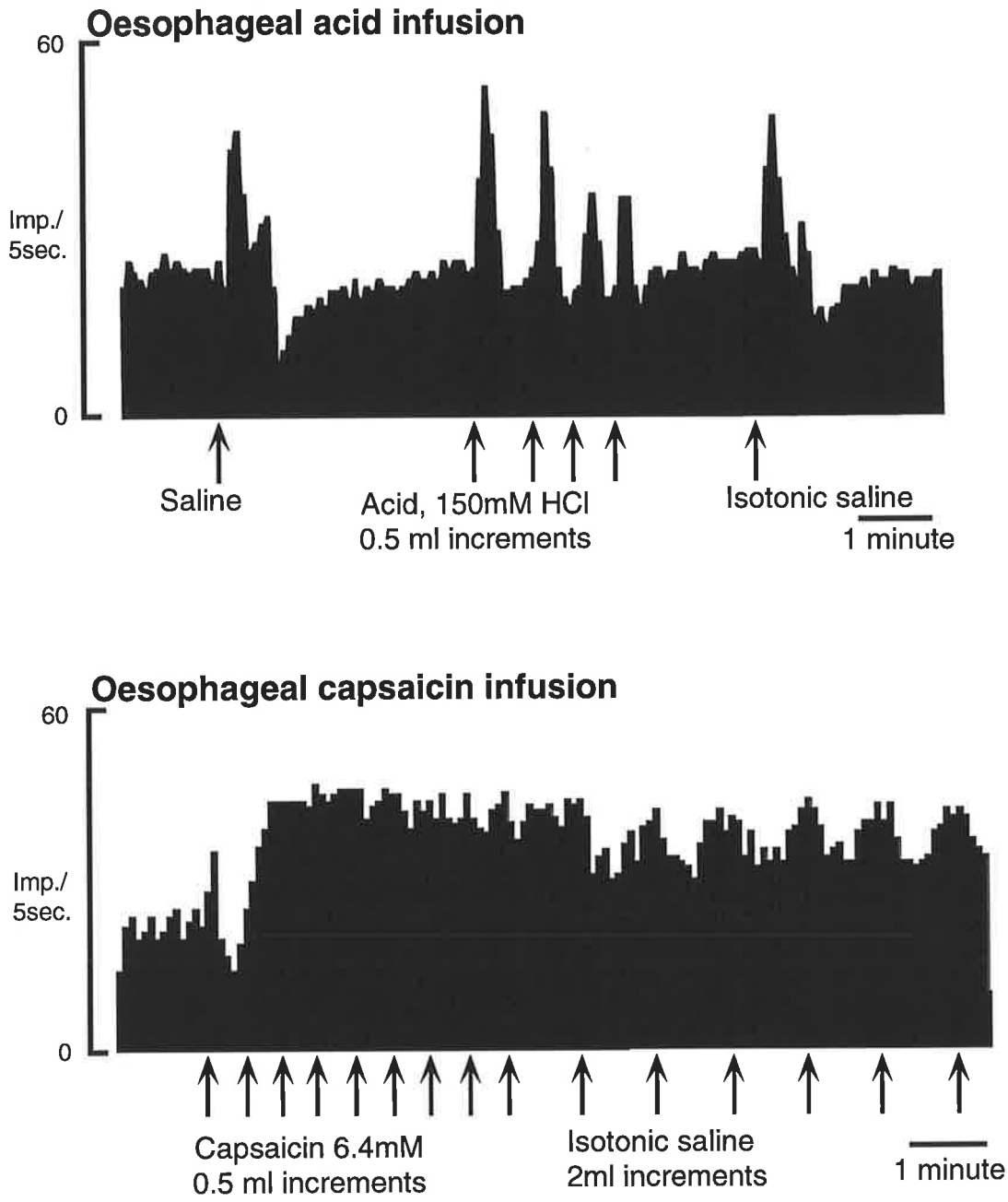


Figure 1-4. Integrated record of a vagal tension receptor response to intra-luminal acid and capsaicin.
The response of this fibre to oesophageal ballooon distension is seen in Figure 1-2.

Saline (isotonic), acid (HCl 150mM), and capsaicin (6.4mM) were infused slowly through different sideholes which were positioned at the level of the receptive field. Arrows indicate the start of the infusion periods.

Top trace: Infusions of saline and acid over the receptive field led to a brief burst of activity which lasted only for the duration of the infusion period.
Bottom trace: Capsaicin infusion yielded a sustained increase in its discharge which slowly returned to basal levels with multiple saline infusions.

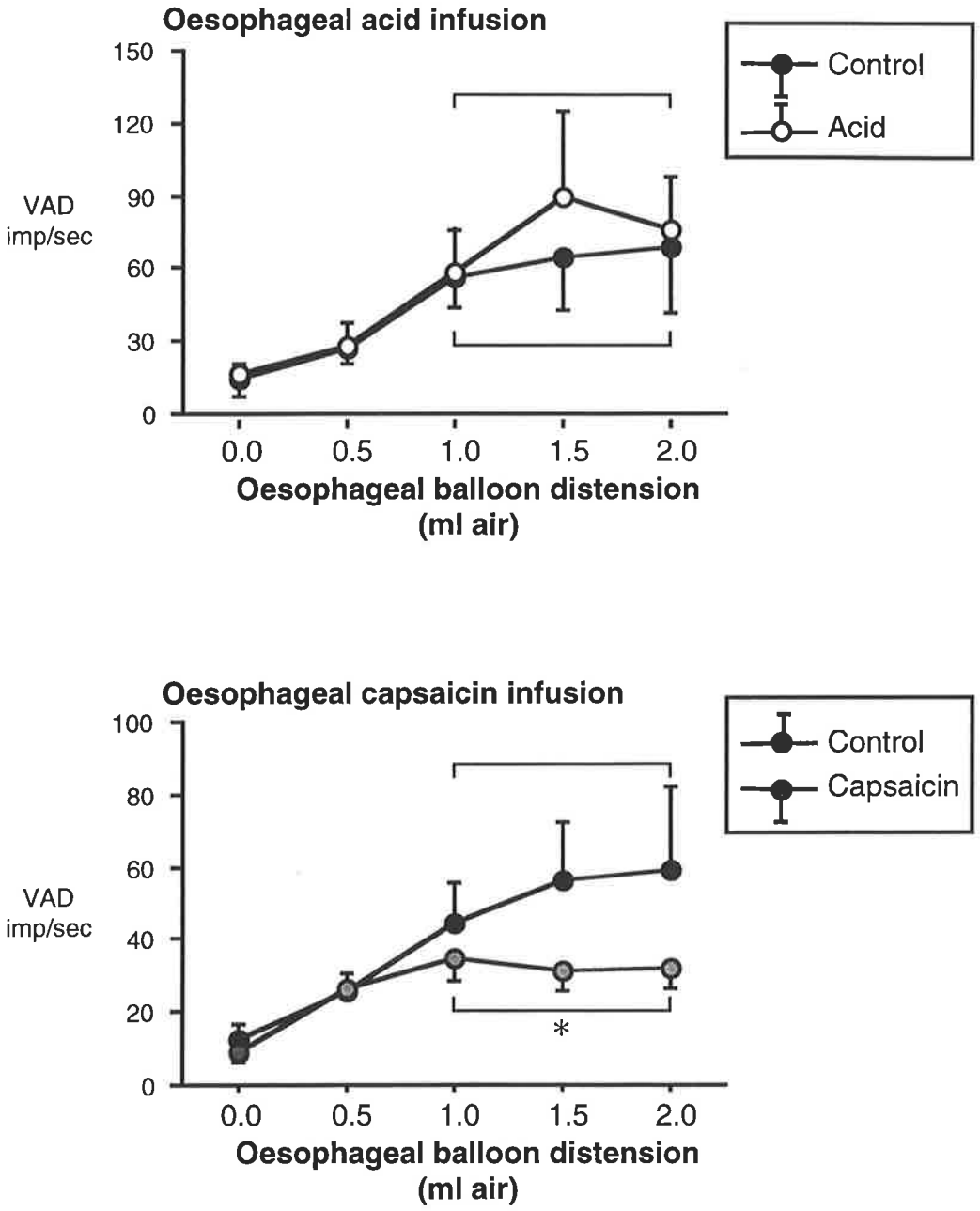


Figure 1-5. Group data representing effect of oesophageal acid and capsaicin infusion on discharge rate (VAD) of oesophageal tension receptors in response to oesophageal balloon distension.

Oesophageal balloon distension (OBD, 0.0-2.0ml in 0.5ml increments) was performed under control conditions, after acid infusion (n=4), and after capsaicin infusion (n=6).

The discharge rate was calculated during the slowly adapting portion, Phase II, of the response at each level of OBD. There was no significant difference in the tension receptor response to distension after acid. Combined data from OBD 1.0-2.0 ml obtained after capsaicin was statistically significant (* $p \leq 0.05$ vs Control using paired t-test).

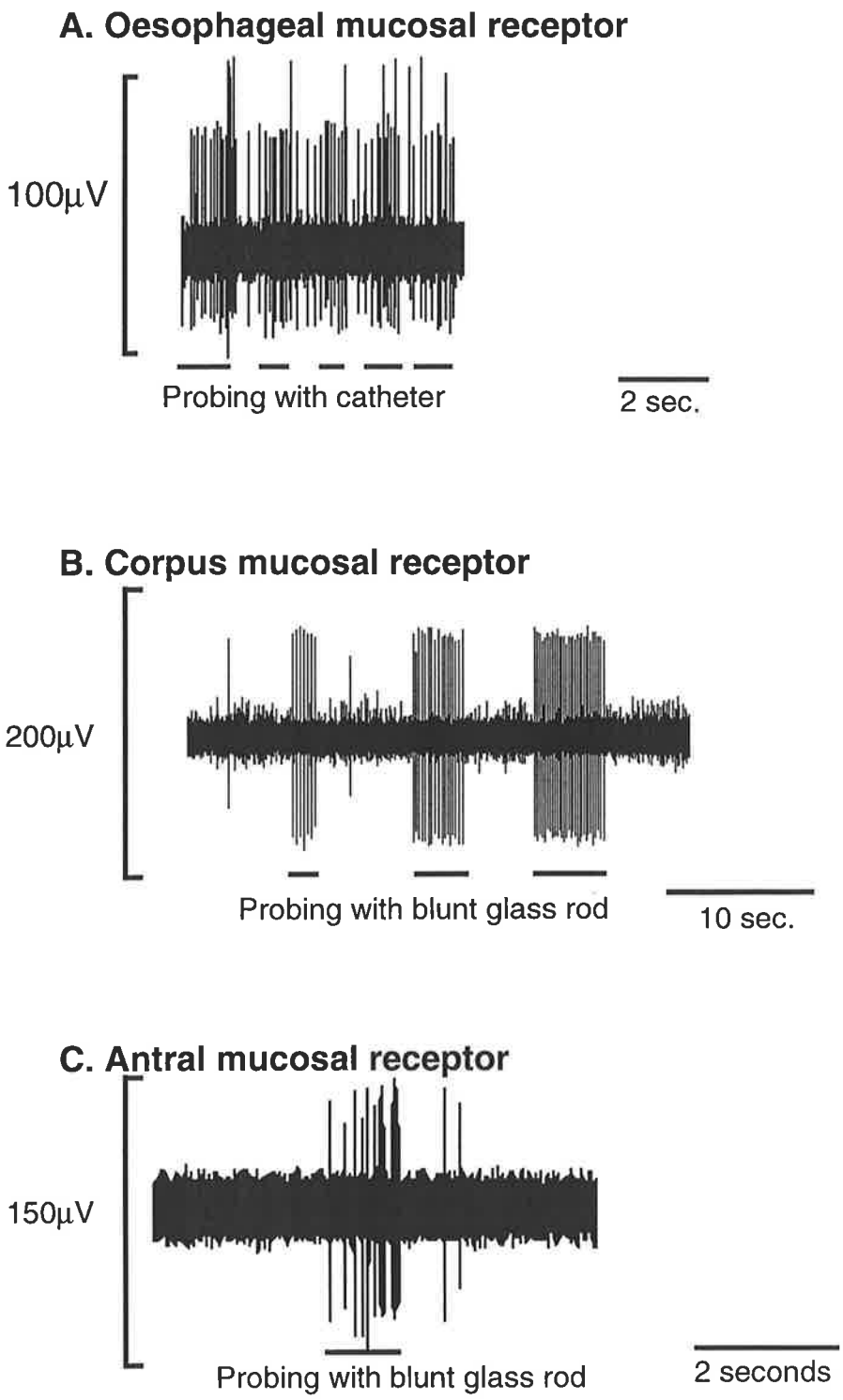


Figure 1-6. Raw record of responses of 3 mucosal receptors to light mechanical stimuli.

A. The oesophageal mucosal fibre responded to movement of the catheter within the distal oesophagus with rapid bursts of discharge.

B-C. Probing of the mucosal surface yielded a short burst of activity in both gastric mucosal fibres.

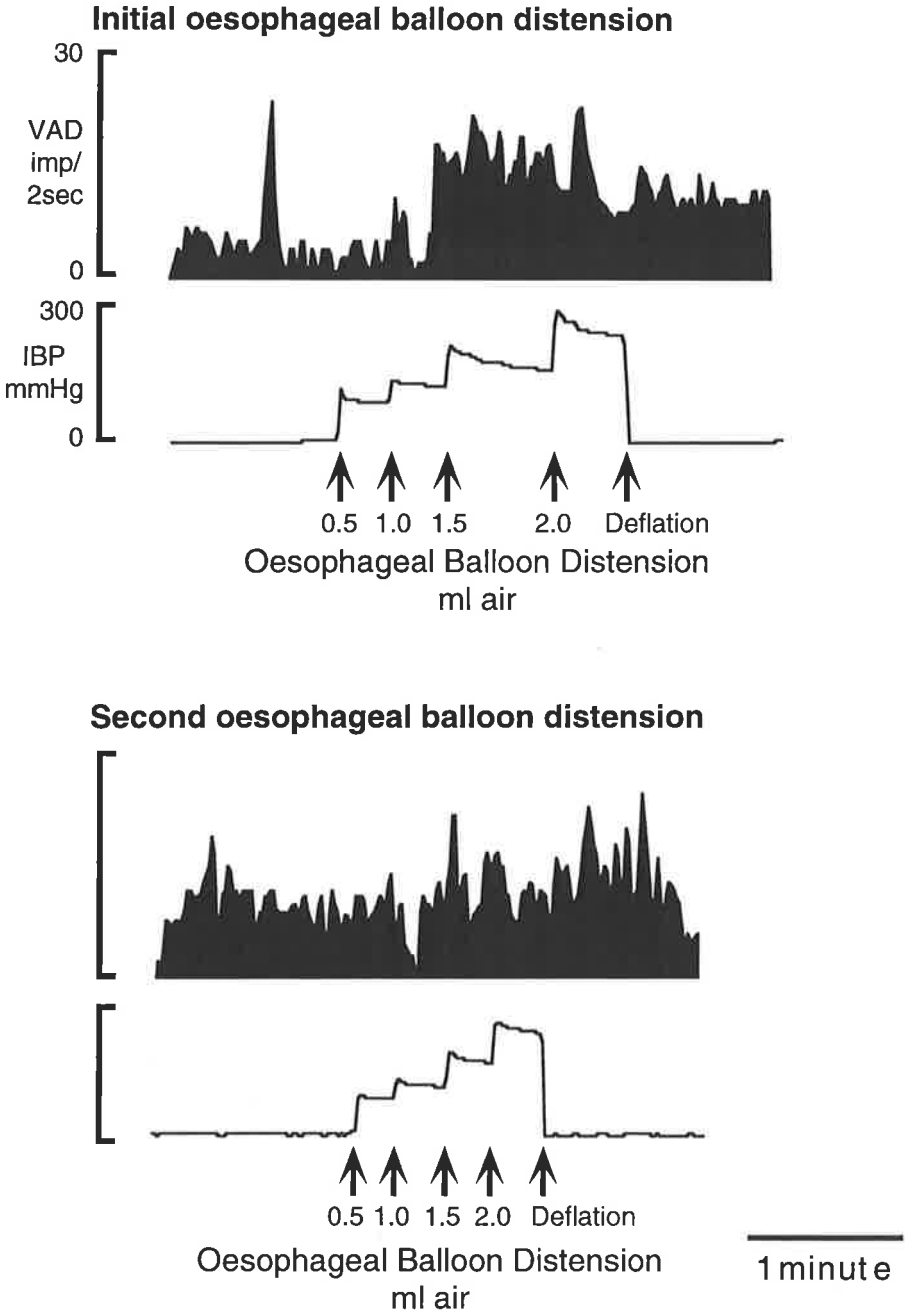


Figure 1-7. Response of an oesophageal mucosal afferent to oesophageal balloon distension.
Top trace: integrated record of the vagal afferent discharge (VAD) of a mucosal receptor response to oesophageal balloon distension showed an increase in discharge which was not linear. However, repetition of the stimulus led to a pattern of response which was distinctly non-linear.
Bottom trace: intra-balloon pressure (IBP) increased with each increment of oesophageal balloon distension.

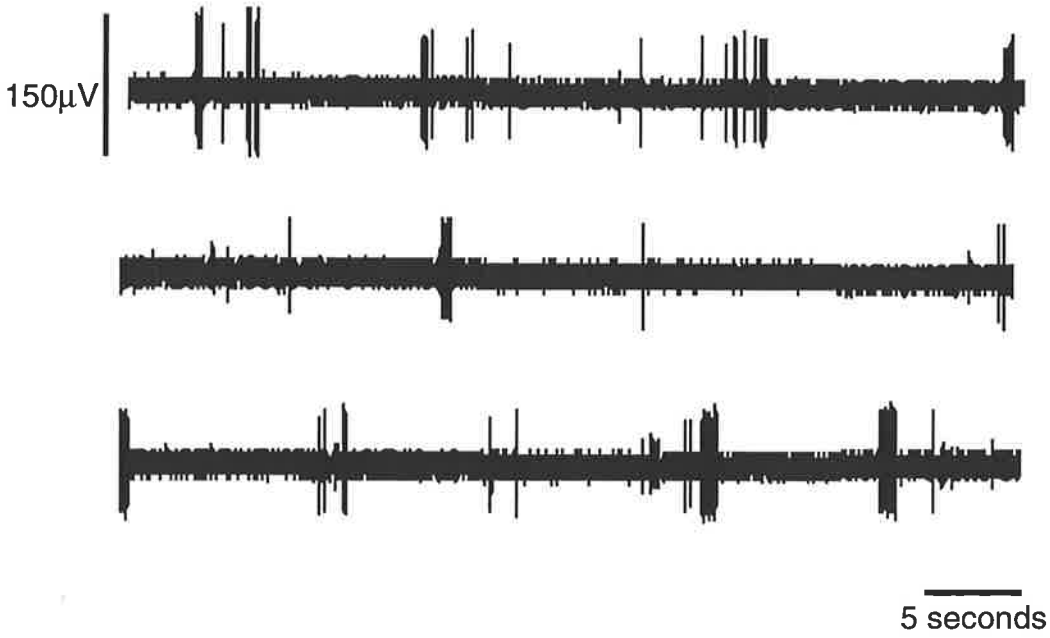


Figure 1-8 Raw tracing of the activity of an antral mucosal receptor (continuous recording).

The receptive field of this unit was located in the antrum along the lesser curvature near the pylorus. Upon opening the stomach along the greater curvature, a piece of food was found lodged within the folds of the mucosa. The 'spontaneous' discharge was due to the food particle rubbing against the mucosa with each antral contraction, which occurred at approximately 6/min. The removal of the particle led to a cessation of the activity.

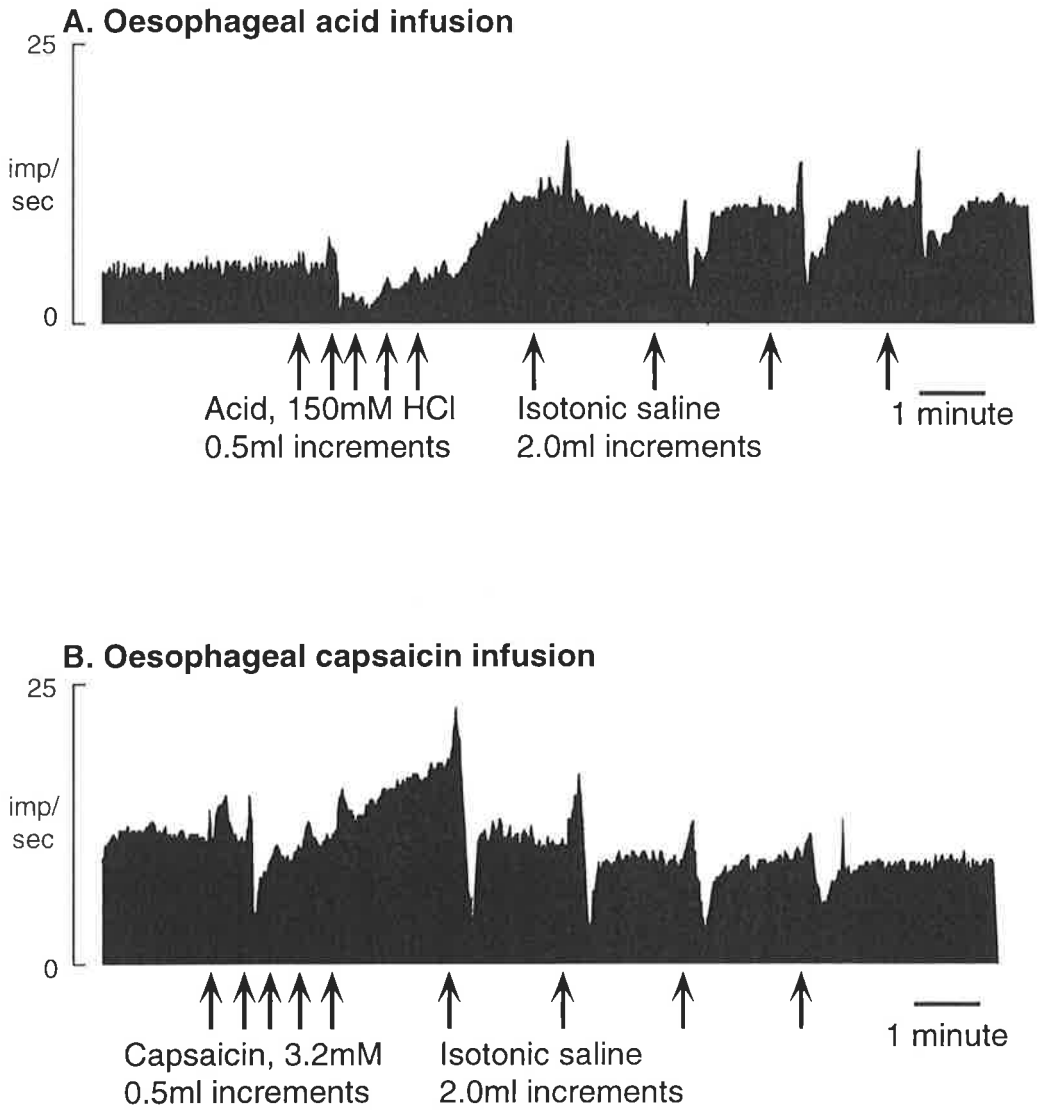


Figure 1-9 Integrated record of the response of an oesophageal mucosal fibre to oesophageal infusions of acid and capsaicin solution.
Arrows indicate the start of each infusion.

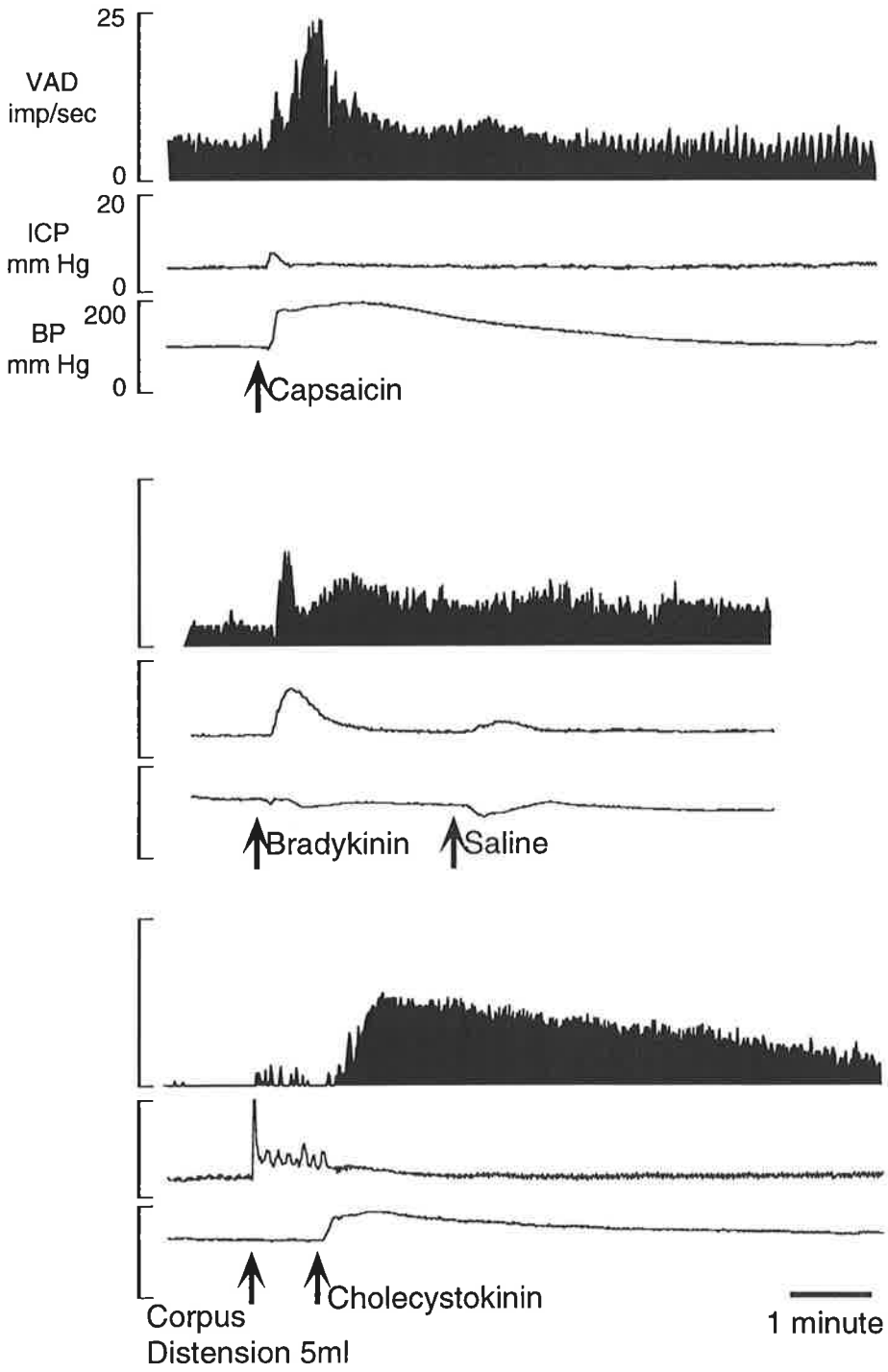
A-B. The flow of fluid over the receptive field led to an increase in discharge which was followed by a silent period at the end of each infusion. Infusions of both acid and capsaicin solution resulted in a prolonged increase in discharge which slowly returned to basal levels with multiple saline infusions. The inhibition of discharge over the period of acid and capsaicin infusions was probably due to the silent period of infusion of the previous bolus as there was not sufficient time between the end of one infusion to the start of the infusion of the next bolus.

Figure 1-10 Effect of capsaicin, bradykinin and cholecystokinin on vagal corpus mucosal receptor discharge, corpus pressure and blood pressure.
Top trace: integrated record of the afferent response.
Middle trace: intracorpus pressure.
Bottom trace: blood pressure.

A. Capsaicin (65nmol close ia) induced an excitation of discharge which commenced at the same time as an increase in blood pressure and a corpus contraction.

B. Bradykinin (18nmol close ia) led to a large corpus contraction simultaneous with an increase in afferent discharge levels. Blood pressure was transiently reduced before returning to basal level. Saline injected through the same port resulted in a response which was similar in profile.

C. The isoloated corpus was slightly distended with 5ml saline before cholecystokinin (100pmol close ia) was administered. The octapeptide induced an increase in discharge, corpus relaxation, and an increase in blood pressure.


25
VAD
imp/sec
0
20
ICP
mm Hg
0
200
BP
mm Hg
0
Capsaicin
Bradykinin
Saline
Corpus
Distension 5ml
Cholecystokinin
1 minute

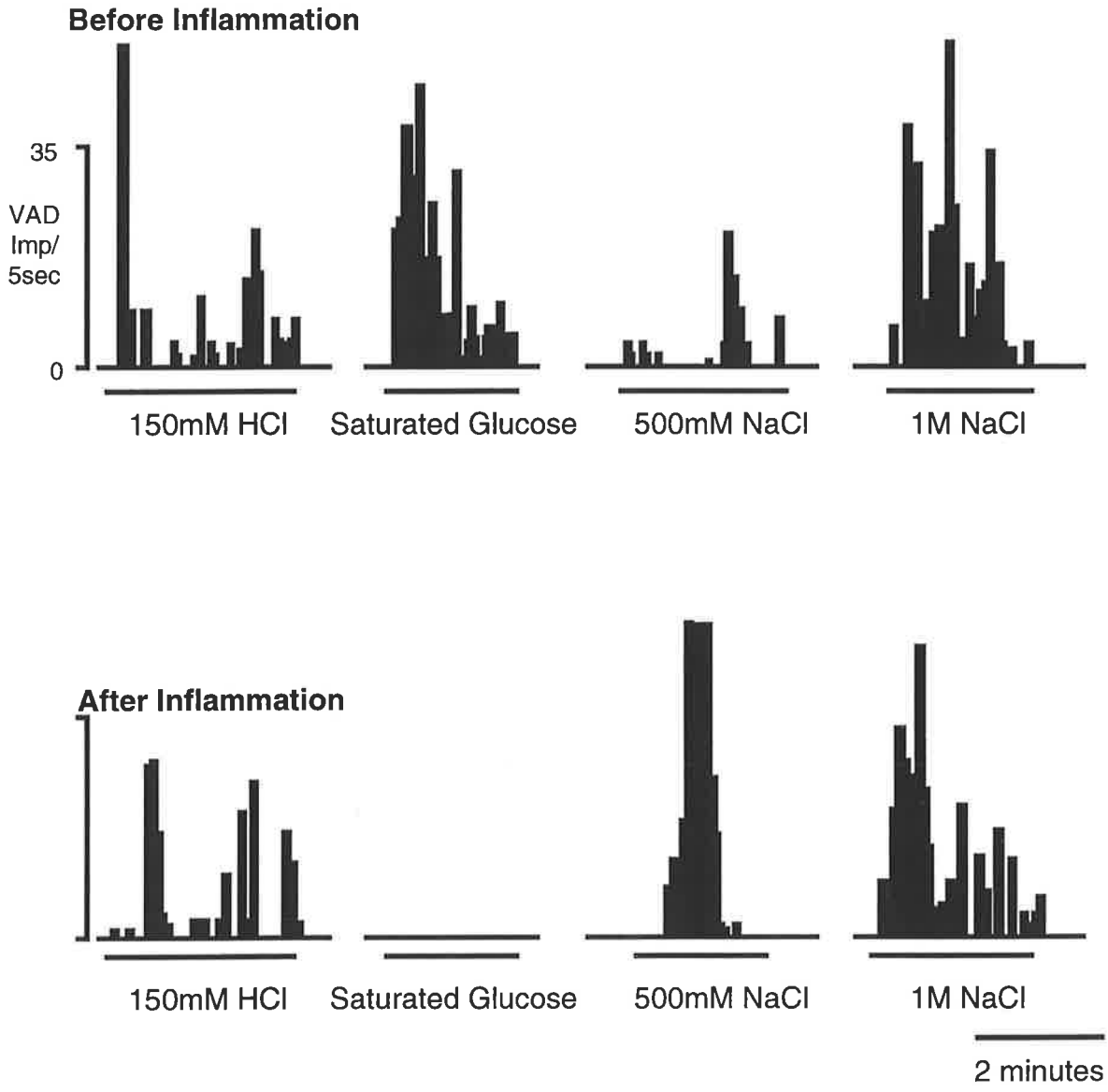


Figure 1-11 Integrated record of the response of an antral mucosal receptor to various chemical stimuli before and after inflammation with acidified aspirin.
The spontaneously silent antral mucosal receptor was activated with acid, saturated glucose and 2 different concentrations of hypertonic saline. The area surrounding the receptive field was irrigated with saline after the response to each stimulus was observed.

After inflammation was induced, the response to each stimulus was again tested. The response of this antral mucosal fibre to acid and 1M NaCl was maintained, while that to saturated glucose was abolished and that to 500mM NaCl was potentiated.

CHAPTER 2.
Modulation of vagal efferent discharge by gastric and oesophageal mechanical and chemical stimuli

1 SUMMARY

1. The properties of gastro-oesophageal afferent inputs onto central vagal neurones were studied in unitary recordings of 90 vagal efferent fibres in the ferret *in vivo*.

2. Responses to oesophageal balloon distension (OBD, 2ml air) and gastric distension (GD, 40-60ml saline) were observed in 68/90 and 80/90 fibres tested respectively. Vagal efferent discharge increased in response to OBD in 64 fibres and decreased in 4 fibres. GD evoked excitation of discharge in 62 fibres and inhibition in 18 fibres. In fibres where both mechanical stimuli evoked changes in discharge, the response was similar in intensity and in the same direction, ie both stimuli either increased or decreased nerve activity, with the exception of 7 fibres that were excited by OBD and inhibited by GD.

3. Responses to oesophageal acid infusion (HCl 150mM) were seen in 18/31 fibres tested. These responses were sensitised with repeated acidification and were mainly excitatory. In 13/31 of these studies, animals were subsequently treated with oesophageal capsaicin infusion, evoking a change in discharge in 12/13 studies. 3/12 fibres which responded to capsaicin infusion did not respond to prior acidification. Where the discharge was modulated in response to both chemical infusions, the direction of response was the same, which in all studies was excitatory. The direction of response to oesophageal chemical infusions was the same as that to oesophageal balloon distension, with the exception of one fibre which was excited in response to the mechanical stimulus but was inhibited by oesophageal acidification.

4. Responses of 67 units to close intraarterial cholecystokinin (CCK, 100pmol), bradykinin (BK, 18nmol), and capsaicin (Cap, 65nmol) were tested. Responses were mainly inhibitory. 15 fibres did not respond to any chemical(s) tested whereas 40 fibres responded to all chemical(s) tested. The remaining 12 fibres responded to at least one chemical but showed a negligible response to at least one other chemical. When responses

to more than one chemical were seen, they were always in the same direction although responses to different chemicals differed in latency, intensity and duration.

5. Bilateral cervical vagotomy performed in 16 studies abolished the basal efferent discharge in 4 studies. In the other 12 studies, responses of vagal efferent fibres to oesophageal mechanical and chemical stimuli and to close intraarterial cholecystokinin were abolished with the exception of 1 fibre whose response to CCK remained unchanged. Responses to close intraarterial bradykinin and capsaicin (n=5) were unaffected after vagal section except for the response of one fibre to BK where it was drastically reduced. In 5/7 fibres, responses to gastric distension were also abolished or drastically attenuated.

7. This study provides evidence that vagal efferent neurones receive convergent inputs from gastric and oesophageal mechano-sensitive afferents, from acid- and capsaicin-sensitive oesophageal afferents, and from cholecystokinin-, bradykinin-, and capsaicin-sensitive afferents within the upper gastrointestinal tract. The oesophageal afferents follow mainly a vagal pathway as do the cholecystokinin-sensitive afferents. There exists also a non-vagal, probably spinal, component which is sensitive to gastric distension, bradykinin, and capsaicin.

2 INTRODUCTION

The afferent innervation of the gastrointestinal tract has been described at length in the overall introduction of this thesis. Briefly, afferent fibres from the upper gut travel within the vagus and spinal nerves to the central nervous system. The majority of electrophysiological work has concentrated on looking at afferents with endings in the stomach, where its sensitivity to both mechanical and chemical stimuli have been studied. Investigation of oesophageal afferents has focussed mainly on the mechanical properties of tension receptors[76, 232, 239], with the exception of 2 studies[76, 131] where the responses of oesophageal afferents to intraluminal chemicals have been recorded. Gastrointestinal spinal afferents also respond to distension, and are sensitive to bradykinin[135, 240, 241].

Vagal efferent fibres receive convergent afferent inputs from many different areas of the gut and from many different types of afferents[16, 39, 41, 43, 92, 93, 105, 126, 219, 277]. Their responses to such a variety of peripheral stimuli demonstrate the complex organisation of vagal sensory and reflex pathways. One of these studies has also investigated the relative organisation and importance of mechano- and chemo- sensitive afferents in the gastro-oesophageal region which converge onto vagal efferent fibres[33]. In this study, 5-hydroxytryptamine (5-HT) was administered close intraarterially via the abdominal aorta, intravenously, and intraarterially via the carotid. 5-HT, administered via any of the 3 routes, strongly activated efferent fibres which also responded to oesophageal and gastric distension. This is in contrast to the weak responses observed in the other studies to intraluminally perfused chemicals[41, 43].

This section examines the convergence of inputs onto vagal efferent fibres from afferents sensitive to mechanical, chemical and pharmacological stimuli applied to different gut regions. The chemical and pharmacological stimuli are applied both intraluminally into the distal oesophagus and close intraarterially to examine the relative contribution of different populations of afferents onto central neurones. The next 3 chapters of this thesis will look at the involvement of different neurotransmitters and neuromodulators along the vagal reflex pathway.

3 METHODS AND PROTOCOL

The methods and protocol followed in this series of experiments are detailed in the Methods section of this thesis.

4 RESULTS

4.1 Patterns of spontaneous activity

All fibres evaluated showed either no basal discharge or a low frequency, irregular pattern of basal discharge (3.42±0.57 impulses/sec). This bore no obvious relationship to respiratory, cardiovascular or gastrointestinal contraction rhythms and remained constant throughout this portion of the study prior to the administration of drug agonists or antagonists when not associated with a particular stimulus. At least 60 seconds were allowed after the cessation of each mechanical stimulus and 3 minutes after the administration of each chemical stimulus to allow the firing rate of the unit to return to pre-stimulus levels. The shape, duration and amplitude of the action potential remained constant throughout the duration of the study. No non-spontaneously active fibres were recruited by any of the stimuli described below.

In 16 ferrets, following characterization of responses in intact animals, bilateral vagotomy was performed caudal to the recording site. This was performed after prior treatment with at least one antagonist drug (see later chapters). The spontaneous activity of each unit was examined for 2 minutes before the response to any stimuli was tested. In 4 units, spontaneous activity ceased immediately upon severing the vagal trunks. The spontaneous activity of the other 12 units was unchanged and their responses to at least 1 stimulus studied.

4.2 Responses to mechanical stimulation

90 vagal efferent units initially responded with a ≥50% change in discharge rate to gastric and/or oesophageal balloon distension, which was the selection criterion used to determine the viability of these units (Tables 2-1 and 2-2). These responses were reproducible under control conditions with the exception of 4 units whose responses to both mechanical stimuli were lost prior to the administration of a drug agonist or antagonist.

4.2.1 Oesophageal balloon distension

Oesophageal balloon distension (OBD) was performed in 90 studies by rapid inflation of the balloon mounted on the oesophageal assembly with 1.5-2.0 ml air for 30 seconds after which the balloon was promptly deflated. This evoked an increase in vagal efferent discharge (VED) in 64 fibres (320±50% increase from basal discharge levels, example Fibre A, Figure 2-1) and an inhibition of discharge (57±12% decrease from basal discharge levels, example Fibre B, Figure 2-1) in 4 fibres. The discharge of the remaining 22 fibres was unchanged (see Tables 2-1 and 2-2).

There were 3 general phases in the response which are clearly demonstrated in the fibre's response in Figure 2-2. These phases were similar to those seen by vagal oesophageal tension receptors in response to the same stimulus (see Chapter 1, Figure 1-2). Phase 1 consisted of a rapidly evoked (<1 second) intense increase in discharge which occurred with the dynamic phase of OBD. This is followed by Phase 2 which lasted throughout the static component of OBD. During this second phase, discharge decreased to a lower level but remained above basal level for the duration of the stimulus. Phase 3 occurred with balloon deflation where discharge abruptly decreased for a period of time up to several seconds to a level lower than resting discharge before returning to predistension levels. In some responses like the one illustrated in Figure 2-2, balloon deflation led to a complete cessation of VED before basal levels were reestablished. In fibres which were inhibited by oesophageal balloon distension, the direction of response was reversed in each phase. The first 2 phases in the inhibitory response of Fibre B in Figure 2-1 to balloon distension were not distinguishable from each other as balloon distension led to a complete cessation of discharge. The third phase can be seen when balloon deflation led to an enhanced discharge before firing returned to predistension levels. These phases were not distinctly identifiable in the responses of all fibres to OBD.

The responses of 6 fibres to OBD were assessed after bilateral vagotomy. All 6 fibres which responded to OBD under control conditions lost their response after acute vagal section (see Table 2-3). Figure 2-6 shows the effect of vagotomy on the response to

OBD. Prior to vagal section, the fibre responded with an increaase in discharge. However, section of both vagal nerve trunks led to the abolition of the OBD response.

4.2.2 Gastric distension

Gastric distension (GD) was performed in 90 studies by the rapid infusion of isotonic saline (40-60ml in the intact stomach in 69 studies or 15-25ml in the isolated corpus and 10ml in the isolated antrum in 21 studies) over 5 seconds. The stomach was distended for 1 minute before drainage. GD increased discharge in 62 fibres (380±66% increase from basal discharge levels, example Fibre A, Figure 2-3), inhibited discharge in 18 fibres (74±7% decrease from basal discharge levels, example Fibre B, Figure 2-3) and failed to evoke a change in the remaining 10 fibres (see Tables 2-1 and 2-2).

The response profile of vagal efferent fibres to this mechanical stimulus was similar to that obtained with OBD in that the response could also be divided into 3 phases. Phase 1 was rapidly evoked with the rapid infusion of saline into the stomach. The increase in discharge is maximal with this dynamic phase of distension. Phase 2 occurred during the static perod of distension where the discharge is still augmented above basal discharge but was lower than the discharge evoked during Phase 1. Phase 3 was not always obvious in all efferent responses to GD and involved a decrease in discharge below basal levels upon gastric deflation before discharge returned to predistension levels. Phases 1 and 2 can be clearly seen in the examples in Figure 2-3 (Fibre A) and Figure 2-4. Like the efferent responses to OBD, the direction of each phase was reversed in fibres which showed an inhibition of discharge with GD. Also, not all phases of the response were distinctly identifiable in the responses of all fibres to GD.

In some studies, intragastric and blood pressure were recorded concurrently with discharge (see example on Figure 2-4). The dynamic phase of gastric distension (Phase 1) led to a sharp rise in intragastric pressure due to the large force generated when saline was administered via a syringe into the stomach. During the static period of gastric distension

(Phase 2), intragastric pressure is slowly reduced over the duration of the distension period due to gastric accommodation. The intragastric pressure tracing was often truncated at the top end in order to more clearly display the intragastric pressure activity during distension. There was no phasic rhythm in the gastric pressure response to distension in this study. Blood pressure was also recorded. A small increase in blood pressure was often seen both during gastric inflation and deflation.

In 21 studies, the stomach was divided into its separate corpus and/or antral parts. The vagal efferent response to corpus distension (20 ml saline) was phasic in nature and strongly correlated with the pattern of intraluminal changes, such that the pressure waves were synchronous with the pattern of nerve discharge (example in Figure 2-5). Corpus distension elicited excitation of discharge in 15/21 studies (194$\pm$67% increase) and inhibition in the other 1 study (50% decrease). The discharge of the other 5 efferent fibres was unchanged by corpus distension. The vagal efferent response to antral distension was similar to most of the responses elicited by whole stomach distension.

The responses of 7 vagal efferent fibres to gastric distension were also assessed after bilateral vagotomy. Of these 7 fibres, the response of 2 fibres was not decreased after vagotomy, that of one was attenuated and that of the remaining 4 fibres was abolished (see Table 2-3). Figure 2-6 shows the effect of vagotomy on the response of a vagal efferent fibre to GD. Prior to vagal section, GD evoked excitation of discharge. After vagotomy, the response to gastric distension was still present, and was actually enhanced.

4.3 Responses to intra-oesophageal chemical stimuli

After characterisation of the responses to oesophageal balloon distension and/or gastric distension, the responses of 31 fibres were tested with oesophageal acid infusion. In 13 of these 31 experiments, capsaicin was also infused into the oesophageal body after at least 3 prior episodes of oesophageal acidification (Tables 1-1 and 1-2).

4.3.1 Oesophageal acid infusion

At least 3 acid infusions per study were performed in 31 studies, and the effect on vagal efferent discharge (VED) observed (Table 2-2). The response of each fibres to an innocuous stimulus was tested by infusing isotonic saline (2ml over 15seconds). This elicited a brief burst of activity which lasted only for the duration of the infusion (see Fibre A in Figure 2-7). The discharge of 18 fibres tested was altered in response to oesophageal acidification (ACID, 2ml HCl), with 15 fibres displaying an excitation of discharge (250±94% increase from basal discharge levels, example Fibre A, Figure 2-7) and 3 fibres showing an inhibition of discharge (51±20% decrease from basal discharge levels, example Fibre B, Figure 2-7). The discharge of the remaining 13 fibres was unchanged by acidification, even after 3 successive infusions. These responses were potentiated with successive acidification, with responses to the second and third series of acidification being significantly greater than the response to the first acidification (Figure 2-8). Of the 18 fibres that responded to acidification, 2 fibres did not display a discernible change in discharge with the initial acid infusion (not shown).

The efferent response to acidification was tested in 3 studies after bilateral vagotomy (Table 2-3). Vagotomy abolished the responses of all 3 fibres tested to acidification (see example in Figure 2-10).

4.3.2 Oesophageal capsaicin infusion

In 13 studies, at least one oesophageal capsaicin infusion (3.2-6.4nM, 2ml) was performed after a minimum of 3 episodes of oesophageal acidification, and the effect on vagal efferent discharge observed (Table 2-2). 12/13 fibres tested were activated by oesophageal capsaicin, with the responses being excitatory in all fibres (see example in Figure 2-9 and Table 2-2). Of the 12 fibres which responded to capsaicin infusion, previous acidification did not evoke a response in 3 fibres (see Table 2-2). In the other 9 fibres which responded to prior acidification, the direction of response to acidification was

the same as that evoked by capsaicin infusion. The one fibre which was unaffected by capsaicin infusion also did not respond to prior oesophageal acidification.

The magnitude of the capsaicin response was similar to that evoked by oesophageal acidification. However, in some studies, the duration of response to capsaicin infusion was longer and required more saline infusions before the discharge levels returned to the pre-infusion levels. The response of 4/8 fibres tested were greatly reduced after the first capsaicin infusion to subsequent infusions of either capsaicin and acid solutions whereas responses of the other 4/8 fibres tested remained essentially unchanged.

The response to capsaicin infusion was not tested after bilateral vagotomy.

4.4 Responses to close intraarterial drugs

Responses of 67 units to close intraarterial cholecystokinin, bradykinin, and capsaicin were tested. 15 fibres did not respond to any of these whereas 40 fibres responded to all three of them. The remaining 12 fibres responded to at least one drug administered but showed negligible responses to other drugs.

4.4.1 Responses to close intraarterial bradykinin

Close intraarterial bradykinin (BK, 18nmol) administered in 60 studies caused a change in vagal efferent discharge in 45 fibres and did not modulate discharge in the remaining 15 (Tables 2-1 and 2-2). Of the 45 studies, BK evoked an excitation of discharge in 16 fibres (414±94% increase from basal discharge levels, example Fibre A, Figure 2-11) and inhibition in 29 fibres (83±5% decrease from basal discharge levels).

The response profile to this stimulus was in general rapidly evoked and of short duration (see example on Figure 2-11). However, in some fibres, the responses were longer in duration, with up to 14 minutes elapsing before the discharge rate returned to

prestimulus levels (see examples on Figure 2-12 & 2-13). BK also caused a brief decrease in blood pressure and evoked an increase in gastric pressure in some studies which was often accompanied by a large intake of breath (described in the previous chapter, see Figure 1-10). The duration of vagal efferent responses were concomitant with the effects on blood pressure and intragastric pressure.

The effects of BK on VED in 5 studies was observed after bilateral vagotomy. Vagal section did not affect efferent responses to BK in 4 studies, while severely reducing the response of the remaining fibre (see Table 2-3 and example on Figure 2-10). In one of these 5 studies, bilateral section of the greater splanchnic nerves, performed after vagotomy, led to a cessation of spontaneous activity. No response to BK was evoked in this study after section of both the vagus and greater splanchnic nerves.

4.4.2 Responses to close intraarterial capsaicin

Capsaicin (Cap, 65nmol) was administered close intraarterially in 53 studies in order to selectively activate small diameter, unmyelinated C-fibres. This compound caused excitation of discharge in 11 fibres tested (280±85% increase from basal discharge levels, example Fibre A, Figure 2-11), inhibition in 20 fibres (73±7% decrease from basal discharge levels) and no response in other 22 fibres tested.

Responses to Cap were generally rapidly and powerfully evoked and lasted for <20 seconds (see example on Figure 2-11). As with responses to BK, there were some exceptions, with vagal efferent responses lasting for longer than 5 minutes in some studies (see example on Figure 2-12). Cap also caused an increase in blood pressure. Some animals responded with a sharp intake of breath which was seen simultaneously with a brief increase in gastric pressure (see example in Figure 1-10).

The responses to Cap were evaluated in 4 studies after bilateral vagotomy. Vagal section did not abolish the efferent responses to Cap in all 4 studies (Table 2-3). In the

efferent response shown in Figure 2-10, vagotomy appeared to enhance the Cap response. Subsequent section of both greater splanchnic nerves in one study abolished both resting discharge and the efferent response to Cap.

4.4.3 Responses to close intraarterial cholecystokinin

The sulphated form of the cholecystokinin octapeptide (CCK, 100pmol) was administered in 37 studies. The discharge of 29 vagal efferent fibres was modulated, with 18 fibres responding with a decrease in discharge (93±3% decrease from basal discharge levels) and 11 fibres with an increase in firing rate (353±77% increase from basal discharge levels, example Fibre A, Figure 2-11). The remaining 8 fibres did not respond to CCK.

The vagal efferent responses to CCK was not as rapidly evoked as its response to capsaicin or bradykinin, and usually lasted for 5-10 minutes before discharge rates slowly returned to pre-stimulus levels (see example on Figure 2-13). CCK caused an increase in arterial blood pressure and a concomitant decrease in intra-gastric pressure. This relaxation of the intact stomach was evident even when the stomach was undistended (see Figure 2-14).

The response of 5 fibres to CCK was tested after bilateral vagotomy. Vagotomy abolished the inhibitory responses of 4/5 fibres tested to CCK. In the remaining fibre, the vagal efferent response to CCK was unchanged after vagal section. In Figure 2-14, the response to CCK was abolished by vagal section. Vagotomy also affected the intragastric pressure response to the octapeptide, inducing a gastric contraction instead of the long lasting relaxation seen prior to nerve section.

4.5 Convergence of inputs

The responses of 90 vagal efferent fibres to various mechanical and chemical stimuli under control conditions are tabulated in Tables 2-1 and 2-2. The group data expressed in Table 2-1 indicate that responses of vagal efferent fibres to mechanical stimuli and oesophageal chemical infusion were predominantly excitatory whereas the majority of those fibres which responded to chemical stimuli applied close intraarteriaally showed inhibition of discharge.

There were trends seen in the direction of response of individual fibres to different subsets of peripheral stimuli. Responses of vagal efferent units to oesophageal balloon distension and gastric distension were similar in intensity and in the same direction with the exception of seven fibres, where an excitation was seen in response to oesophageal distension and inhibition of discharge seen with gastric distension. Responses to oesophageal stimulation, whether mechanical or chemical, were in the same direction with the exception of one fibre which showed an increase in discharge with oesophageal distension but was inhibited by oesophageal acidification. Where responses were seen to more than one chemical administered close intraarterially, they were always in the same direction (examples on Figure 2-11, 2-12, and 2-13).

Vagal efferent fibres did not respond to all stimuli with the same intensity. In general, the intensity of response in most studies was similar for both mechanical stimuli, ie oesophageal and gastric distension. The fibre in Figure 2-12 responded in an excitatory manner to oesophageal and gastric distension, and to close intraarterial CCK, bradykinin and capsaicin. The increase in discharge due to oesophageal and gastric distension and CCK was 2.02imp/sec, 3.10imp/sec and 3.3imp/sec respectively. However, the increases in discharge evoked by bradykinin and capsaicin were fivefold that evoked by oesophageal and gastric distension and CCK, with increases of 16.23imp/sec and 14.11imp/sec respectively.

5 DISCUSSION

This study shows that vagal efferent neurones receive extensive convergent inputs from afferents with mechanoreceptive and chemoreceptive endings within the oesophagus and the upper GI tract. Previous work has concentrated mainly on the convergence of mechanoreceptive inputs from different regions of the GI tract onto vagal central neurones and has highlighted the minor role of chemoreceptor inputs[33, 41, 43, 126]. This study is the first to describe 1) the extensive convergence from oesophageal acid- and capsaicin-sensitive afferents and the sensitisation of these inputs onto vagal efferent neurones, 2) the influence of cholecystokinin-, bradykinin- and capsaicin-sensitive receptors on vagal efferent activity, and 3) the variation in the degree of contribution of these and distension-sensitive inputs onto vagal efferent neurones.

5.1 Convergence of oesophageal and gastric mechanosensitive inputs

Previous electrophysiological studies on single vagal efferent fibres at the cervical level have shown that they respond to mechanical distension of viscera as proximal as the oesophagus and as distal as the colon within the gastrointestinal tract. Vagal efferent responses to distension of the corpus, antrum, duodenum, jejunum and colon have also been recorded[16, 33, 40, 41, 43, 94, 126, 129]. The afferent arm of this reflex pathway is mainly vagal in the stomach, and non-vagal in the duodenum and colon as bilateral vagotomy abolishes or markedly reduces the majority of the responses to gastric distension but not to duodenal and colonic distension[126, 129].

In this study, 88% and 77% of vagal efferent fibres tested received inputs from gastric distension-sensitive and from oesophageal distension-sensitive afferents respectively. The afferent limb of the reflex involved in mediating oesophageal mechanosensitive inputs onto vagal efferent fibres follows the vagal pathway as all responses to oesophageal balloon distension were abolished following bilateral cervical vagotomy. This is despite studies showing that non-vagal (sympathetic) oesophageal mechanoreceptors exist in the cat and oppossum[75, 240, 241]. These non-vagal

oesophageal mechanoreceptors require higher threshold levels in order to evoke a response to oesophageal balloon distension and possess higher saturation pressures than their vagal counterparts[240] and are proposed to be nociceptors which are activated by high, noxious levels of distension. On the other hand, the response of vagal oesophageal mechanoreceptors to distension or increased intraluminal pressure are saturated within the normal physiological ranges. Thus, the vagal oesophageal tension receptors are thought to be involved in the autonomic regulation of normal gastrointestinal functions.

In the case of gastric mechanoreceptive inputs, not all afferents that converge onto vagal central neurones follow the vagal pathway as bilateral vagotomy led to a reduction or abolition of response of 71% of efferent fibres. This is comparable to the findings by Grundy et al[126], where vagal section completely abolished 68% of the efferent responses to the same stimulus. The portion of the efferent response that was not abolished by vagotomy is probably mediated via the splanchnic nerve. In the dog, splanchnic mechano-receptors in the corpus and antrum which respond to maintained mechanical stimuli in a tonic manner have been identified[110]. The vagus may exert a tonic inhibitory influence as in one fibre, bilateral vagotomy led to a potentiation of the inputs received from gastric distension-sensitive afferents.

In four studies where bilateral vagotomy was performed, the basal efferent discharge was abolished immediately after section of the second nerve trunk. This abolition in discharge was due to the loss of tonic vagal afferent input onto the efferent fibre. This is in comparison to the other 12 studies where the efferent discharge was not abolished, indicating that the non-vagal inputs to the efferent fibre being recorded was sufficient to maintain a level of basal activity despite the removal of the tonic vagal input.

The main afferent population which respond to distension have their peripheral terminations within the muscular layers, ie they are tension receptors. Although gastric and oesophageal mucosal receptors also respond to distension, these afferents are activated primarily during the dynamic phase of inflation and during deflation, where flow of fluid

across the mucosal surface, in the case of fluid distension, or the friction generated by the balloon rubbing against the mucosa, in the case of balloon distension, could briefly activate the mucosal units. Indeed, the pattern of response of vagal efferent fibres to oesophageal balloon and gastric distension was very similar in nature to those of oesophageal and gastric tension receptors respectively. They were rapidly evoked, slowly adapting and persisted for the duration of the stimuli (see Chapter 1 and [12, 45, 73]). The brief inhibitory period seen with rapid deflation of the balloon has also been described in vagal tension receptor responses to oesophageal distension by fluid, air or balloon.

The similarity in the response profile to distension obtained by oesophageal and gastric tension receptors and efferent fibres suggests that there is likely to be very little central processing, ie that the pathways may be monosynaptic or involve only a few strong synaptic connections. Indeed, monosynaptic vagovagal circuits have been identified in the NTS of the rat with ultrastructural studies. These exist between fibres from central subnucleus of the NTS and those from compact formation of the NA[264], the main site of termination of vagal oesophageal afferents and efferents respectively. They have also been found between vagal afferents within the subnucleus gelatinosus of the NTS, the site where the majority of gastric vagal afferents terminate, and the DMVN, the central site of vagal gastric motoneurones[221, 229]. These monosynaptic connections between vagal afferents and soma or dendrites of motoneurones of the DMVN are numerous, occuring in 13% of labelled terminals[229]. These direct circuits probably are involved in timing of gastric contractions and provide short latency ("beat by beat") modulation of motility whereby gastric afferent information is relayed directly to appropriate vagal efferent factors.

In addition, the existence of short vago-vagal circuits which involve one or a few synapses have been confirmed electrically[40, 201]. In these studies, afferents were electrically stimulated orthodromically and the latency of response recorded. Both studies found that the variability in latency was either very small (less than 1ms) or varied substantially (>40ms). The small latency variability and short latency in the first group

suggests that the connections between the afferent and the preganglionic neurones involve only a few synapses whereas a larger variability of longer latencies indicates the presence of a polysynaptic pathway.

5.2 Convergence and sensitisation of oesophageal chemo-sensitive inputs

Of the plethora of studies in which chemosensitivity of gastrointestinal afferents has been examined, only two have looked at the response of oesophageal afferents to chemical stimuli[76, 131]. In our laboratory, we have attempted to gain a better understanding of the chemosensitivity of oesophageal afferents in 3 ways. Firstly, a ferret *in vitro* model was established whereby the lower oesophagus with the attached vagi was opened longitudinally and pinned out flat. Mucosal units found using this technique exhibited fine tactile discrimination and a low proportion were responsive to superfusion of bradykinin, acid, capsaicin, and 5-hydroxytryptamine[207]. The second method used in this laboratory is detailed in Chapter 1, where recordings were made from single vagal afferents using an *in vivo* anaesthetised ferret model. Only 1 oesophageal mucosal receptor was identified and characterised due to the technical difficulties involved with this preparation. This mucosal receptor responded to both acid and capsaicin infusions. Finally, the afferent sensitivity to oesophageal chemicals were investigated using an established *in vivo* ferret vagal efferent preparation. It is this technique which forms the basis for the results outlined in this chapter.

Vagal efferent units have been shown to receive convergent inputs from more than one afferent[33]. The afferent input that converge onto vagal central neurones need not neccessarily be homogenous and can arise from more than one population of afferent fibres. For example, the same vagal efferent fibre can modulate their discharge in response to a mechanical stimulus, eg gastric distension, and to a chemical stimulus, eg 5-hydroxytryptamine applied close intraarterially[41]. These 2 stimuli activate 2 different populations of afferents, with gastric distension primarily activating gastric tension receptors[45] and 5-hydroxytryptamine directly stimulating gastroduodenal mucosal

afferents[37]. As vagal efferent fibres has been shown to respond to distension of the distal oesophageal lumen[33], the property of convergence was exploited to explore the sensitivity of these same efferents to chemical stimuli applied to the same region, ie within the distal oesophageal lumen. Using this method, the need to pinpoint the exact location of the receptive field of individual mucosal fibres (approx. 5-10mm^2) is bypassed.

Vagal efferent responses to oesophageal acid were observed in 58% of the fibres tested (18/31 fibres). The predominant inputs are likely to follow the vagal route as all responses to acid infusion were abolished after cervical section of the vagal nerve trunks. These afferent inputs are likely to be mediated by mucosal receptors and not by tension receptors due to the following reasons. Firstly, in one study, the response to oesophageal distension was in the opposite direction to that evoked by oesophageal acid infusion. Secondly, in 13 other fibres, oesophageal distension evoked a strong response whereas acidification of the lumen did not. Thirdly, none of the vagal oesophageal tension receptors recorded in Chapter 1 and in other studies[76, 131] modulated their discharge in response to acid infusion whereas mucosal afferents were activated by the same stimulus. Finally, the sensitisation in the efferent responses to repeated acidification was specific to that stimulus as the magnitude of response to other stimuli such as gastric or oesophageal distension was unchanged (see below). These pieces of evidence implies that the afferents activated by oesophageal distension are not neccesarily the ones stimulated by oesophageal acidification.

Responses to oesophageal acidification grew significantly larger with successive infusions, indicating sensitisation. The sensitisation seen is likely to be mainly peripherally mediated. Firstly, the sensitisation seen in response to oesophageal acidification is specific to that stimulus as the efferent responses to the other stimuli were unaffected. This implies that it may be specific to peripheral endings of mucosal fibres and may result from changes in the permeability of the squamous epithelium[205, 257]. Secondly, other studies[34] using the same model in our laboratory have shown that the vagal efferent activation is concomitant with a decrease in lower oesophageal sphincter

(LOS) pressure. Repeated acid infusions led to the sensitisation of both efferent and LOS responses, where a deeper relaxation of the LOS was evoked with successive acid infusion. The sensitisation is proposed to be peripherally mediated via an axon collateral as the LOS response to oesophageal acidification is still present after bilateral vagotomy whereas here, efferent responses to the same stimuli were abolished after vagotomy.

However, there may be a central component to this sensitisation. A study performed by Willing and Berthoud[268] found that degree of c-fos expression, which is used as a marker for neuronal activation, within NTS and the DMVN neurones is significantly greater when the stomach was distended with 18mls of water than when 9mls of water is used for gastric distension. This may mean that the population of vagal afferents have different thresholds of activation, with more afferents being activated by a higher volume of distension. It therefore may be indicative of a recruitment process which is dependent on the magnitude of the stimulus. The same phenomenon of recruiting may play a role in explaining the sensitisation seen in my responses to oesophageal acidification. I had hoped to further explore this line of reasoning by recording the responses of vagal mucosal afferents to the same stimulus, the results of which are reported in Chapter 1. While I made a recording from only a single oesophageal mucosal afferent unit, due to the technical difficulties incurred in the process, this unit did not respond to the initial episode of acid infusion but responded to the second episode of acid infusion. Further work needs to be performed in order to elucidate whether there is a central component to the sensitisation that occurs with repeated oesophageal acidification.

Vagal efferent responses to oesophageal capsaicin infusion were evoked in 12/13 fibres tested. In the one study where capsaicin infusion failed to evoked an efferent response, prior acidification also did not modulate the efferent fibre discharge. This may mean that this individual efferent fibre did not receive inputs from any oesophageal mucosal afferents. In 3/12 studies where capsaicin elicited a response, prior acidification failed to modulate efferent discharge. This implies that capsaicin is a more potent stimulus than acid.

Responses to acid and capsaicin were always in the same direction and similar in intensity, although the capsaicin response was generally longer in duration. This suggests that there is a common site of action. It has even been proposed that the hydrogen ion may act as the endogenous activator of the vaniloid receptor[31, 162, 212], the receptor that is activated by capsaicin. Indeed, a recent study indicates that the membrane site responsible for the effects of capsaicin is the same as that mediating the response to hydrogen ions[247]. Also, a capsaicin antagonist, capsazepine, has been shown to selectively block the effects of both protons and capsaicin, but not bradykinin, in stimulating single C-fibres with endings in the guinea pig trachea and bronchi[111]. Whether or not capsaicin and protons act via the same receptor, protons can markedly potentiate capsaicin-evoked responses[65], which probably occurs by increasing the potency of capsaicin.

4/8 fibres tested responded equally to repeated capsaicin infusion, while the responses of the other 4/8 fibres were lost after the initial capsaicin infusion. The acute desensitisation caused by repeated capsaicin infusion in some, but not all, fibres suggests that the dose used for these studies is around the threshold necessary for inducing desensitisation. Capsaicin is also able to overcome the initial acid induced sensitization in these studies.

5.3 Convergence of gastro-intestinal chemosensitive inputs

Studies have demonstrated that vagal efferent fibres receive inputs from afferents in the upper gastrointestinal tract sensitive to chemicals administered both intraluminally and close intraarterially[33, 41, 43]. Efferent fibre responses in this study were studied after activation of selected groups of afferents by close intraarterial injection of cholecystokinin, bradykinin and capsaicin. The afferent pathways of the reflex involved in mediating these inputs is also investigated.

5.3.1 Convergence of cholecystokinin-sensitive inputs

The vagal efferent responses to cholecystokinin in these studies are likely to be due to activation of mucosal afferents in the stomach and duodenum as these have been shown to exhibit a direct sensitivity to the administration of close intraarterial CCK in the ferret[38]. These CCK-induced responses are unlikely to be due to activation of gastric distension-sensitive receptors. If the efferent responses were due to tension receptor activation, the response evoked by CCK would be in the opposite direction to that evoked by gastric distension as CCK generally caused an inhibition in gastric pressure whereas gastric distension increased intraluminal pressure[236]. Instead, some fibres responded in the same direction to both gastric distension and CCK. Also, recordings of vagal muscular afferents have shown that CCK exert its effects on these tension-sensitive afferents indirectly through changes in intraluminal pressure[38].

From the results of the experiments where responses to cholecystokinin were abolished in 4/5 studies after bilateral vagotomy, it is probable that the afferent arm of the reflex is mediated predominantly via vagal pathways. This is confirmed by vagal afferent studies in which mucosal fibres from the gastro-duodenal region were activated by CCK[38]. Also, CCK induced c-fos expression in neurones contained witihin the NTS and the AP, two regions in the brain stem where vagal afferents terminate[114]. However, there is also likely to be a non-vagal component as the response of 1 efferent fibre to CCK was unchanged after vagal section.

Bilateral vagotomy also reversed the decrease in intracorpus pressure caused by CCK into an excitatory response. This inhibition of gastric motility in vagally intact ferrets has been shown to be a direct vagal effect mediated by non-cholinergic, non-adrenergic pathways, resulting in corpus relaxation. When the vagal inputs are removed, CCK then exerts a direct excitatory effect on the corpus[39].

5.3.2 Convergence of bradykinin-sensitive inputs

Bradykinin is produced and acts at the site of tissue injury and inflammation, where it produces an acute inflammatory response, causing pain, oedema and increased blood flow at the site of injury[262]. Bradykinin excites fine myelinated and unmyelinated fibres that may be involved in nociceptive functions from the viscera as well as those afferents which are not involved in nociception[147]. It has been shown to stimulate splanchnic afferents in the cat with endings in the serosal and muscular layers[174]. Bradykinin also directly activates vagal mucosal afferents located within the upper gastrointestinal tract of the ferret[35] as well as splanchnic oesophageal tension receptors in the oppossum[240] and splanchnic colonic afferents in the cat[135]. Bradykinin also activates some classes of afferents indirectly through changes in motility. A report by Floyd et al [109] indicates that splanchnic and hypogastric nerves arising from the cat bladder respond indirectly to bradykinin, as do vagal duodenal tension receptors in the sheep[84], vagal oesophageal mechanoreceptors in the opossum[240] and vagal gastroduodenal tension receptors in the ferret[35].

The close intraarterial injections of bradykinin administered in the present vagal efferent study were given at the coeliac axis so that they would reach mainly afferent endings located within the upper gastrointestinal tract. Bradykinin may activate more than one class of afferents listed above as the duration of efferent response to bradykinin varied from study to study. In some studies, the efferent response lasted longer than the simultaneously evoked gastric contraction. In other studies, the efferent response lasted less than the duration of the gastric motility response. Therefore, an indirect effect due to gastric contraction is unlikely. Obviously, the efferent response may be secondary to mechanoreceptor activation following changes in muscle activity in regions other than the stomach, which was the only organ from which intraluminal pressure was measured. However, the efferent responses to bradykinin were tonic in nature and in some studies, lasted for >5minutes. The timecourse of response indicates that the effect on the afferents is most likely to be direct as bradykinin has not been seen to increase muscular activity for this period of time in any area of the gastrointestinal tract.

Responses to bradykinin in this study were unaffected by bilateral vagotomy in 4/5 fibres tested. This implies that the predominant input from bradykinin-sensitive afferents is non-vagal in origin. The non-vagal input is probably via the greater splanchnic nerves as section of these nerves subsequent to vagotomy in 1/1 study resulted in the loss of basal discharge and the bradykinin response.

5.3.3 Convergence of capsaicin-sensitive inputs

Capsaicin was administered close systemically in order to stimulate as large a population of sensory C-fibres to serve as a positive control procedure. Even though capsaicin also excites Aδ-fibres, a study has shown that it does activate all C-fibres tested, but roughly only a third of Aδ-fibres[174]. In the current study, capsaicin evoked a response in 58% of vagal efferent fibres tested, possibly indicating that both populations were activated.

The latency and duration of response of vagal efferent fibres to capsaicin were similar to those observed for bradykinin. In some fibres, responses were very rapidly evoked and activation occured almost instantaneously. The duration of response ranged from less than 15 seconds (see example in Figure 2-11) to over 5 minutes (see example in Figure 2-12). As with bradykinin-induced responses, capsaicin-evoked efferent responses were either shorter or longer in duration than the changes in intragastric pressure. Responses to capsaicin and bradykinin, when observed, were always in the same direction in individual fibres. Together with my data in which bilateral vagotomy did not affect the responses of vagal efferents to both bradykinin and capsaicin in all 4 fibres which were tested with both chemicals, it may indicate that both bradykinin and capsaicin activate the same population of afferents.

The unchanged responses after vagotomy also infer that at least part of the afferent arm of this reflex is likely to be non-vagal. Indeed, splanchnic afferents with endings within the abdominal viscera have been shown to be activated by capsaicin[174]. In

Chapter 1, I have shown that capsaicin also activates gastric vagal mucosal fibres. This mucosal fibre was also activated by bradykinin. There was one efferent study in which the response to bradykinin was drastically reduced after vagotomy. Unfortunately, the effect of capsaicin was not tested in this study after vagotomy. Nevertheless, these capsaicin sensitive vagal fibres may only play a minor role in the overall input to vagal efferent fibres. These observations further highlight the role of non-vagal input to vagal reflexes.

5.4 Relative contribution of afferent inputs

There is great heterogeneity in the relative contribution of different afferent inputs from the various parts of the GI tract onto the individual vagal efferent neurones. Some fibres studied responded very powerfully to mechanical distension while having no or little response to any chemical stimuli tested. Other fibres responded minimally to distension when contrasted to the responses evoked by chemical stimuli. For example, the intensity of response of the vagal efferent fibre in Figure 2-12 to bradykinin and capsaicin was fivefold to that evoked by oesophageal balloon distension, gastric distension, and cholecystokinin. The difference in the magnitude of excitation evoked by the various afferent stimuli may be due to the relative contribution of different numbers, synaptic strength, neurotransmitters involved in populations of afferents. This particular fibre may receive a strong input from several bradykinin- and capsaicin-sensitive afferents, with a weaker input from only a few oesophageal and gastric distension-sensitive and cholecystokinin-sensitive afferents. The neurotransmitters utilised by these different types of afferents are dealt with in the next chapters.

The strong response evoked by chemical stimuli in this and other experiments contradicts previous findings[33, 41, 43] where vagal efferent fibres using a similar model received a greater input from mechanoreceptors than they did from chemoreceptors. The difference may be due to 2 reasons. Firstly, in the earlier experiments, the greater splanchnic nerves were routinely sectioned, thereby interrupting any possible flow of information via these nerves to vagal central neurones. In my experiments, the greater

splanchnic nerves were left intact with the exception of one study. In this study, the greater splanchnic nerves were sectioned subsequent to cervical bilateral vagotomy, and resulted in the abolition of basal efferent discharge together with responses to all stimuli tested. The presence of non-vagal chemosensitive inputs onto efferent neurones is further confirmed when bilateral vagotomy did not affect the efferent responses to chemicals in most of my experiments. Secondly, different routes of drug administration were used. In the earlier studies, chemicals were infused intraluminally through the duodenum or stomach, with the exception of 5-HT, which was administered intravenously, intraarterially and close intraarterially. Intraluminal perfusion of sodium hydroxide, acid, hypertonic saline, glucose and tryptophan resulted in a weak efferent response in a few fibres whereas the magnitude of response obtained by 5-HT via either route was at least equal to those evoked by oesophageal or gastric distension. In my study, chemicals were also infused intraluminally, although this infusion was performed in the distal oesophagus, not the stomach or duodenum. It should be noted that in these studies, the initial acid infusion evoked small responses in less than half the number of fibres tested. It was only with repeated acidification that the magnitude of responses was substantially increased. This is in contrast with the amplitude of responses obtained by over 76% of fibres tested with bradykinin, capsaicin and cholecystokinin, all three of which were administered close intraarterially. The difference in the magnitude of response and the proportion of fibres which responded to chemicals administered close intraarterially compared to those infused intraluminally can be attributed to the ease with which the chemicals is able to reach the peripheral endings of the relevant afferents. Chemicals infused intraluminally need to overcome the diffusion barrier before reaching peripheral afferent endings in the mucosal layer whereas it takes less time and effort for intraarterially administered chemicals to reach the peripheral afferent endings. This is reflected in the latency of efferent responses to chemicals. The responses to oesophageal acidification may take 30 seconds to occur whereas some responses to intraarterial bradykinin and capsaicin are evoked almost instantaneously. Also the number of afferents that intraluminal perfusion would reach is relatively small as it would only be limited to a small portion of gut. On the other hand, close intraarterially administration would allow the chemical to activate a larger number of

afferents from different regions within the upper gut. Thus, the differences in the degree and frequency of responses seen in this study when compared to previous studies is due to the route of drug administration as well as an involvement of non-vagal, and probably splanchnic, afferent inputs onto central vagal neurones.

The degree of input from non-vagal afferents onto vagal efferent neurones is quite extensive. This implies that both vagal and non-vagal inputs are processed by the dorsal motor vagal nucleus (DMVN) which then modulates the vagal efferent outflow to the abdominal viscera. This is further supported by Blackshaw and Grundy[42] in a later study where vagal efferents which respond to gastric and duodenal distension modulated their discharge in response to electrical stimulation of central ends of cut splanchnic nerves. The evidence points to the splanchnic afferents as having an influence on vagal reflexes to the gastrointestinal tract. This is supported by the one study where the section of the greater splanchnic nerves after bilateral vagotomy led to the cessation of basal efferent discharge and the loss or response to any peripheral stimuli tested.

5.5 Conclusions

The amount of information received by vagal efferents is extensive. The convergence occurs from afferents located in different regions of the gut, with peripheral endings in different layers of the gut wall and following different pathways. This represents an economical method of transmitting information from the CNS to the periphery with a small number of fibres and is consistent with histological data which confirm that less than 10% of vagal fibres are efferent in origin[18]. The degree of convergence means that the efferent fibres projecting to the periphery to exert changes in motility and secretory patterns receive information from a representative number of afferents. This ensures that the action exerted by these efferent is based on accurate information arising from changes in the periphery and CNS that have occurred.

	OBD	GD	ACID il	Cap il	CCK ia	BK ia	Cap ia
Total number of fibres	**90**	**90**	**31**	**13**	**37**	**60**	**53**
No. fibres- excited	64	62	15	12	11	16	11
inhibited	4	18	3	0	18	29	20
no response	22	10	13	1	8	15	22

Table 2-1. Summary of vagal efferent responses to peripheral mechanical, chemical, and pharmacological stimuli.

The responses of 91 fibres were tested with oesophageal balloon distension (OBD 1.5-2ml air), gastric distension (GD, 40-60ml saline in intact stomach or 5-20ml saline in isolated corpus), oesophageal acid infusion (ACID il, 150mM), oesophageal capsaicin infusion (Cap il, 3.2-6.4mM), close intraarterial cholecystokinin (CCK ia, 100pmol), close intraarterial bradykinin (BK ia, 18nmol) and close intraarterial capsaicin (Cap ia, 65 nmol). The majority of efferent responses to mechanical stimuli were excitatory, as were the responses to oesophageal infusions of acid and capsaicin solution. On the other hand, inhibition was most commonly observed where a response was evoked by close intraarterial drug administration.

Date		Treatment									Peripheral Stimuli						
	Nb	Basal	NK1	CGRP	NMDA	non-NMDA	M1	M2	GABAc	GABA iv	BIVX	OBD	GD	ACID	Capi	CCK	BK	Cap
12/5/94	N1		1								2	+	+	-				
18/5/94	N2		1								2	+	-	0	0			
7/6/94	N3	X										+	++	0				
21/6/94	N4	X										+	+	0				
23/6/94	N5		1									-	--	-				
4/7/94	N6	X										+	-	+				
14/7/94	N7		1									+	+	+				
27/7/94	N8	X										+	++	0				
3/8/94	N9		1									+	+	+			--	--
12/8/94	N10	X										++	0	+				
24/8/94	N11		1									++	+	+			0	0
14/9/94	N12		1									+	++	+				
21/9/94	N13		1									+	++	0			--	-
23/9/94	N14		1								2	+	-	0	+		--	0
7/10/94	N15		1									+	+	0				-
12/10/94	N16		1									+	0	0			--	0
19/10/94	N17		1									++	+	+	+		0	0
31/10/94	N18		1								2	--	--	--				
8/11/94	N19		1									+	+	0			0	
15/11/94	N20		1								2	+	+	+	+		+	0
2/12/94	N21	X										+	-	0	+			
3/1/95	N22		1									+	-	+	+		0	0
6/1/95	N23		1									+	+	+	+		+	+
10/3/95	N24	X										+	-	0			+	+
17/3/95	N25	X										+	+					
21/3/95	N26	X										+	+	+	+			
29/3/95	N27	X										++	++					
15/8/95	N28		1									+	+	+	++		0	0
23/8/95	N29		1									+	+	+	+		-	0
24/8/95	N30		1									+	+	0	+		+	0
24/10/95	N31	X										+	+	++	++			
2/2/96	N32				1							+	++					
12/3/96	N33	X										+	+					
22/3/96	N34											+	-	0				
28/5/96	N35										1	0	++					
24/6/96	N36										1	++	++				--	-
12/7/96	N37										1	0	--				--	--
19/7/96	N38										1	0	++				-	0
29/7/96	N39											0	+				+	+
7/8/96	N40										1	+	+				0	0
13/8/96	N41										1	0	++					
26/9/96	N42						1	2				+	+				--	-
3/10/96	N43						2	1				++	+				++	++
22/10/96	N44											0	+*				+	0
22/10/96	N45						2	1				+	+*				--	--

Total no of fibres	45	45	30	12	0	23	23
No fibres- excited	37	33	14	11	-	7	4
inhibited	2	10	3	0	-	10	7
no response	6	2	13	1	-	6	12

Date		Treatment									Peripheral Stimuli						
	Nb	Basal	NK1	CGRP	NMDA	non-NMDA	M1	M2	GABAc	GABA iv	BIVX	OBD	GD	ACID	Capi	CCK	BK	Cap
24/10/96	N46						1	2				+	0 *				0	0
31/10/96	N47						1	2				0	+*				-	--
4/11/96	N48				1							0	0 *			+	+	+
6/11/96	N49	X										0	--				--	--
7/11/96	N50									1		+	+*			+	+	+
8/11/96	N51									1		0	++*			++	+	+
14/11/96	N52	X										+	+			+		++
25/11/96	N53	X										+	+*				0	
25/11/96	N54									1		0	+*			++		
29/11/96	N55				1							+	+*			+	+	+
6/12/96	N56	X										0	+					
11/12/96	N57	X										+	0	+	+			
16/12/96	N58				1							0	0 *			--		
17/12/96	N59				1							0	0 *			-	-	-
20/2/97	N60		1		2							+	+*				0	
6/3/97	N61		2		1							+	+*			++	++	
21/5/97	N62	X										+	+			0	0	0
22/5/97	N63				1							+	0			0	0	0
30/5/97	N64											+	+			0	0	0
4/6/97	N65	X										-	-			0	--	-
16/6/97	N66				1							+	+*			0	0	0
20/6/97	N67				1							+	+*			++	+	
24/6/97	N68	X										++	+			+	++	
8/7/97	N69				1							+	+			--	--	
9/7/97	N70									1		0	--			--		
14/7/97	N71				1					2		+	+			0	0	0
16/7/97	N72									1		0	--			--		
18/7/97	N73		1		2							+	+			--	--	0
24/7/97	N74											+	+			---	--	
28/7/97	N75		1		2							--	--*			++	++	++
29/7/97	N76		1		2							++	+*			--	--	--
31/7/97	N77	X										++	+				--	0
6/8/97	N78	2	1									0	0 *			--	--	--
7/8/97	N79									1		0	--			--		
8/8/97	N80	X										+	0			0	-	--
11/8/97	N81		1									+	+*			--	-	0
12/8/97	N82		1								2	0	+*			--	--	-
18/9/97	N83	2		1						3		+	+			--	---	
9/10/97	N84		2		3			1				0	+			---	--	--
10/10/97	N85				2			1				+	++			0	0	0
14/10/97	N86				2			1				0	--			--	--	--
15/10/97	N87	2						1				+	+			++	++	++
16/10/97	N88		2					1		3		0	--			--	--	-
21/10/97	N89	2	5		3	4		1				+	+			--	--	--
23/10/97	N90	2	5		3	4		1		6		++	++			--	--	--

Total no of fibres	45	45	1	1	37	37	30
No fibres- excited	27	29	1	1	11	9	7
inhibited	2	8	0	0	18	19	13
no response	16	8	0	0	8	9	10

Table 2-2 Responses of individual vagal efferent fibres to peripheral stimuli.
The peripheral stimuli used were oesophageal balloon distension (OBD, 1-2ml air), gastric distension (GD, 40-60ml saline in intact stomach or * indicates 5-20ml saline in corpus), oesophageal acid infusion (ACID, 150mM HCl 2ml), oesophageal capsaicin infusion (Cap il, 3.2-6.5mM 2 ml), and close intraarterial cholecystokinin (CCK, 100pmol), bradykinin (BK, 18nmol) and capsaicin (Cap, 65nmol).

The direction and intensity of response under control conditions is indicated by the following symbols:
0 denotes no response: + denotes a 50-100% increase in discharge rates from basal levels:
++ denotes a >100% increase: - denotes a partial inhibition: -- denotes a complete inhibition.

Treatments used were: no treatment (Basal), NK-1 receptor antagonism with CP99994 or CP96345 (NK-1, at 12 and 8μmol/kg iv respectively), CGRP receptor antagonism with hCGRP8-37 (CGRP, 3.2-6.4nmol/kg icv), NMDA receptor antagonism with CGS19755 (NMDA, 13μmol/kg iv), non-NMDA receptor antagonism with CNQX (non-NMDA, 75-155nmol/kg icv), M1 cholinoceptor antagonism with pirenzepine (M1, 2.5-5.0μmol/kg iv), M2 cholinoceptor antagonism with methoctramine (M2, 7-14μmol/kg iv), GABAb receptor activation and antagonism centrally and systemically with baclofen (3-7nmol/kg icv) and CGP35348 (100nmol/kg icv) respectively (GABAic), and GABAb receptor activation and antagonism systemically with baclofen (7-14mmol/kg iv) and CGP35348 (100mmol/kg iv) respectively (GABA iv), and bilateral vagotomy (BIVX). Where more than 1 treatment was administered, the order of the treatments is indicated by the numbers in the treatment column. See Chapters 3-5 for more details.

Unit ID	OBD		GD		ACID		CCK		BK		Cap	
		%Con		%Con		%Con		%Con		%Con		%Con
12/5/94	NO BASAL VED PRESENT											
31/10/94	NO BASAL VED PRESENT											
15/11/94	NO BASAL VED PRESENT											
23/9/94	NO BASAL VED PRESENT											
18/5/94	+	0	-	50	+	0						
26/5/94	+	0	-	100	+	0						
15/8/95			+	0								
23/8/95			+	0								
3/1/95	+	0										
6/1/95	+	0	+	100					+	100	+	100
3/8/94	+	0			+	0			-	20		
12/8/97			+	0			--	0	+	100	-	100
23/10/97	+	0	-	0			-	0	-	100	-	100
24/10/97							--	0				
16/10/97							--	0	-	100	-	100
7/8/97							--	100				
Total No. of Responses Studied	**6**		**7**		**3**		**5**		**5**		**4**	
Drug Effect -None	0		2		0		1		4		4	
-Reduced >50%Control	0		0		0		0		0		0	
-Reduced ≤50%Control	0		1		0		0		1		0	
-Blocked	6		4		3		4		0		0	

Table 2-3. Responses of individual vagal efferent fibres to peripheral stimuli after bilateral vagotomy

The direction and intensity of response is indicated by the symbol in the grey boxes:
+ denotes a 50-100% increase in discharge rates from basal levels: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes.

Prior to bilateral vagotomy, oesophageal balloon distension (OBD 1.5ml air), gastric distension (GD, 40-60ml saline) and oesophageal acidification (ACID,150mM HCl 2ml) elicited mainly excitatory responses whereas close intraarterial administration of cholecystokinin (CCK, 100pmol), bradykinin (BK, 18nmol) and capsaicin (Cap, 65nmol) yielded mainly inhibitory responses.

Bilateral vagotomy abolished the basal efferent discharge of 4/15 fibres. No responses to any stimuli were elicited in these fibres. Bilateral vagotomy abolished all vagal efferent responses to OBD and ACID, most responses to CCK and GD, while leaving the responses to BK and Cap in 4/5 fibres unaffected.

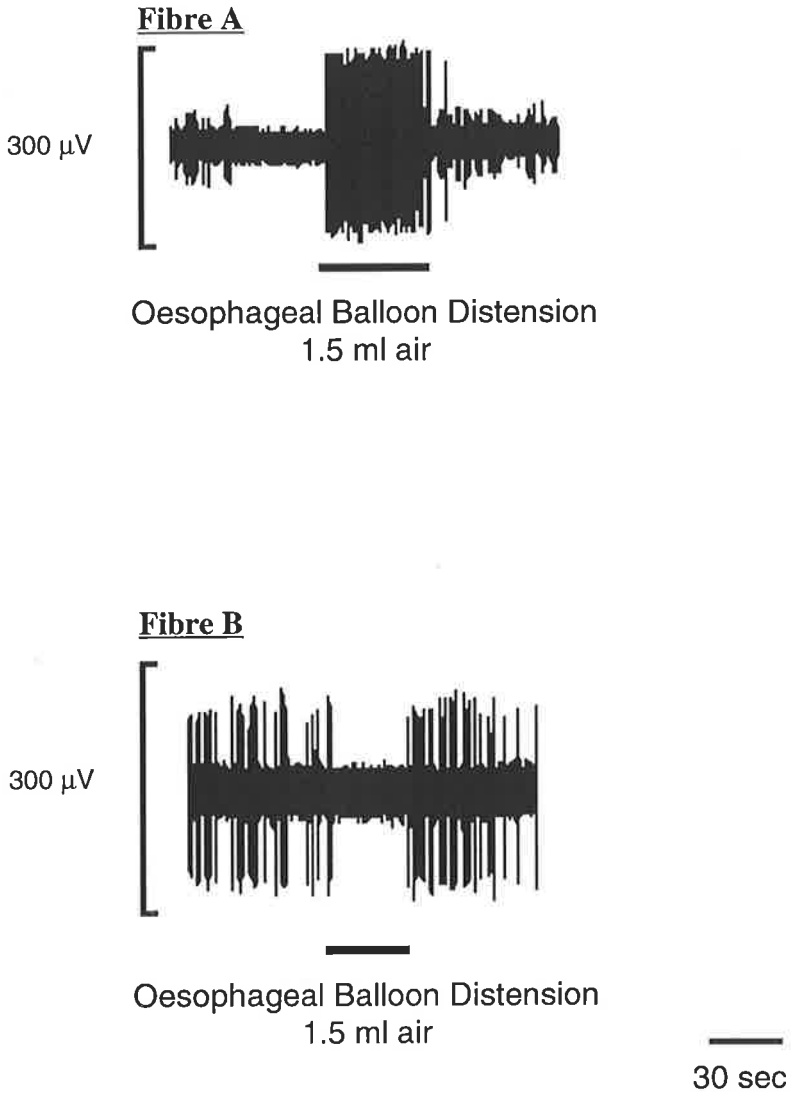


Figure 2-1. Raw record of 2 vagal efferent fibre responses to oesophageal balloon distension.
Fibre A showed no resting activity during the period of recording shown, except in response to experimental stimuli. Fibre B showed a low frequency, irregular pattern of discharge in the absence of mechanical stimuli.

Oesophageal balloon distension (1.5 ml air) Fibre A This evoked a prompt excitatory response which was maintained until the balloon was deflated, whereupon activity rapidly returned to pre-distension level. Fibre B This evoked a total inhibition of fibre activity which lasted for the duration of the stimulus.

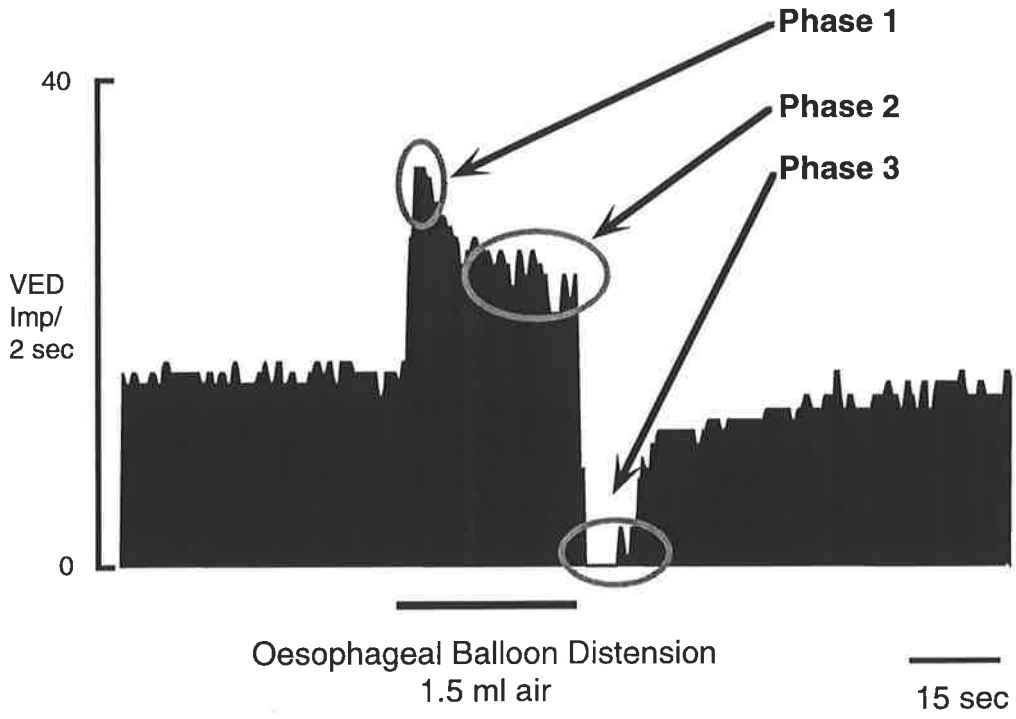


Figure 2-2. Integrated record of vagal efferent discharge of a fibre in response to oesophageal balloon distension, 1.5ml air.

The fibre possessed a basal level of spontaneous discharge. The efferent response to balloon distension can be divided into 3 phases: a sharp increase in vagal efferent discharge (VED) is seen during the dynamic phase of distension (Phase 1). Phase 2 is the sustained increase in VED during the static period of distension. Phase 3 occurs immediately upon balloon deflation, when an "off-loading" phenomenon, or silent period, is observed before discharge levels returned to pre-stimulus levels.

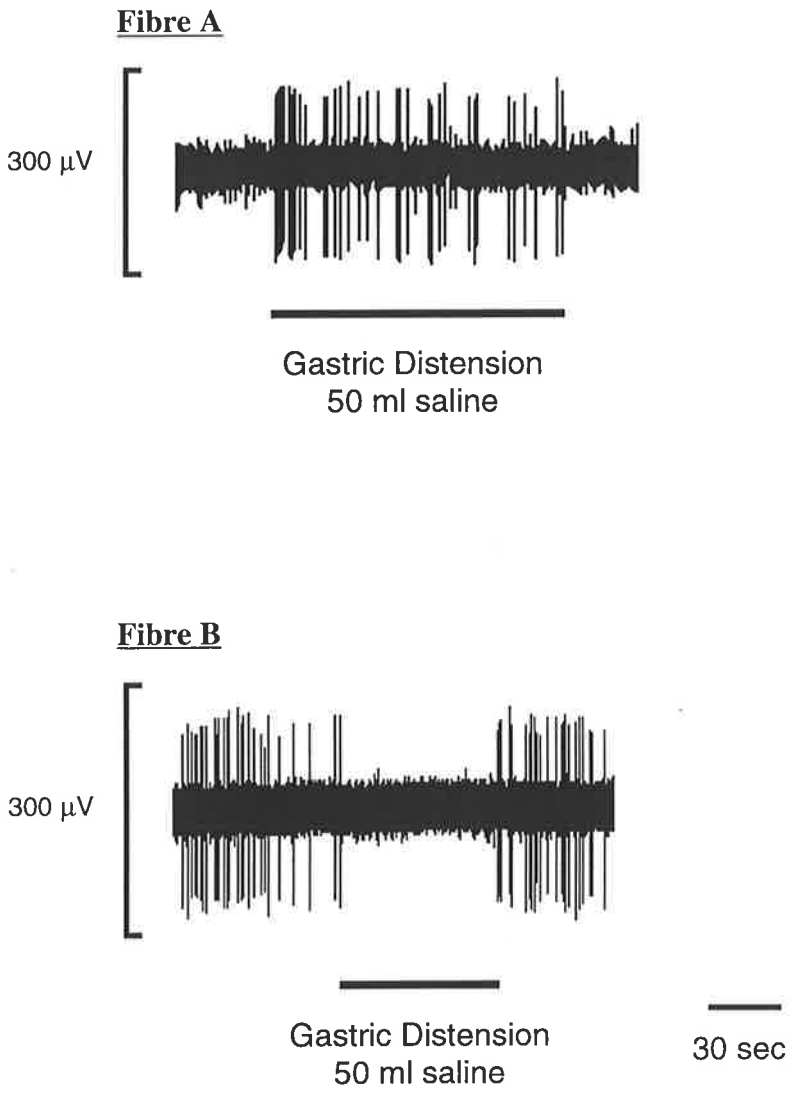


Figure 2-3. Raw record of 2 vagal efferent fibre responses to gastric distension
Fibre A and Fibre B are the same 2 fibres shown in Figure 2-1.

Gastric Distension (50 ml saline) Fibre A. An excitatory response was also seen in this fibre during gastric distension via the pyloric cannula. Fibre B. Activity was completely inhibited by distension. In both fibres, the response was prompt, maintained for the duration of the stimulus and ceased immediately upon saline removal.

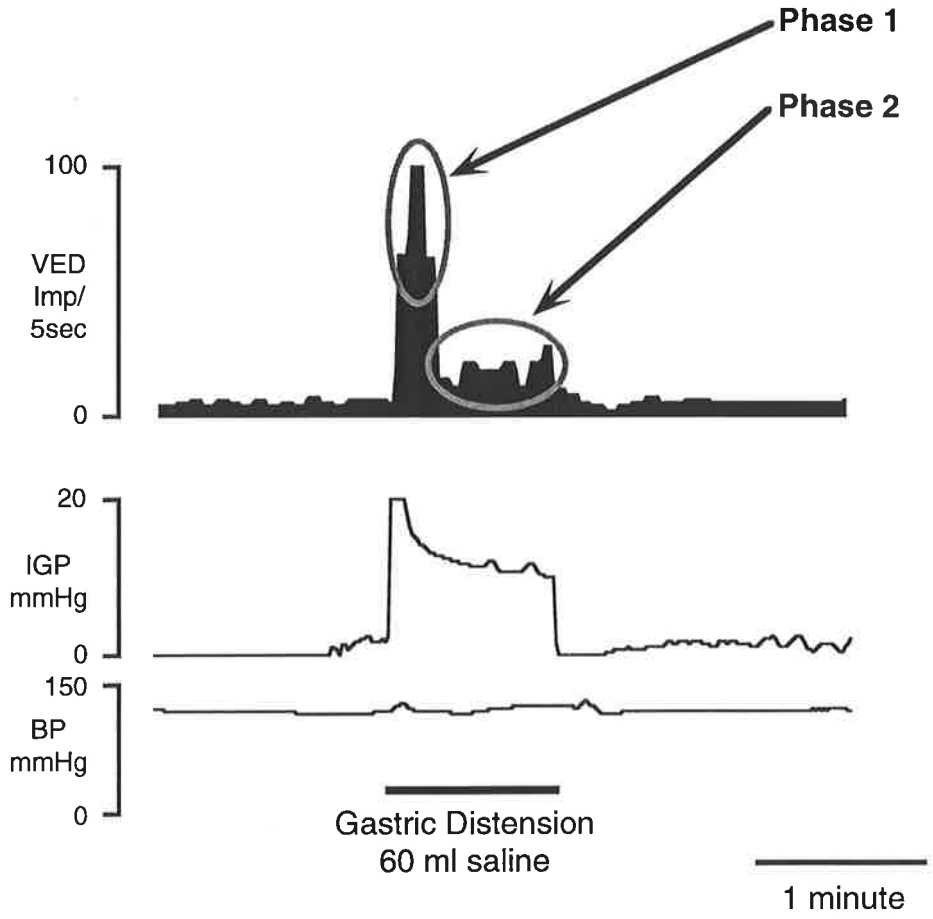


Figure 2-4. Effect of gastric distension on discharge of a vagal efferent fibre, intragastric pressure and blood pressure.

Upper trace: integrated record of firing rate in an efferent fibre (5second reset). This fibre responded in a biphasic manner to gastric distension (60ml saline). Phase 1 occurred during the dynamic phase of the inflation and was approximately 5 times the intensity of the Phase 2. Phase 2 occurred during the static portion of the distension period.

Middle trace: the dynamic phase of gastric distension led to a sharp increase in intragastric pressure (IGP). The tracing was truncated at the top during this phase in order to more clearly display the IGP during distension. Adaptation was seen during the static period of distension. IGP rapidly returned to pre-stimulus levels after aspiration of gastric contents.

Lower trace: blood pressure showed a slight increase with both inflation and deflation of the stomach.

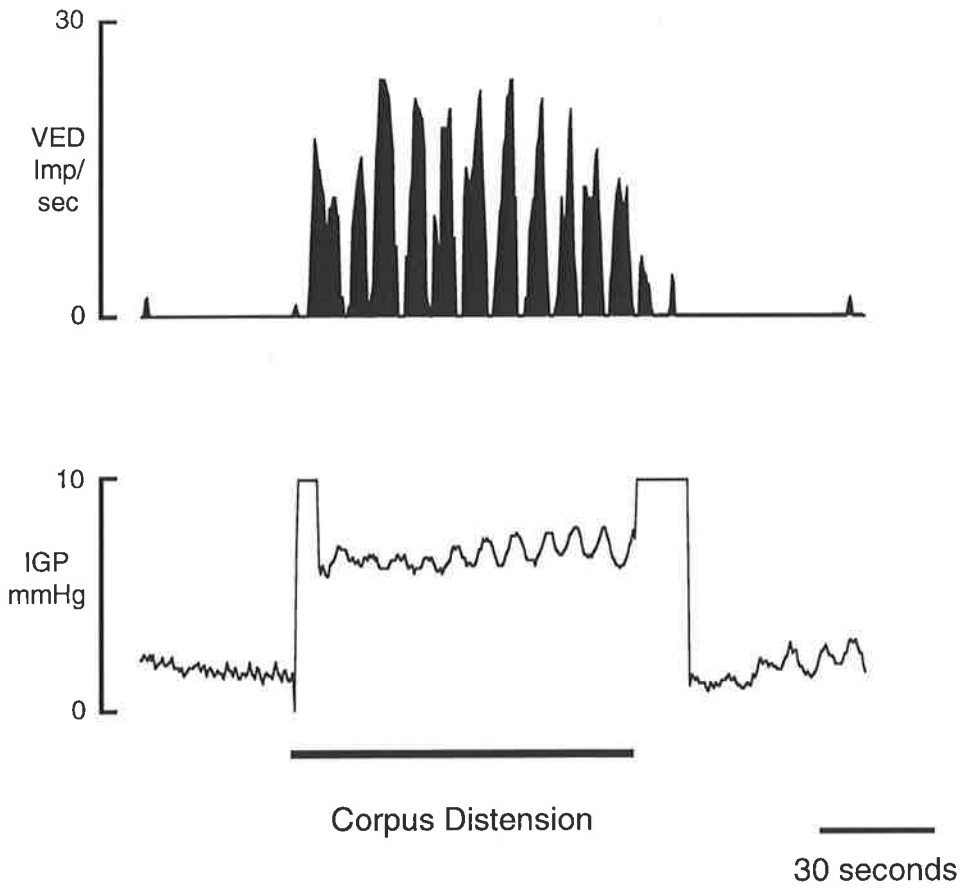


Figure 2-5. Effect of corpus distension on the discharge of a vagal efferent fibre and intra-corpus pressure.

Upper trace: integrated record of firing rate of an efferent fibre (5 second reset). This spontaneously silent efferent unit showed a strong phasic response to corpus distension (20ml saline) which was strongly correlated with corpus motility. Upon deflation of the stomach, the efferent discharge quickly returned to pre-stimulus levels.

Lower trace: intra-corpus pressure was increased during corpus distension. The phasic activity was correlated with the efferent fibre response. The large increases (off scale) at the start and end of the distension period are generated when the three way tap between the pressure tranducer and the corpus cannula is closed. The tracing was truncated during inflation and deflation in order to display the intra-corpus pressure response during distension more clearly.

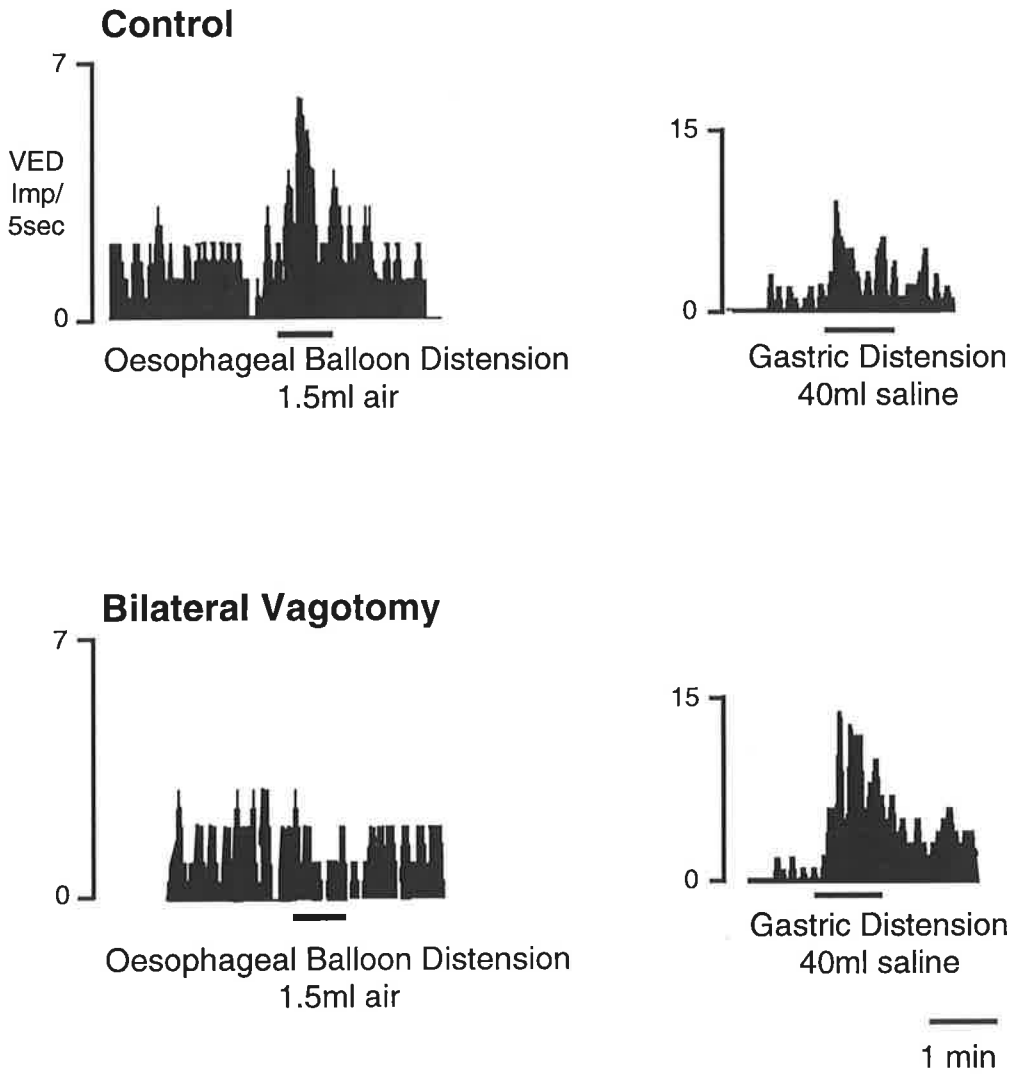


Figure 2-6. Effect of bilateral vagotomy on the vagal efferent response to mechanical stimuli.
Prior to bilateral vagotomy, oesophageal balloon distension (1.5ml air) and gastric distension (40ml saline) evoked excitatory responses in this fibre.

Bilateral vagotomy caudal to the recording site led to a decrease of the basal discharge rate of this fibre. It also abolished the efferent response to oesophageal distension but potentiated the response to gastric distension.

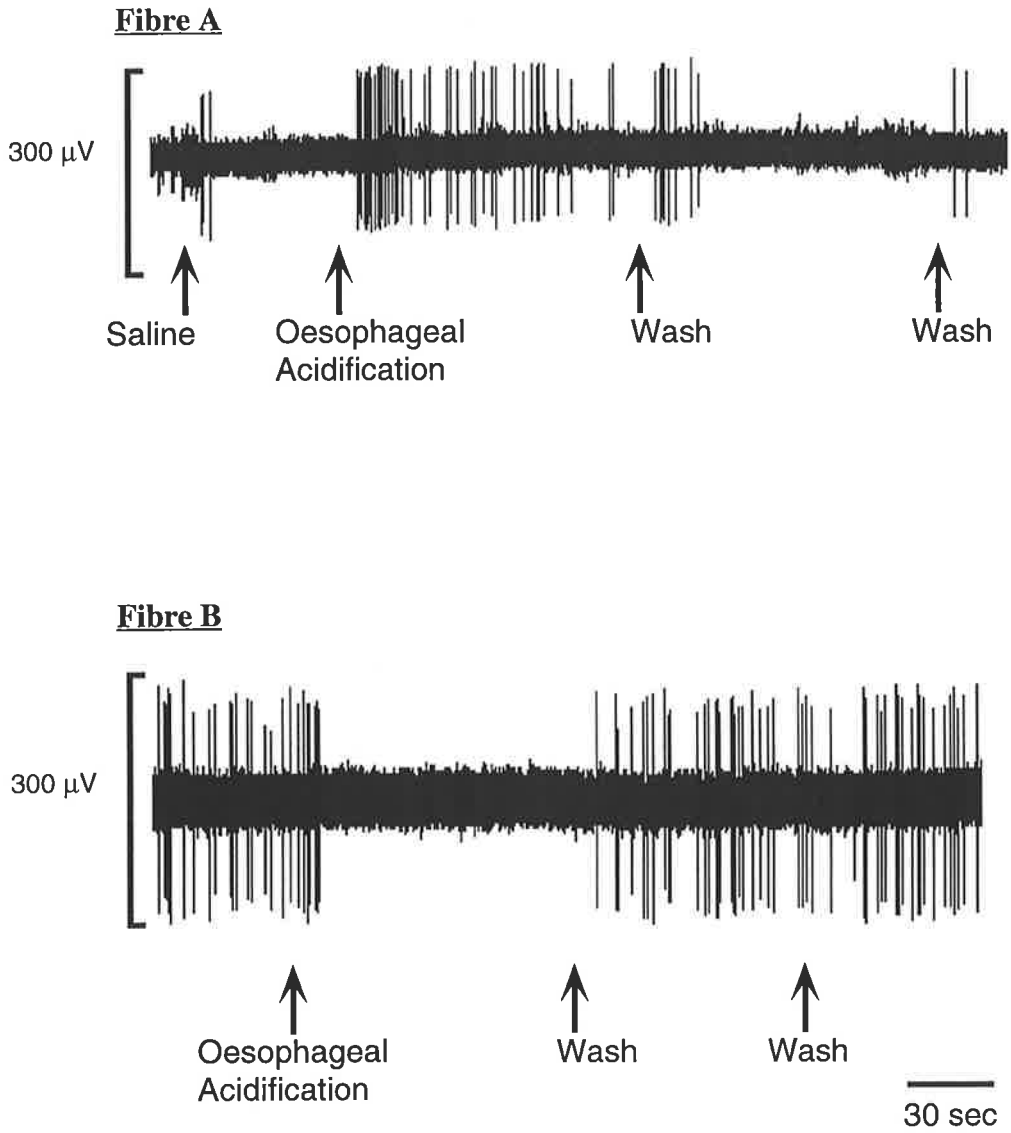


Figure 2-7. Raw record of 2 vagal efferent fibre responses to oesophageal acidification.
Fibre A and Fibre B are the same 2 fibres shown in Figures 2-1 & 2-3.

Oesophageal acidification (150mM HCl, 2ml) Fibre A Saline infusion led to brief burst of activity that lasted only for the duration of the infusion. The infusion of acid into the distal portion of the oesophagus led to a burst of action potentials which persisted until the lumen was bathed with subsequent saline infusion. Fibre B Acid infusion led to complete inhibition of the fibre activity which resumed only when the oesophageal lumen was washed with isotonic saline.

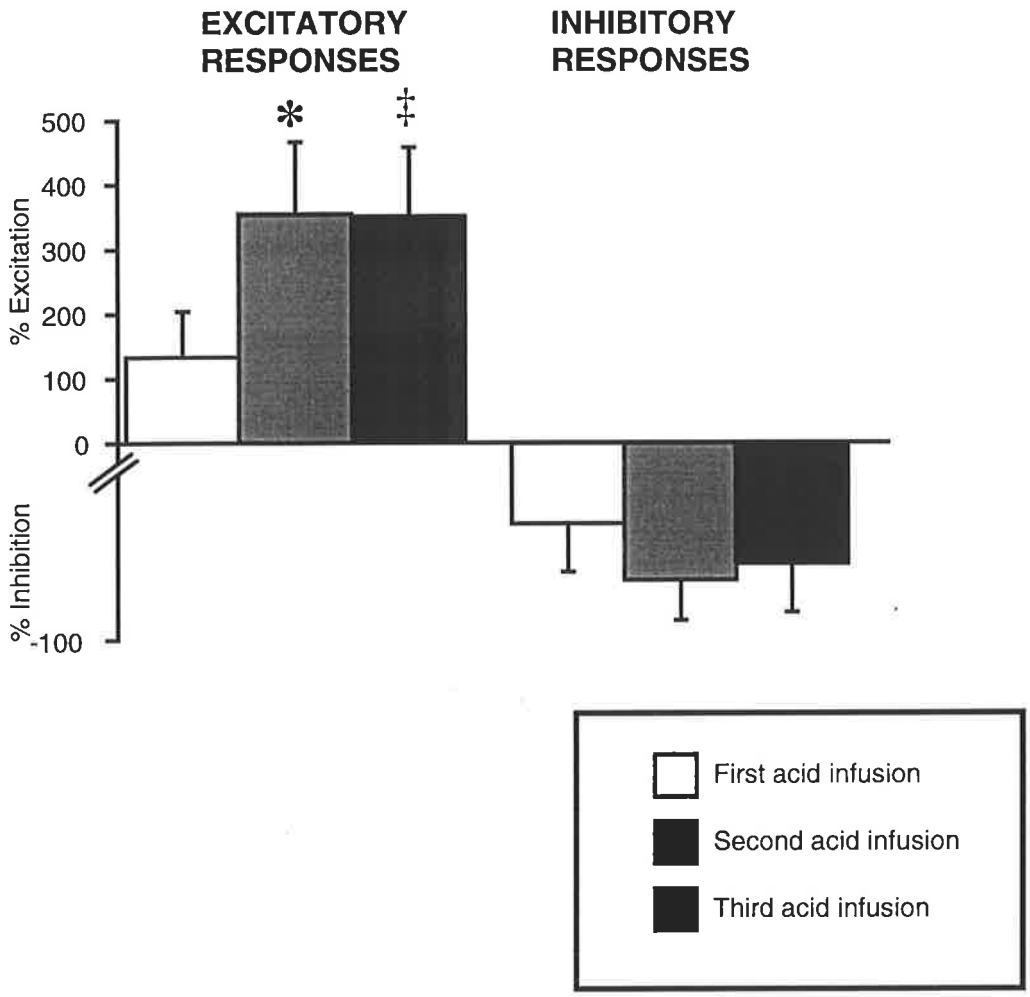


Figure 2-8 Group data showing change in vagal efferent discharge with repeated oesophageal acidification.

Oesophageal acid infusions (2ml, HCl 150mM) led to either excitation (n=15) or inhibition (n=3) of vagal efferent discharge. Excitatory responses were significantly larger with repeated acid infusion.

Data presented as mean$\pm$sem. * $p<0.05$ vs control, ‡ $p<0.01$ vs control

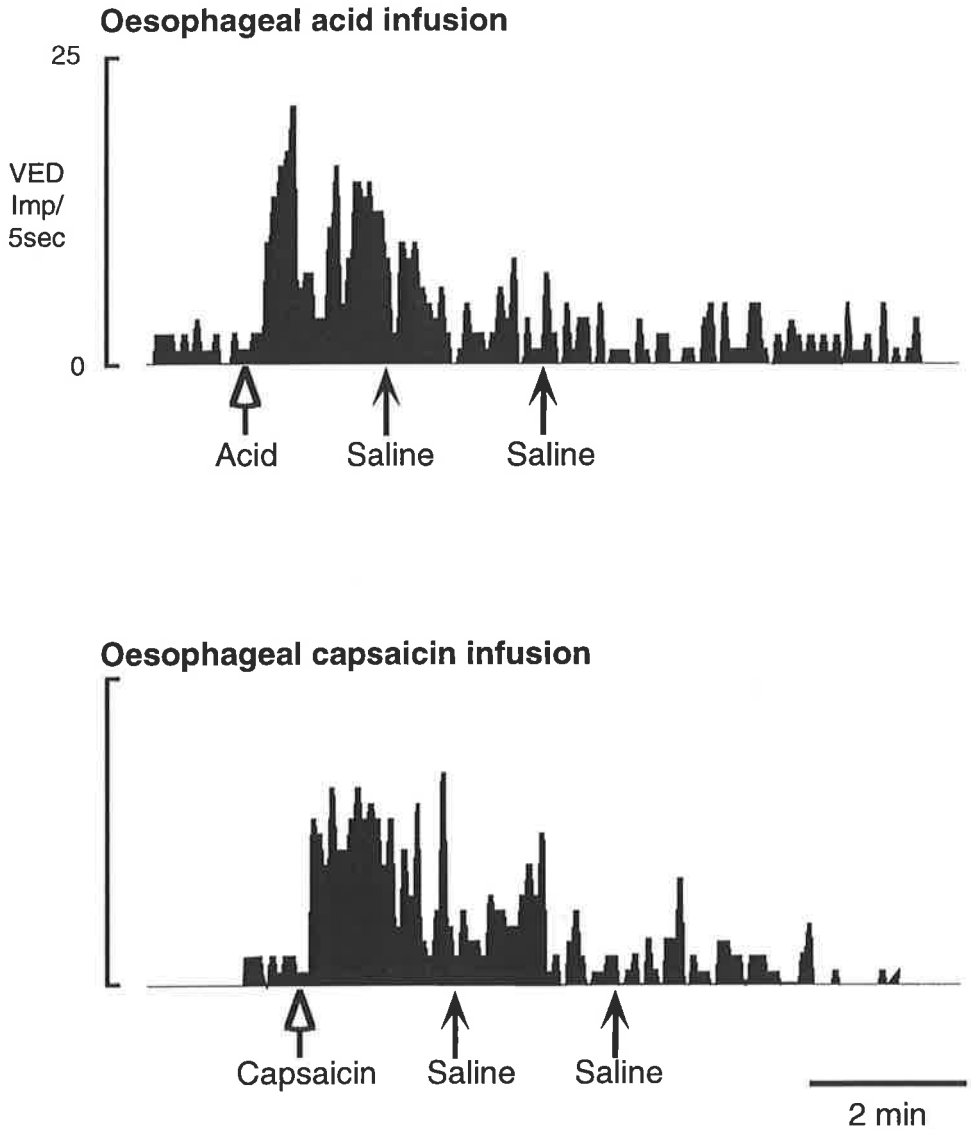


Figure 2-9. Integrated record of a vagal efferent fibre response to intra-luminal oesophageal stimuli
Acid (150mM HCl, 2ml) and capsaicin (6.4mM, 2ml) were infused slowly into the distal portion of the oesophagus. Each stimulus was washed out with subsequent isotonic saline infusions.

The efferent fibre responded to both acid and capsaicin with an increase in activity which was sustained until the oesophageal body was washed out with saline.

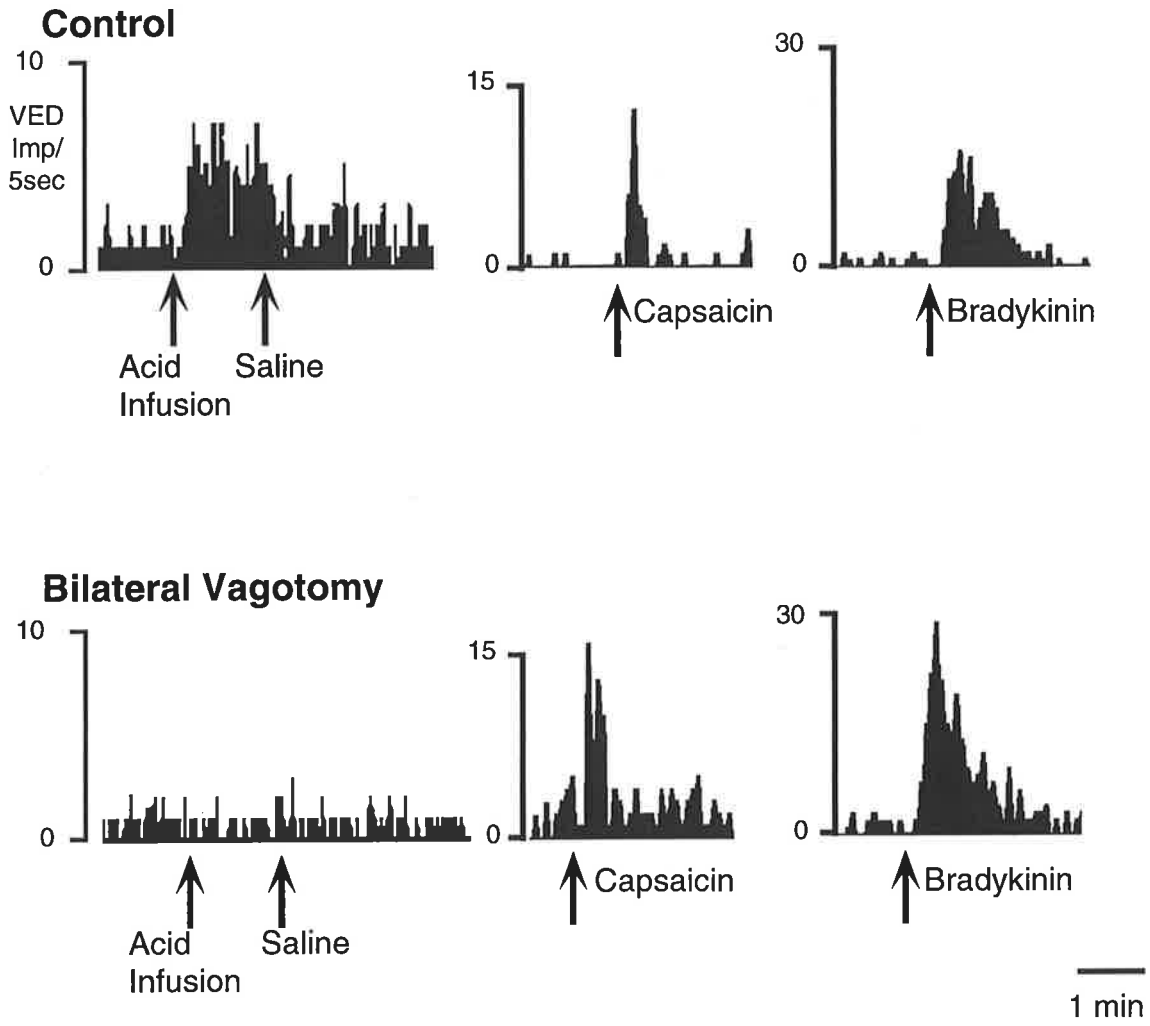


Figure 2-10. Effect of bilateral vagotomy on the vagal efferent responses to chemical stimuli.
This recording is taken from the same fibre as Figure 2-6.

Prior to vagotomy, acid infusion (150mM HCl, 2ml), close intraarterial capsaicin (65nmol) and bradykinin (18nmol) evoked excitatory responses in this fibre.

Bilateral vagotomy caudal to the recording site abolished the efferent response to oesophageal acidification and enhanced the responses to close intraarterial chemical stimuli.

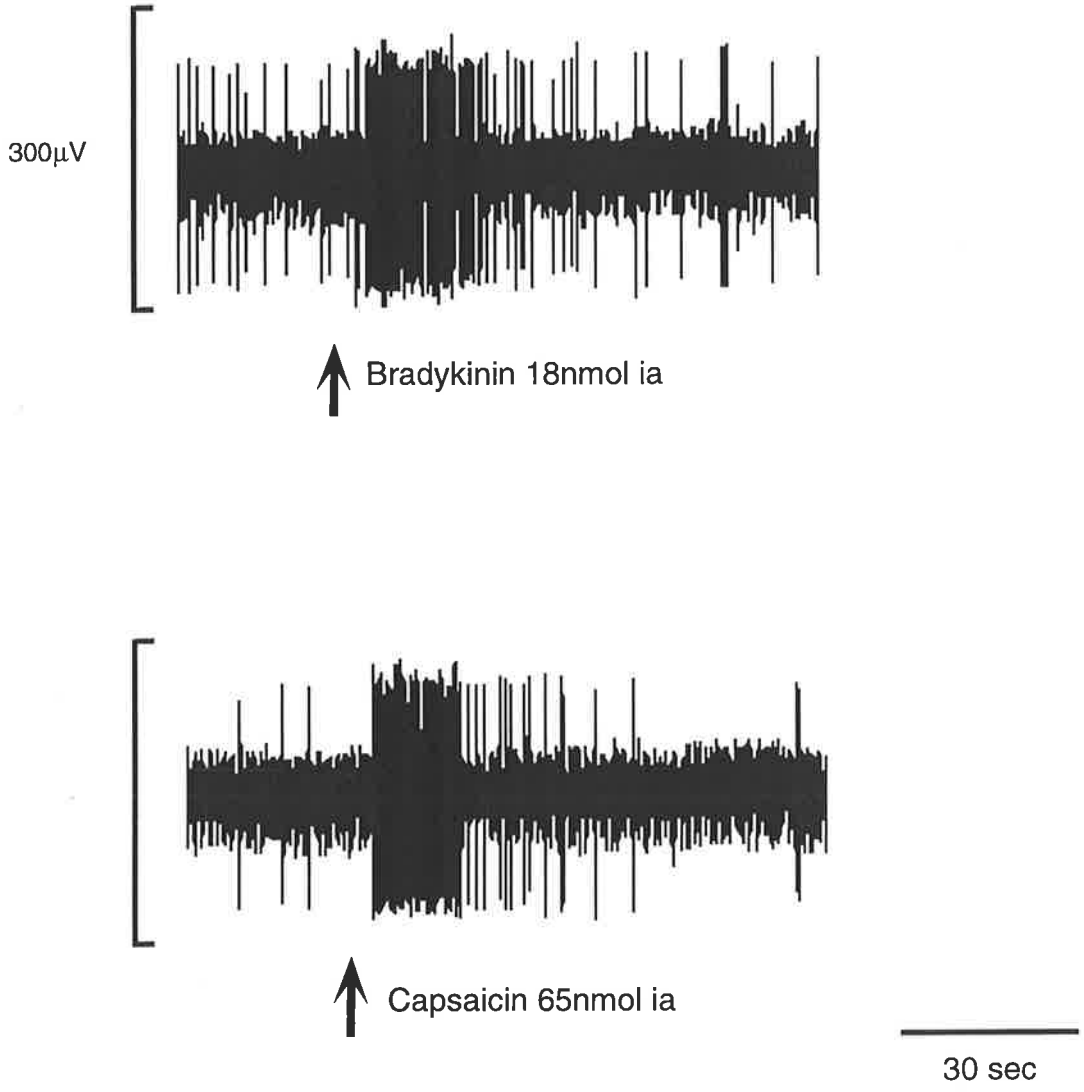


Figure 2-11 Raw record of a vagal efferent fibre response to chemical stimuli
This tracing is obtained from the same fibre as Fibre A in Figures 2-1, 2-3 and 2-7.

The resting activity of the efferent fibre was rapidly excited by close intraarterial bradykinin (18nmol). The intense burst of activity was rapidly evoked (<5seconds), lasted for ~30seconds, after which activity returned to pre-stimulus level. A similar response was seen in this fibre to close intraarterial capsaicin (65nmol).

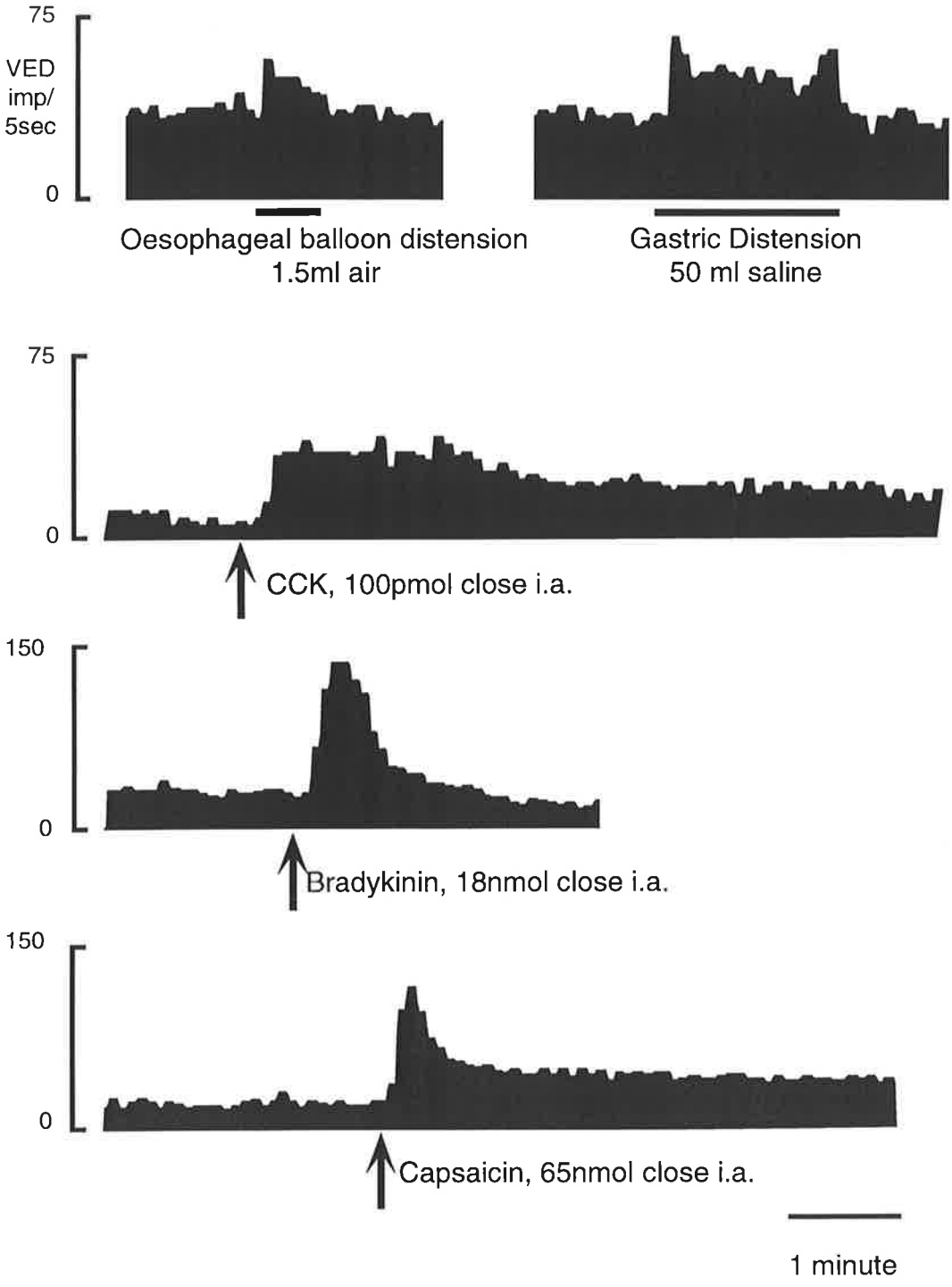


Figure 2-12. Integrated record of a vagal efferent fibre response to peripheral stimuli.

The discharge rate of the efferent fibre was increased by all peripheral stimuli. The efferent fibre response to the different stimuli varied in intensity and profile. The responses to mechanical stimuli were rapidly evoked and maintained for the duration of the stimuli. The responses to chemical stimuli were not correlated with changes in gastric or blood pressure (not shown) in terms of amplitude, direction or duration.

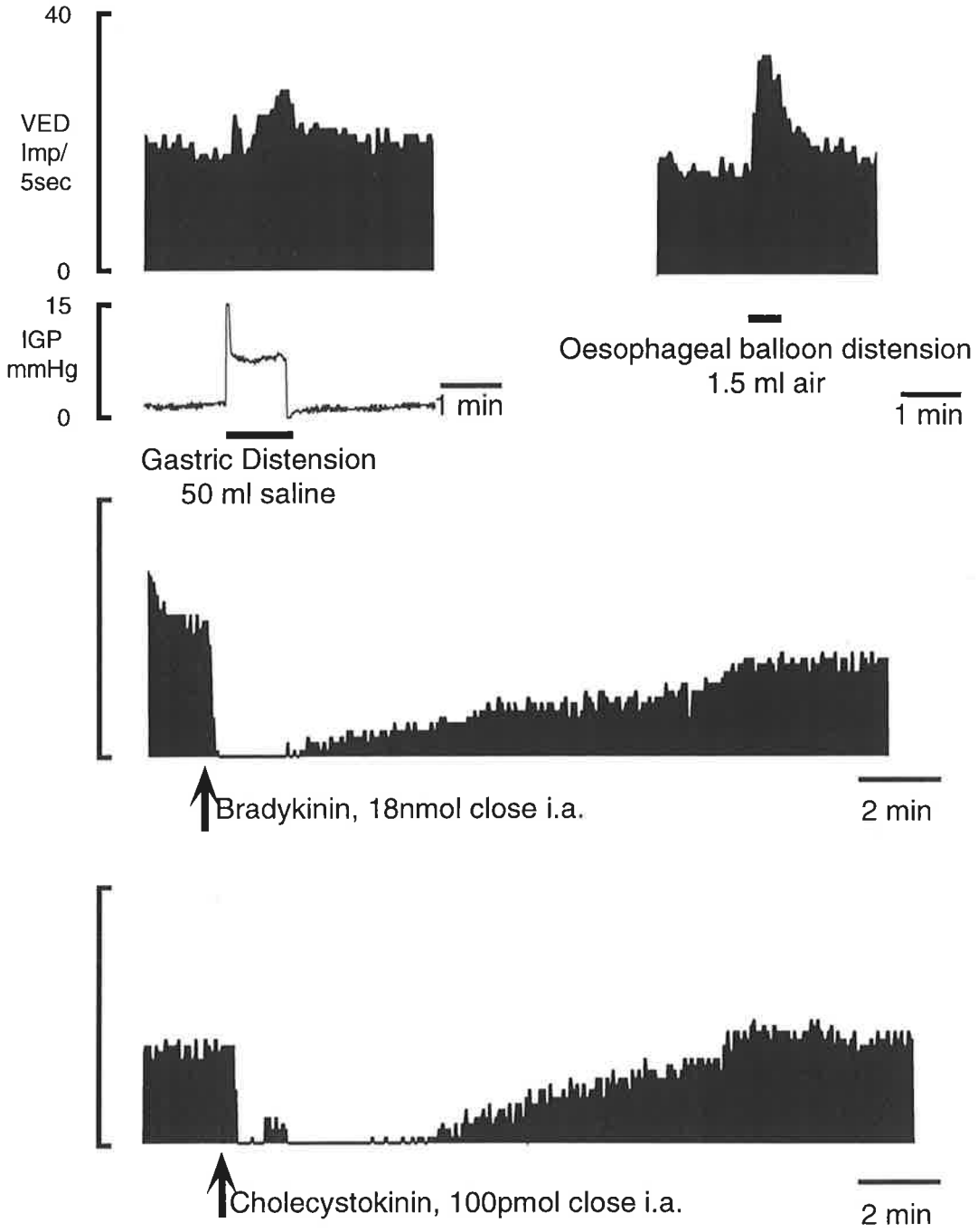


Figure 2-13. Effect of various peripheral stimuli on the discharge of a vagal efferent fibre.
Solid trace: integrated record of the firing rate of a vagal efferent fibre. *Bottom trace* (gastric distension only): intragastric pressure.

The spontaneously active fibre reponded in opposite directions to mechanical stimuli and to chemical stimuli, ie it responded with excitation to gastric distension and oesophageal balloon distension, and with inhibition to close intraarterial bradykinin and cholecystokinin. The responses to bradykinin and CCK were not as rapidly evoked as those to mechanical stimuli. The time taken for efferent activity to return to basal levels was more than 10 minutes.

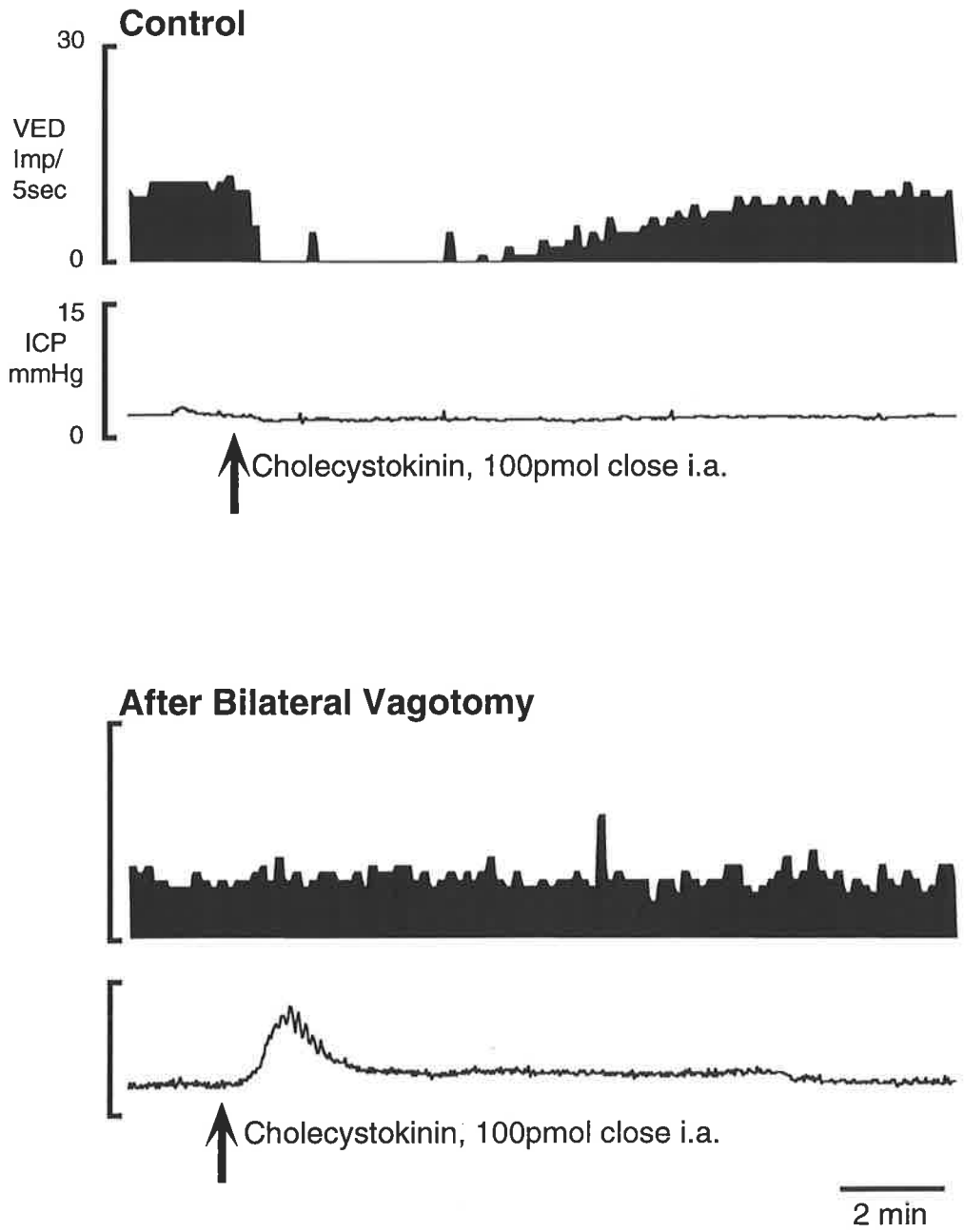


Figure 2-14. Effect of bilateral cervical vagotomy on the vagal efferent fibre and corpus response to cholecystokinin.
Top tracing: Integrated record of the discharge rate of a vagal efferent fibre (5sec). *Bottom trace*: intra-corpus pressure recording.

Close intra-arterial administration of cholecystokinin induced a complete and long lasting inhibition of efferent discharge which is mirrored by a slight decrease in intra-corpus pressure in the deflated stomach. After bilateral vagotomy, cholecystokinin contracted the stomach. The efferent response to CCK was abolished after vagal nerve section.

CHAPTER 3
Central neurotransmitter mechanisms modulating gastrointestinal reflexes

1 SUMMARY

1. The involvement of various neurotransmitters in the vagal efferent responses to peripherally administered chemical and mechanical stimuli were assessed.

2. The effects of muscarinic M1 and M2, CGRP1, NK-1, NMDA and non-NMDA receptor antagonism were investigated. The antagonists used were pirenzepine (2.5-5.0μmol/kg iv), methoctramine (7-14μmol/kg iv), hCGRP8-37 (3.2-6.4nmol/kg icv), CP96345 (8μmol/kg iv) or CP99994 (12μmol/kg iv), CGS19755 (13μmol/kg iv), and CNQX (75-155nmol/kg icv) respectively. Their effects on vagal efferent responses to oesophageal balloon distension (OBD, 1-2ml air), gastric distension (GD, 40-60ml saline), corpus distension (CD, 5-20ml saline), oesophageal acidification (ACID, 150mM HCl, 2ml), cholecystokinin (CCK, 100pmol, close ia), bradykinin (BK, 18nmol close ia) and capsaicin (Cap, 65nmol close ia) were investigated in 41 ferrets.

3. Methoctramine, CGS19755, CP99994 and CP96345 did not affect the majority of vagal efferent responses to OBD, ACID, GD, CCK, BK and Cap. M2, NMDA and NK-1 receptors, respectively, therefore play a minor role in mediating inputs from oesophageal and gastric mechanoreceptors, oesophageal mucosal receptors, and from CCK, BK- and Cap-sensitive afferents within the upper gastrointestinal tract onto vagal central neruones.

4. Systemic administration of the M1 cholinoceptor antagonist pirenzepine reduced efferent responses to GD in 75% of fibres tested, but did not affect any responses to OBD, CCK, Cap and BK. Thus, M1 receptors are selectively involved in mediating inputs from gastric mechanoreceptors onto vagal efferent neurones.

5. Central administration of the selective CGRP1 receptor antagonist hCGRP8-37 modulated >50% of efferent responses to GD and BK. It also influenced a minority of efferent responses to OBD, CCK and Cap. Thus, oesophageal and gastric mechanoreceptor

inputs and chemosensitive inputs from the upper GI tract onto vagal efferent neurones can be mediated via CGRP1 receptors.

6. Central administration of the selective non-NMDA receptor antagonist CNQX, by itself, reduced 4/5 efferent responses to OBD and 3/3 responses to GD. It did not affect 2/2 responses to CCK or BK. When administered in combination with hCGRP8-37 or baclofen and CGP35348, CNQX reduced most responses to OBD, GD, CCK, BK, and Cap. Hence, the non-NMDA receptor may interact with the CGRP1 and GABAb receptors in vagal reflex pathways involving inputs from oesophageal and gastric mechanoreceptors, CCK-, BK- and Cap-sensitive afferents.

7. While there appears to be some specificity in the receptor mechanisms involved in vagal reflexes, there is also great heterogeneity in the effects of receptor antagonists on the efferent responses to peripheral stimuli as no one receptor antagonist was able to block all efferent responses to any single stimulus.

2 INTRODUCTION

The vagus nerve, one of the major sources of extrinsic innervation to the gut, is involved in receiving and relaying vast quantities of afferent information in order to provide integrative control mechanisms necessary for the interaction between various organs and systems within the body and with the external environment. Vagal afferents contain a large number of neuroactive substance within their central and peripheral terminals. These extrinsic neurones that supply the gut usually contain more than one neurotransmitter substance, with some neurones containing up to four or more different ones[186]. These substances include the classical neurotransmitters such as dopamine, 5-hydroxytryptamine, acetylcholine, adrenaline, and noradenaline, neuropeptides such as substance P, calcitonin gene-related peptide (CGRP), galanin, and cholecystokinin, and amino acids such as glutamate, γ-amino-butyric acid (GABA), glycine and aspartate (see Introduction).

It is known that more than one transmitter substance is often involved in mediating an effect, for example, nitric oxide (NO) and vasointestinal peptide (VIP) are often coreleased peripherally from the same neurones and contribute to relaxation in the gastrointestinal tract. This idea of having more than one substance involved with the transmission process is called the concept of plurichemical transmission[186]. However, even though multiple neurotransmitters may be involved, the degree of participation of the different substances can vary and are grouped into three categories. Primary transmitters are those substances that can significantly alter the excitability of the target cells. Primary transmitters tend to be the same in neurones that serve similar functions in different regions of the gastrointestinal tract and in different species. Subsidiary, or secondary, transmitters act in the same way as primary transmitters although their role may be minor by comparison. In the example given above, NO is usually the primary transmitter and VIP the subsidiary transmitter. Neuromodulators, on the other hand, modify transmission either by regulating the amount of neurotransmitter released or by changing the long term level of excitability. In contrast to primary transmitters, subsidiary transmitters and neuromodulators can vary substantially between different regions of the gut and different species.

It is not known if transmitter content in the CNS is responsible for providing functional specificity of the system. For example, different peptides may be contained in mucosal or muscular afferents. With this in mind, I undertook to investigate the involvement of several neurotransmitters which are present in the dorsal vagal complex, the main site of central termination of vagal afferents and efferents. Due to the large number of neuroactive compounds present in the brainstem, it was impossible to look at the involvement of all the neurotransmitters listed above and their respective receptors in modulating gastrointestinal inputs onto vagal central neurones. On the basis of the available pharmacological, anatomical and physiological data, I have chosen to investigate only the involvement of the primary neurotransmitters acetylcholine (via M1 and M2 muscarinic receptors) and glutamate (via N-methyl-D-aspartate (NMDA), and non-NMDA, or α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainate, receptors), and of the subsidiary neurotransmitters substance P (via neurokinin-1 (NK-1) receptors) and

CGRP (via CGRP1 receptors) in the responses of vagal efferents to peripheral stimuli. The involvement of the neuromodulator γ-amino butyric acid (GABA) via the GABAb receptor in vagal reflex pathways has also been investigated and will be reported in Chapters 4 & 5. The effect of each receptor antagonist on the efferent responses will be discussed independently. The possible interactions between different receptors in mediating mechano- and chemo-sensitive afferent inputs in vagal reflexes will then be briefly outlined.

3 PROTOCOLS

Antagonists to the NK-1, NMDA, M1 and M2 receptors were administered intravenously at doses which were considered to be centrally active. Antagonists to the non-NMDA and the CGRP1 receptors were administered centrally into the fourth ventricle, which juxtaposes the various subnuclei in the brainstem where vagal afferents and efferents are known to terminate[26, 78, 203]. The effects of these antagonists on vagal efferent responses to oesophageal balloon distension (OBD, 1-2ml air), gastric distension (GD, 40-60ml saline), corpus distension (CD, 20ml saline), oesophageal acidification (ACID, 2ml 150mM HCl), cholecystokinin (CCK, 100pmol close ia), capsaicin (Cap, 65nmol close ia), and bradykinin (BK, 18nmol close ia) were determined. The pattern of efferent responses to these stimuli have been discussed previously in Chapter 2 and therefore will not be reiterated here.

Results will be initially presented according to the effects of each antagonist on the efferent responses to various stimuli. The effect of each antagonist, usually administered as the first treatment in the study, will be used to illustrate this section.

However, in some studies, more than 1 receptor antagonist was administered. This was done cumulatively in random order and the efferent responses to peripheral stimuli were assessed after administration of each antagonist. To determine whether an order effect exists in modulating efferent responses to the above stimuli, a few studies in which different antagonists were administered will be discussed. As there are numerous combinations and

sequences in which different antagonists can be administered, the number of observations for each group was usually insufficient to provide enough data for statistical analysis. Thus, the descriptions in this section are mainly qualitative, and not quantitative in nature.

The effects of muscarinic receptor blockade were evaluated in a total of 5 studies. In 4/5 studies, both pirenzepine (2.5-5.0μmol/kg iv) and methoctramine (7-14μmol/kg iv) were administered, with pirenzepine being administered prior to methoctramine in 2 of these studies and the reverse order being followed in 2 other studies. In the other experiment on muscarinic receptor antagonism, pirenzepine was given without methoctramine. In this study, pirenzepine was administered after non-NMDA, NK-1 and GABAb receptor blockade.

The CGRP1 receptor antagonist hCGRP8-37 (3.2-6.4nmol/kg icv) was administered in 11 studies (Table 3-2). It was administered as the first treatment in 7 of these studies. In 1 study, it was administered subsequent to CNQX administration. In 2 other studies, it was the third treatment administered after baclofen and CGP35348 administration. In the remaining study, CGRP antagonism was performed as the last treatment after administration of baclofen, CGP35348, CP99994, CNQX and pirenzepine.

The effects of NK-1 receptor antagonism with either CP96345 (8μmol/kg iv) or CP99994 (12μmol/kg iv) were investigated in 22 studies. CP96345 was used in 16 studies prior to the availability of CP99994 in our laboratory. In all 16 studies, it was the only drug antagonist administered. CP99994 was used in the other 6 studies. In these later experiments, CP99994 was the first treatment in 2 studies. It was administered after hCGRP8-37 in 1 study and after CGS19755 in 1 other study. In the remaining 2 studies, CP99994 was given after GABAb receptor activation and blockade. CP99994 has reduced affinity for the L-type calcium channel than does CP96345[187], and therefore has fewer potential non-specific effects.

The effects of the NMDA receptor antagonist CGS19755 (13μmol/kg iv) on vagal efferent responses to peripheral stimuli were investigated in 7 studies. In all 7 studies, it was the first treatment administered.

The influence of the non-NMDA receptor antagonist CNQX (75-155nmol/kg icv) in efferent responses to peripheral stimuli was investigated in 12 studies. In 5 of these studies, it was administered as the first treatment. In 4 other studies, CNQX was administered after CGRP receptor antagonism. In the remaining 3 studies, GABAb receptor activation and blockade with baclofen and CGP35348 respectively was administered as the first treatments prior to CNQX administration. Of these 3 studies, the CGRP receptor antagonist had also been administered prior to CNQX in 1 study and the NK-1 receptor antagonist prior to CNQX in 1 study.

In five studies, both hCGRP and CNQX were administered. These antagonists were not administered simultaneously. Rather, they were given in a cumulative manner, such that the effects of each antagonist on the efferent response to peripheral stimuli could be ascertained. In four of these studies, hCGRP8-37 was administered prior to CNQX. The order was reversed in the remaining study. The changes in efferent responses due to administration of hCGRP8-37 and CNQX are tabulated in Table 3-6.

4 RESULTS

All efferent responses reported here are reproducible within each treatment group. In some studies, responses to certain stimuli were lost prior to treatment with antagonists. These responses were not included in the tables.

4.1 M1 and M2 cholinoceptor antagonism

4.1.1 Effects on motility

The M1 and M2 cholinoceptor antagonists, pirenzepine and methoctramine respectively, were administered intravenously at doses which were effective in modulating intraluminal pressure responses to gastric and corpus distension. The illustration on Figure 3-1 depicts the effect of both drugs on gastric pressure during gastric distension (GD, 50ml saline). Under control conditions, GD evoked an increase in mean gastric pressure of 7.96mmHg which was also accompanied by phasic activity. Methoctramine administration (14μmol/kg iv) lowered this increase to 5.56mmHg. Subsequent pirenzepine administration (5μmol/kg iv) further attenuated this response to 3.85mmHg. Morever, the phasic rhythm seen under control conditions was abolished with M1 cholinoceptor antagonism.

The intraluminal pressure during distension was monitored in 5 studies. The stomach was divided into its corpus and antral portions in 3 of these studies and the response to corpus distension (CD, 20ml saline) was investigated. In the other 2 studies, the intact stomach was distended with 50ml saline (GD). Group data shown in Figure 3-2 indicate that both antagonists inhibited the increase in intraluminal pressure with both gastric and corpus distension, although the inhibition was statistically significant only with pirenzepine administration. As both drugs readily cross the blood brain barrier, these peripheral effects were assumed to also reflect parallel effects on receptors that may be located in the CNS.

4.1.2 Patterns of spontaneous activity

Basal vagal efferent discharge and intragastric pressure were monitored in 5 studies in which the muscarinic receptor antagonists were administered (not shown). Group data indicated that there were no significant effects of either pirenzepine or methoctramine on basal vagal efferent discharge levels. Basal intragastric pressure was also not affected by methoctramine administration. However, pirenzepine significantly decreased basal intraluminal pressure.

4.1.3 Modulation of efferent responses to peripheral stimuli

Pirenzepine (2.5-5.0μmol/kg iv) was administered in 6 studies and its influence on vagal efferent responses to OBD (n=4), GD (n=4), CCK (n=1), BK (n=4) and Cap (n=4) evaluated. The effect of pirenzepine (5.0μmol/kg iv) can be seen in the example on Figure 3-3. In this study, the efferent response to GD was completely abolished by M1 receptor antagonism. This abolition of efferent discharge was not secondary to changes in intraluminal pressure as there was no change in discharge even during the dynamic phase of distension when a high mean gastric pressure was elicited. The effect of pirenzepine was also specific to the efferent response to GD as efferent responses to OBD, BK, and Cap were unaffected. This is true of the other 4 studies where pirenzepine was administered (Table 3-1A). Pirenzepine reduced or abolished the efferent responses to GD in 3/4 fibres tested while leaving the efferent responses to OBD, BK, Cap, and CCK unaffected.

The effect of methoctramine (7-14μmol/kg iv) in 4 studies on vagal efferent responses to OBD (n=3), GD (n=2), BK (n=3) and Cap (n=3) was evaluated. In the study illustrated in Figure 3-4, the initial responses to OBD and CD were small but reproducible under control conditions. Methoctramine (14μmol/kg iv) in this study did not affect the efferent responses to GD, OBD, Cap and BK. This trend was consistent with the data obtained from the other 3 studies, where the M2 cholinoceptor antagonist did not modulate the response to any peripheral stimulus tested (Table 3-1B).

4.2 CGRP1 receptor antagonism

4.2.1 Patterns of spontaneous activity

Central administration of the CGRP1 receptor antagonist hCGRP8-37 (3.2-6.4nmol icv) led to a consistent decrease in resting efferent discharge. This can be seen in both in the example and group data presented on Figure 3-5. In the raw tracing, a decrease in basal vagal efferent discharge was seen within 1 minute of hCGRP8-37 administration. Group data showed that this reduction in basal efferent discharge due to hCGRP8-37 administration

was statistically significant. The other 2 parameters measured, intragastric pressure and blood pressure, were relatively unchanged by CGRP1 receptor antagonism.

4.2.2 Modulation of efferent responses to peripheral stimuli

hCGRP8-37 (3.2-6.4nmol/kg icv) was administered in 11 studies and its influence on vagal efferent responses to OBD (n=6), GD (n=7), CCK (n=9), BK (n=10) and Cap (n=7) were evaluated. The effect of hCGRP8-37 (3.2-6.4nmol/kg icv) on an efferent fibre activitiy can be seen in the example on Figure 3-6, where the response to BK was reduced by CGRP1 receptor antagonism. The efferent responses to OBD, GD, and CCK were unaffected in this study.

In Table 3-2, the effects of hCGRP8-37 on individual efferent responses to the stimuli above are listed. This CGRP1 receptor antagonist reduced efferent responses to OBD (2/6 studies), GD (4/7 studies), and BK (5/10 studies). The efferent responses to CCK and Cap were largely unaffected.

4.3 NK-1 receptor antagonism

4.3.1 Patterns of spontaneous activity

The influence of NK-1 receptor antagonism with CP99994 (12μmol/kg iv) on resting blood pressure, intragastric pressure and efferent discharge was measured in 5 studies. In all of these studies, at least one treatment had been administered prior to NK-1 receptor antagonism. An example of the effect of NK-1 receptor antagonism on the 3 variables is shown in Figure 3-7. The NMDA receptor antagonist CGS19755 (13μmol/kg iv) had been administered prior to CP99994 (12μmol/kg iv) in this study. NK-1 receptor antagonism decreased basal efferent discharge from 6.25 to 0.35imp/sec, mean gastric pressure from 7.94 to 3.88mmHg and mean arterial pressure from 75.05 to 47.16mmHg.

Group data compiled from 5 studies show a general trend towards reduction in the 3 parameters that were measured (Figure 3-7A-C). However, this reduction was only statistically significant with mean arterial pressure and was consistently seen in all 5 studies. In one of these studies, blood pressure was so severely reduced that this particular experiment was terminated before the effects of NK-1 receptor antagonism on efferent responses could be ascertained. The overall change in basal efferent discharge was not statistically significant as it was reduced in 2 studies, increased in 2 studies and unchanged in the 1 study. Likewise, mean gastric pressure was not significantly reduced as it was decreased in 3 studies, and increased in 2 studies. The effects of NK-1 receptor antagonism on basal VED were not secondary to changes in intragastric pressure as the changes were in opposite directions in 2/5 studies. Nevertheless, as both these drugs readily cross the blood-brain barrier[187], these peripheral effects were also assumed to reflect parallel effects on NK-1 receptors that may be located in the CNS.

4.3.2 Modulation of efferent responses to peripheral stimuli

The influence of NK-1 receptor antagonism on vagal efferent responses to OBD (n=16), GD (n=19), ACID (n=11), CCK (n=3), BK (n=9) and Cap (n=8) were evaluated.

The effects of NK-1 receptor antagonism on the responses of each efferent fibre to the peripheral stimuli tested is listed in Table 3-3. Neither antagonist used affected any of the efferent responses to OBD, ACID, or BK. The response to GD was slightly attenuated (60% response prior to CP96345 administration) in 2/20 fibres tested. The responses to CCK in 1/3 fibres tested were modulated with CP99994.

In the study illustrated in Figure 3-8, GABAb receptor activation and blockade with baclofen and CGP35348 respectively had been achieved prior to NK-1 receptor antagonism. CP99994 reduced the efferent response to CCK by <50%, but did not affect the efferent responses to OBD, GD, BK and Cap. In the second study where the efferent response to CCK was attenuated, CP99994 was administered after CGRP receptor antagonism with hCGRP8-37. In this study, the response to Cap was also severely reduced (not illustrated).

4.4 NMDA receptor antagonism

4.4.1 Patterns of spontaneous activity

Basal vagal efferent discharge, intragastric pressure, and blood pressure were monitored in 7 studies in which the NMDA receptor antagonist was administered. Group data indicated that there were no significant effects of CGS19755 (13μmol/kg iv) on the 3 parameters measured (Figure 3-9 A-C). The effect of NMDA receptor antagonism on basal efferent discharge in individual studies were varied, ie it led to an increase in resting discharge in 1 study, a decrease in 4 studies, and no change in 2 studies. Likewise, the effect on mean arterial pressure was not consistent. Blood pressure was decreased in 2 studies, increased blood pressure in 4 and unchanged in 1 study. Mean gastric pressure was unchanged by CGS19755 in 5 studies, increased in 1 study, and decreased in another. In the study illustrated in the top part of Figure 3-9, NMDA receptor antagonism caused a gradual increase in the resting efferent discharge and blood pressure. CGS19755 did not modulate resting intragastric pressure in this study.

Although CGS19755 did not lead to significant changes in resting vagal efferent discharge, intragastric pressure or blood pressure, it modulated basal efferent discharge and blood pressure to a small extent in most experiments. These peripherally manifested effects are assumed to reflect any parallel changes on NMDA receptors which may be located in the CNS.

4.4.2 Modulation of efferent responses to peripheral stimuli

The effects of CGS19755 (13μmol/kg iv) on vagal efferent responses to OBD (n=3), GD (n=2), CCK (n=6), BK (n=5), and Cap (n=3) were investigated in 7 studies. The effect of NMDA receptor antagonism on the responses of 2 vagal efferent fibres to peripheral stimuli is illustrated in Figure 3-10. The first efferent fibre responded to OBD and GD. CGS19755 did not affect the response to either stimulus. The second efferent fibre responded consistently only to CCK, BK and Cap. After CGS19755 administration,

the response to CCK was slightly attenuated. Responses to the other 2 chemicals were unchanged.

The effect of NMDA receptor antagonism on the responses of each efferent fibre to the peripheral stimuli tested is tabulated in Table 3-4. CGS19755 did not modulate any efferent responses to distension of either the distal oesophagus or the stomach. It also did not affect the efferent responses to BK. In 1/3 studies, the response to Cap was abolished. It attenuated the efferent response to CCK in 2/6 studies.

4.5 non-NMDA receptor antagonism

4.5.1 Patterns of spontaneous activity

Basal vagal efferent discharge, mean gastric pressure and mean arterial pressure were monitored in 11 studies. There were no significant changes in basal efferent discharge and intragastric pressure in response to CNQX administration (75-155nmol/kg icv) (Figure 3-11 A-B). Central non-NMDA receptor blockade achieved with CNQX (75-155nmol icv) led to a small but statistically significant decrease in resting blood pressure (Figure 3-11 C). This is illustrated in the example on Figure 3-11. In this study, administration of CNQX (155nmol icv) decreased resting efferent discharge within 1 minute, while leaving basal intragastric pressure unchanged. The decrease in efferent discharge was followed closely by a small decrease in blood pressure.

4.5.2 Modulation of efferent responses to peripheral stimuli

The effect of CNQX on vagal efferent responses to OBD (n=11), GD (n=9), CCK (n=8), BK (n=8) and Cap (n=4) were evaluated. In the example on Figure 3-12, CNQX (75nmol/kg icv) attenuated the efferent responses to OBD and CD but did not affect efferent responses to both chemical stimuli tested.

The influence of CNQX on the responses of individual fibres to each stimulus can be seen in Table 3-5. When CNQX was administered as the first treatment in the study, it reduced or abolished the responses to OBD in 4/5 studies and to GD in 3/3 studies. Responses to CCK and BK were unaffected. In 7 studies, other drugs were administered prior to CNQX. The effects of CNQX on efferent responses in these studies will be discussed in detail in the section below and in Chapter 5. Overall, CNQX modulated the efferent responses to OBD in 7/11 fibres tested, to GD in 6/9 fibres tested, to CCK in 6/8 fibres tested, to BK in 4/8 fibres tested and to Cap in 3/4 fibres tested. There did not appear to be a dose related effect as the responses to certain stimuli were reduced with the lower dose of the antagonist whereas responses to the same stimuli were unaffected by the higher dose used in other studies (see Table 3-5).

4.6 Cumulative effects of multiple receptor antagonism

The cumulative effects of CNQX and hCGRP8-37 on vagal efferent responses to OBD (n=5), GD (n=4), CCK (n=4), BK (n=4) and Cap (n=2) were evaluated. In the example on Figure 3-13, hCGRP8-37 (3.2nmol/kg icv) did not modulate the responses to gastro-oesophageal mechanical stimuli but reduced the period of complete inhibition caused by CCK as well as attenuating the response to BK. Subsequent administration of CNQX (155nmol/kg icv) abolished the efferent responses to gastro-oesophageal distension and reversed the direction of responses to both CCK and Bk from one of inhibition to one of excitation.

The data from four studies where hCGRP8-37 was administered prior to CNQX, the combined effect of both antagonists on efferent responses to close intraarterial chemicals were consistent. With the exception of 1 efferent response to BK, all responses to CCK, BK and Cap were either completely abolished (n=2) or had their direction of response reversed (n=1). In 3 fibres, administration of CNQX did not reduce the responses to either oesophageal or gastric distension that had already been attenuated by prior administration of hCGRP8-37.

In the single study where CNQX was administered as the first treatment, CNQX did not modulate the efferent response to OBD, CCK, or BK. However, subsequent central blockade of CGRP receptors with hCGRP8-37 reduced the responses to CCK and BK. The response to OBD was not affected by hCGRP8-37 administration.

5 DISCUSSION

This study provides evidence of the involvement of three major transmitter mechanisms in vagal reflex pathways. However, antagonism of a specific receptor did not led to a modulation in all efferent responses to a specific stimulus.

5.1 Involvement of acetylcholine in gastrointestinal vagal reflexes

5.1.1 Acetylcholine and muscarinic receptors

Both methoctramine and pirenzepine were administered at physiologically relevant doses as seen by the effects of both antagonists on intraluminal pressure during gastric distension. Although only the M1 receptor antagonist pirenzepine caused a significant reduction in mean gastric pressure and gastric contractile activity, the M2 receptor antagonist methoctramine consistently decreased the intraluminal pressure during distension. This finding is in agreement with earlier studies using both pirenzepine and atropine in the ferret[11, 13].

From the results of this study, the M1 receptor may be selectively located on the central terminals of those neurones which are involved in mediating inputs from gastric mechanoreceptors as pirenzepine selectively attenuated the efferent response to gastric distension in 75% of fibres tested, but did not affect responses to any other peripheral stimuli tested. The change in the efferent response to gastric distension caused by pirenzepine is not secondary to the reduction of mean gastric pressure as changes in efferent responses were not proportional to the effect of pirenzepine on intragastric pressure during

gastric distension. If the efferent responses were secondary to changes in intraluminal pressure, pirenzepine would have exerted similar effects on all fibres tested. Instead, in one experiment, the efferent response to gastric distension was abolished after pirenzepine. M1 receptor antagonism had no affect on the efferent response to distension in another study and reduced the efferent responses to varying degrees in 2 other studies..

The involvement of the M2 receptor in the vagal reflex pathways studied here appears to be relatively minor at best, as no changes in efferent responses to any peripheral stimulus tested was seen with methoctramine.

Acetylcholine is the neurotransmitter found in pre- and post-ganglionic neurones of parasympathetic nerves. M1 receptors are generally found in autonomic ganglia and various secretory glands whereas M2 receptors are predominantly found in the myocardium and in smooth muscle. Centrally, the presence of acetylcholinesterase, the degradative enzyme for acetylcholine, has been found in the gelatinous and commissural subnuclei of the NTS. Later studies looking for the presence of the synthesizing enzyme, choline acetyltranseferase, has demonstrated the existence of neurones containing this enzyme within the nodose ganglion[108], DMVN[17], NTS[245], NA[160] and dorsal root ganglion (DRG)[231]. Studies looking for the presence of muscarinic receptors in the CNS have not differentiated between different subtypes. An autoradiographic study has found muscarinic receptors in the rat and cat brainstem, with binding sites found in the NA, NTS, AP and DMVN[169, 263].

In rats, oesophageal peristalsis can be generated by applying muscarine to subnucleus centralis of NTS[264]. In patients suffering from gastro-oesophageal reflux disease, atropine, the non-selective muscarinic receptor antagonist, inhibits the rate of transient lower oesophageal sphincter relaxations (TLOSRs) and affects primary oesophageal peristalsis by reducing peristaltic success and wave amplitude and increasing peristaltic velocity[171]. Atropine has also been shown to affect triggering of TLOSRs,

gastric pressure and contractility[7, 11, 165]. This implies that muscarinic receptors are involved in the generation of oesophageal peristalsis and TLOSRs.

Modulation of efferent responses by pirenzepine in the present study may be the neurophysiological correlates of actions such as these. The concentration of muscarinic binding sites in the subnucleus gelatinosus of the NTS, the site of termination of vagal afferent fibres with sensory endings in the gastric wall[169], may explain the results of the present study. I have shown that M1 cholinoceptor antagonism selectively reduced the inputs from gastric mechanoreceptors onto vagal efferent neurones. This implies that activation of some gastric vagal afferents probably releases acetylcholine centrally. My data also stresses the possibility that cholinergic fibres could play a role in areas such as nausea, satiety or emesis. However, further autoradiographic work is needed to confirm the existence of the specific M1 receptors in the dorsal vagal complex as both atropine and muscarine, the non-selective muscarinic antagonist and agonist respectively, act on both muscarinic M1 and M2 subtypes.

5.2 Involvement of peptides in gastrointestinal vagal reflexes

5.2.1 CGRP and the CGRP1 receptor

The present study demonstrates that the release of endogenous CGRP is involved in mediating gastrointestinal afferent inputs to vagal preganglionic neurones. These inputs were predominantly from gastric mechanoreceptors and bradykinin-sensitive afferents. However, 1) the CGRP1 receptor antagonist was not effective in modulating responses of all efferent to any particular stimulus, eg not all responses to bradykinin were attenuated, 2) it did not block the responses of any individual efferent fibre to all stimuli tested, 3) prior administration of other antagonists did not reveal a more consistent effect of CGRP antagonism, and 4) hCGRP8-37 attenuated, rather than blocked, the efferent responses to peripheral stimuli. These observations indicate 1) heterogeneity in the types of receptors is present on vagal efferent neurones, 2) although CGRP is evidently released centrally by

afferent stimulation, other transmitters must be involved in each type of afferent fibre, and 3) the role of CGRP is not masked by that of a primary transmitter.

As the CGRP1 receptor antagonist was administered into the fourth ventricle, it was assumed to be only effective centrally. CGRP immunoreactivity has been identified in the nodose ganglion[225], and the medial and commissural regions of the NTS, a region which received inputs from most visceral organs[250]. CGRP-immunoreactive fibres have been demonstrated in the substantia gelatinosa (dorsal horn) of the spinal cord, probably arising from dorsal root ganglion cells which in turn belong to splanchnic afferents[231]. CGRP-stained motoneurones were also found in the rostral part of the NA [226]. CGRP receptors have been located within the CNS, where they have been identified in the rat and human DMVN, NTS and substantia gelatinosa of the spinal trigeminal tract[145].

Peripheral administration of CGRP, either systemically or locally to tissue, can produce either contraction or relaxation of the gut smooth muscle, although it is more likely to inhibit gastrointestinal muscle function[217]. It inhibits peristalsis and gastric emptying of liquids, which are both direct effects on smooth muscle. CGRP is also released locally in response to gastric acid backdiffusion in order to mediate gastric hyperaemia in the rat gastric mucosa[142]. This hyperaemia serves to limit acid damage to the superficial portion of the mucosa. The protective effect of CGRP is seen when gross mucosal damage in the rat stomach induced by ethanol and aspirin is inhibited by close arterial infusion of the peptide[173].

A role for CGRP in CNS control of gastrointestinal function has been postulated as central administration of CGRP into the dorsal vagal complex inhibits pentagastrin- or baclofen-stimulated gastric acid secretion[254]. Centrally applied CGRP also inhibits gastric emptying[217] and contractility via an increase in sympathetic outflow. These effects on gastric secretory and motility patterns may explain the reduction of responses to gastric distension by central CGRP1 antagonism seen in my study.

CGRP is also involved in mediating pain transmission in the gut ranging from the oesophagus[177] to the colon[215]. This is transmitted via the spinal nociceptive visceral afferents and terminate centrally on neurones within the dorsal horn. The involvement of CGRP in pain transmission may lead to the reduction in efferent responses to bradykinin in 50% of fibres tested when central CGRP receptors are blocked. The main afferent pathway followed by bradykinin-sensitive afferents is non-vagal as seen by the lack of change in efferent response to bradykinin after bilateral vagotomy (Chapter 2) and is further confirmed by studies where bradykinin has been shown to activate spinal afferents[135, 174] and can exert nociceptive and algesic responses. Bradykinin also releases neuropeptides, such as CGRP, substance P and neurokinin A (NKA), from the peripheral teminals of afferents[117].

The question that arises from our data is whether bradykinin releases the same neuropeptides centrally, and if so, whether the antagonist, which was administered at the level of the fourth ventricle, was able to exert its effects further down at the level of the dorsal horn of the spinal cord. The bradykinin-stimulated release of substance P from cultured nodose ganglion neurones[179] suggests that the bradykinin-induced peptide release is not limited to local release of CGRP, NKA and substance P from the peripheral terminals of afferent. Along with the convergence of non-vagal inputs onto central vagal neurones[42, 126, 219], it appears plausible that bradykinin, in my study, activates spinal afferents to cause the release of CGRP either within the spinal cord or via long ascending projections[66] to the brainstem.

5.2.2 Substance P and the NK-1 receptor

My studies indicate that NK-1 receptors are not involved in mediating inputs from gastric mechanoreceptors and oesophageal mechano- and mucosal receptors onto vagal efferent fibres as the efferent responses to GD, OBD and ACID were not modulated by NK-1 receptor antagonism. NK-1 receptors are also not involved in altering inputs from BK-sensitive afferents located within the upper GI tract onto vagal central neurones. The pathway taken by CCK- and Cap-sensitive afferents onto vagal efferents may require either

CGRP or NK-1 receptors as central blockade of both receptors led to a marked reduction in responses to both chemicals in one study. However, in another experiment where both receptors were blocked along with the M1 muscarinic, GABAb and non-NMDA receptors, responses to both stimuli were unchanged.

Substance P has been found in both peripheral and central ends of sensory nerves[86, 119]. In the periphery, substance P immunoreactivity was found in dorsal root ganglia[118]. Together with CGRP, Substance P is the most abundant peptide found within the GI tract[248]. However, in the vagus over 95% of substance P from the nodose ganglion undergo rapid transport peripherally with less than 5% of peptide being transported centrally[58]. In the CNS, only a small percentage of vagal afferents was found to contain substance P immunoreactivity: this was not specifically localised in any region of the rat dorsal medulla, although the majority is found within the medial portion of the rostral NTS[250]. Substance P containing nerve terminals have also been identified by light and electron microscopy to make axo-somatic and axo-dendritic contacts with neurones in the ventrolateral NA, the site of cardiac motoneurones[182]. An autoradiographic study has found substance P receptors in the rat brainstem[271] and, more specifically, NK-1 receptors in the ferret area postrema and NTS[265].

Experiments on involvement of the NK-1 receptor in vagal reflex pathways used both competitive non-peptide NK-1 receptor antagonists available, CP-99994 and its earlier prototype, CP96345. CP96345 was used in the earlier portion of the experiments until CP99994 was made available. Similar results were obtained with both antagonists despite the comparatively high affinity of CP96345 for the L-type calcium channel as well as its effects as a local anaesthetic at high doses[187]. Both were administered systemically as they were shown to freely cross the blood-brain barrier[187, 213, 260]. In one study, CP96345, when injected either intracerebroventricularly or intraperitoneally, eliminated the inhibition of colonic motility induced by rectal balloon distension[150]. Also, the reduction in mean arterial bloood pressure seen here is at least partially centrally mediated as centrally

administered CP96345 has been shown to attenuate the arterial pressure increase evoked by exogenous substance P administered centrally[260].

Both CP99994 and CP96345 blocked emetic responses induced by a wide range of emetic agents, including intraduodenal hypertonic saline, copper sulphate, and cisplatin[95, 265]. The emetic response is vagally mediated as substance P administered to the area postrema induces retching[6]. Also, intraduodenal hypertonic saline induces c-fos immunoreactivity in the NTS which is substance P mediated as well[48]. Therefore, the 'vomiting centre' is most likely located within dorsal vagal complex, which is the site of central terminations of vagal efferent and afferent fibres[249]. This may mean that substance P and the NK-1 receptors are involved only with the generation and coordination of emesis, and not with the physiological responses in normal conditions such as the innocous stimuli applied in the present study.

The substance P present in the dorsal vagal complex is also involved with vagal cardio-vascular reflexes as 1) NK-1 antagonism in this and other studies led to a significant reduction of mean arterial pressure, 2) topical application of the NK-1 receptor antagonist CP99994[211] on NTS neurones in the working heart-brainstem preparation in mice attenuated excitatory post-synaptic potentials evoked by vagal nerve stimulation and chemoreceptor input from left ventricular receptors to the NTS, and 3) the neurones showing substance P immunoreactivity in the CNS arise from the heart and make contact with NA neurones located in the region of origin of cardiovascular motor neurones[182].

Substance P and the NK-1 receptor, like CGRP, is involved in pain transmission[64, 96, 213]. Combined with my other data that CGRP1 receptor antagonist attenuated responses to bradykinin, I presumed that a similar attenuation would be seen with NK-1 receptor antagonism. This is reinforced by evidence that substance P is often colocalised with CGRP[118, 215] and, like CGRP, is likewise released by bradykinin[117, 179]. However, this was not the case.

Results from the present study demonstrate that substance P and the NK-1 receptor do not appear to play a major role in the vagal central mechanisms involved in mediating the peripheral mechano- and chemo-sensitive inputs from the upper gastrointestinal tract onto vagal central neurones. This appears to conflict with data which suggests that substance P and the NK-1 receptor is involved with emesis[48, 95, 265], in motor responses to afferent activation at the periphery[34], in pain transmission[64, 96, 213], and in cardiovascular reflexes[182, 211, 260]. However, areas in the brainstem and dorsal vagal complex which possess substance P-immunoreactivity and the NK-1 receptor may be involved solely with cardiovascular reflexes and the generation and coordination of the emetic response. Bradykinin, the only potentially noxious stimulus applied in our study, may not have been administered in sufficiently high doses as tachykinins have been shown to be important in mediating moderate to intense pain, and not to mildly painful stimuli[64]. The involvement of the NK-2 and NK-3 receptors in gastrointestinal vagal reflexes has not been studied here as the cost of the non-peptidal antagonists for these receptors is prohibitive.

5.3 Involvement of glutamate in vagal reflexes

5.3.1 NMDA and non-NMDA receptors

My experiments demonstrate that the NMDA receptor is not involved in mediating inputs from oesophageal and gastric mechanoreceptors or from BK-sensitive afferents onto vagal central neurones. NMDA receptor antagonism with CGS19755 was able to modulate a minority of efferent responses to Cap and CCK. By contrast, and in keeping with the prevalence of glutamate as an afferent transmitter, it was not surprising to find that central blockade of non-NMDA receptors was effective in modulating a proportion of efferent responses to all the peripheral stimuli tested. However, like the CGRP1 receptor antagonist hCGRP8-37, CNQX did not reduce or block all responses to a particular stimulus nor did it affect all responses of any single vagal efferent fibre.

Glutamate is the main excitatory amino acid found in the mammalian CNS. Glutamate immunoreactivity is found throughout the NTS, AP, DMVN and hypoglossal

nucleus[97, 229]. A proportion of these NTS afferent terminals also demonstrate co-existence with SP and NKA. Glutamate is released in the NTS in response to electrical stimulation of the vagus[1]. The presence of NMDA receptor subunits has been revealed throughout the brainstem, including on the somata and dendrites of neurones within the NTS, on the membranes of NTS interneurons and on DMVN neurones projecting to the stomach[62, 79]. Most DMVN neurones which express NMDA receptor subunits also coexpress GABAa receptor subunits. Bidirectional transport of the NMDA receptor complex is seen along the vagal nerve trunk, implying that NMDA receptors can be found on all vagally innervated organs[102].

Both non-NMDA and NMDA receptors are implicated in the synaptic transmission of information in the rat brainstem between vagal sensory neurones and vagal motoneurons[264]. They play an important role in the solitarii-ambigual pathway which is essential in generating oesophageal peristalsis in the rat. Oesophageal peristalsis and contractile rhythm generated from the NTS was blocked by the injection of either an NMDA receptor antagonist (AP7) or a non-NMDA receptor antagonist (CNQX) on the compact formation of the NA_c, the main site of vagal central neurones involved with oesophageal motility.

The NMDA receptor is involved in the triggering of transient lower oesophageal sphincter relaxations (TLOSRs) due to gastric distension with air and nutrient[165]. CGS19755, the same antagonist used in my experiments, reduced the number of TLOSRs in the first 90 minutes after administration. However in the first 45 minutes, the number of TLOSRs was increased with CGS19755 in 2 of 5 studies, and reduced in 3 of 5 studies. The NMDA antagonist also reduced the number of contractions simultaneous with TLOSRs but did not affect the duration of the TLOSRs. Because NMDA receptors are located at various sites considered important in the triggering of TLOSRs, the author concluded that it was the balance of negative and positive influences of NMDA receptors in the individual that determined the outcome of NMDA receptor antagonism.

On the other hand, the non-NMDA receptor is heavily involved in the CNS control of respiration and cardiovascular responses[4, 55, 56, 269, 276]. Firstly, injection of NMDA and quisqualic acid (QUIS), NMDA and non-NMDA receptor agonists respectively, into an area of the NTS which contain neurones needed for the production of the Breuer-Hering reflex, produced apnoea which imitated the response to lung inflation[56]. This apnoea could be impaired by injection of either CNQX, the non-NMDA antagonist used in my studies, or kynurenic acid, a broad spectrum excitatory amino acid antagonist. In contrast, AP5, an NMDA receptor antagonist, did not alter the reflex apnoea evoked by lung inflation. In a second study, extracellular recordings were made from single units in the commissural NTS with inputs from vagal cardiopulmonary C fibre endings supplied by pulmonary circulation[269]. These units were activated by phenylbiguanide, a sensory C fibre stimulant which evoked rapid shallow breathing. The activation was mimicked by NMDA and QUIS. Responses to QUIS were blocked by NBQX, a non-NMDA receptor antagonist, and that to NMDA slightly attenuated by AP5. The use of kynurenic acid also abolished responses to both agonists. This implies that non-NMDA receptors play a larger role in the neural transmission of cardiopulmonary afferents in the NTS. The third study investigated the differential roles of non-NMDA and NMDA receptors in the integration of baroreceptor afferent inputs in the NTS[276]. Here, they found that rapid synaptic events are selectively mediated by non-NMDA receptors whereas both non-NMDA and NMDA receptors are involved in slower synaptic events.

The involvement of non-NMDA receptors in primary synaptic transmission is also seen in recordings from medullary slices of rat brainstem where CNQX suppressed excitatory post synaptic potentials (EPSPs)[4]. AP5, on the other hand, only slightly diminished the EPSPs. Taken together, these studies imply that non-NMDA receptors are important in the fast transmision of primary sensory input in the NTS. The NMDA receptor is only likely to play a minor role in this area.

The involvement of non-NMDA receptors, but not NMDA receptors, in my study parallels the work performed in the area of the central control of respiratory and

cardiovascular responses. The inability for central non-NMDA receptor antagonism in the present study to reduce or block all responses to a particular stimulus or affect all responses of any single vagal efferent fibre highlights the heterogeneity and redundancy of multiple transmitters in afferent inputs from the gastrointestinal tract.

5.4 Multiple receptor involvement

To obtain maximum information from each experiment, the effects of more than 1 receptor antagonist on efferent responses to peripheral stimuli were often observed in a single study. This may have led to interactions between more than 1 receptor type.

In experiments where both non-NMDA and CGRP receptors were blocked centrally, most responses to close intraarterially adminstered stimuli were reduced, or in the case of one experiment, were reversed in direction. This implies that neurones involved in mediating inputs from gastrointestinal afferents may contain more than one transmitter. In fact, the involvement of 3 receptors in these vagal reflexes is intimated in the study where the responses to 2 of 4 stimuli were blocked and that to the other 2 were reversed in direction. This change in response from an inhibitory one to one of excitation suggests that both CGRP and non-NMDA receptors were collectively involved in causing the initial decrease in nerve discharge seen in response to both bradykinin and cholecystokinin under control conditions. However, with the central blockade of both receptors, a previously masked excitatory component of the response was revealed. This latent excitatory component may be mediated by the NK-1 receptor, but obviously futher work needs to be done in order to shed more light on this.

Evidence for involvement of multiple receptors can be found in several studies. In one study, administration of either an NMDA or NK-2 receptor antagonist is able to inhibit the capsaicin evoked responses in the dorsal horn *in vitro* by 65%. However, when antagonists to both receptors are perfused simultaneously, the capsaicin evoked depolarisation is almost completely abolished. This suggests that both NMDA and NK-2

receptors may be co-activated by appropriate afferent stimuli and act in concert to generate an effect in the rat dorsal horn[197]. Another study has found that NMDA and GABAa receptors are often colocalised within DMVN neurones of the rat[62]. Still another study has found interactions between substance P and excitatory amino acids. Here, substance P, administered by itself into the apnoea producing region of the NTS, had no effect on basal repiratory patterns. However, when it was administered simultaneously with a broad spectrum excitatory amino acid (EAA), the reflex apnoea produced by the EAA was potentiated by substance P.

5.5 Conclusions

Considering the heterogeneity of receptors present on vagal efferent neurones studied here, it was not surprising that our results could not be categorised neatly. However, the data obtained here is useful in providing a rough guideline in the receptor subtypes involved in mediating the afferent inputs that were investigated. As stated before, although not all responses in any individual fibre was affected by any one receptor antagonists and not all responses to any stimulus was changed by a single receptor antagonist, there were some generalisations that could be ascertained from our data.

These were as follows 1) the best candidate neurotransmitter involved in mediating inputs from vagal oesophageal mechanoreceptors is glutamate, which is likely to exert its effects via the non-NMDA receptor; 2) the majority of vagal afferent inputs from distension-sensitive afferents in the stomach to vagal central neurones appear to be mediated via the M1 muscarinic receptors; 3) non-vagal gastric mechanoreceptors seem to involve CGRP and glutamate as neurotransmitters; 4) bradykinin-sensitive fibres, which are mainly spinal serosal afferents, also seem to act via the non-NMDA and CGRP receptors; 5) CCK-sensitive afferents, which are mainly vagal gastroduodenal mucosal afferents, require glutamate and substance P to act via the NK-1 and non-NMDA receptors respectively; 6) capsaicin, which is supposed to activate all small diameter nerve fibres, probably activates both vagal and non-vagal pathways mainly via the non-NMDA receptor.

The information acquired in this chapter is useful knowledge for future treatment of upper gastrointestinal tract conditions. For example, blocking CGRP receptors may serve to attenuate the transmission of pain-related information arising from the gut. Our data on the heterogeneity of receptors present in the pathway from different populations of afferents means that the antagonism of a particular receptor subtype may not block all inputs from that particular population, but rather attenuate the influence of neurotransmitter action via the specific receptor. This may therefore redress the balance of information transferred from the CNS to the periphery.

At the moment, my work has concentrated on investigating the the influences of various neurotransmitters on vagal reflexes originating from the gut under normal healthy conditions. The next stage would be to examine any changes in neurotransmitter content and function that occurs in animals with upper gastrointestinal conditions such as oesophagitis or dysphagia.

A. Effect of the M1 cholinoceptor antagonist, pirenzepine (2.5-5.0μmol/kg iv), on vagal efferent responses to peripheral stimuli.

Unit ID	Dose	Pretreat	OBD		GD		CCK		BK		Cap	
				%Con		%Con		%Con		%Con		%Con
26/9/96	5.0		+	100	+	0			-	100	-	100
22/10/96	2.5	Methoc.	+	100	+*	30			--	100	--	100
24/10/96	2.5		+	100								
31/10/96	2.5	Methoc.			+*	100			-	100	--	100
21/10/97	5.0	Baclofen, CGP35348, CNQX	+	100	+	50	-	100	-	100	+	100
Total number of fibres tested			**4**		**4**		**1**		**4**		**4**	
Drug Effect		-None	4		1		1		4		4	
		-Reduced >50%Control	0		0		0		0		0	
		-Reduced ≤50%Control	0		2		0		0		0	
		-Blocked or Reversed	0		1		0		0		0	

B. Effect of the M2 cholinoceptor antagonist, methoctramine (7-14μmol/kg iv), on vagal efferent responses to peripheral stimuli.

Unit ID	Dose	Pretreat	OBD		GD		BK		Cap	
				%Con		%Con		%Con		%Con
26/9/96	7	Pirenz	+	100			-	100	-	100
22/10/96	14		+	100	+*	100	--	100	--	100
24/10/96	14	Pirenz	+	100						
31/10/96	7				+*	100	--	100	--	100
Total number of fibres tested			**3**		**2**		**3**		**3**	
Drug Effect		-None	3		2		3		3	
		-Reduced >50%Control	0		0		0		0	
		-Reduced ≤50%Control	0		0		0		0	
		-Blocked or Reversed	0		0		0		0	

Table 3-1 Responses of individual fibres treated with the M1 and M2 cholinoceptor antagonists pirenzepine and methoctramine respectively.
The direction and intensity of response prior to muscarinic receptor antagonism is indicated by the symbol in the grey boxes:
+ denotes 50-100% increase from basal discharge rates; - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes. * denotes corpus distension (20ml saline) was performed instead of whole stomach distension. Doses are given in μmol/kg.

Oesophageal balloon distension (OBD, 1.5ml air) and gastric distension (GD, 40-60ml saline) elicited excitatory responses whereas close intraarterial administration of cholecystokinin (CCK, 100pmol), bradykinin (BK, 18nmol) and capsaicin (Cap, 65nmol) yielded mainly inhibitory responses.

Pirenzepine (2.5-5.0μmol/kg iv) reduced most vagal efferent responses to GD but had no effect on the responses to the other stimuli tested. Methoctramine (7-14μmol/kg iv) did not affect any efferent responses to the stimuli tested.

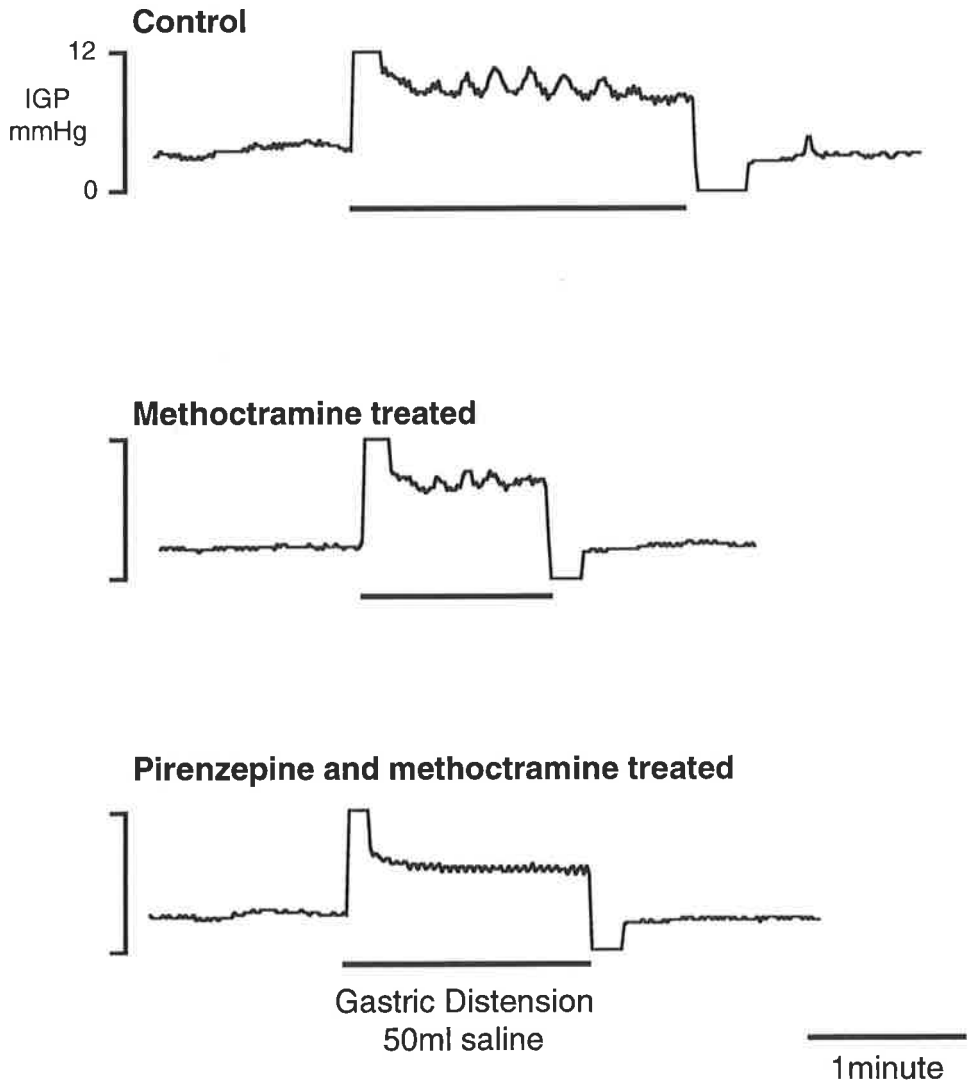


Figure 3-1. Effect of cholinoceptor antagonism on intraluminal pressure during gastric distension.

The intraluminal pressure response during gastric distension (50ml saline) after methoctramine (14μmol/kg iv) was decreased slightly. Subsequent M1 antagonism with pirenzepine (2.5μmol/kg iv) led to a marked attenuation in the intraluminal pressure response to gastric distension. The phasic component of the contractile rhythm (~5/minute) was also abolished after M1 antagonism.

The pressure tracings were truncated at the start and end of the distension period in order to display the changes in intragastric pressure during distension more clearly. The increase at the start of distension was caused by the high positive pressure generated when fluid was infused via the pressure transducer into the stomach. The decrease at the end of the period was due to the negative pressure caused by the rapid removal of gastric fluid with a large syringe.

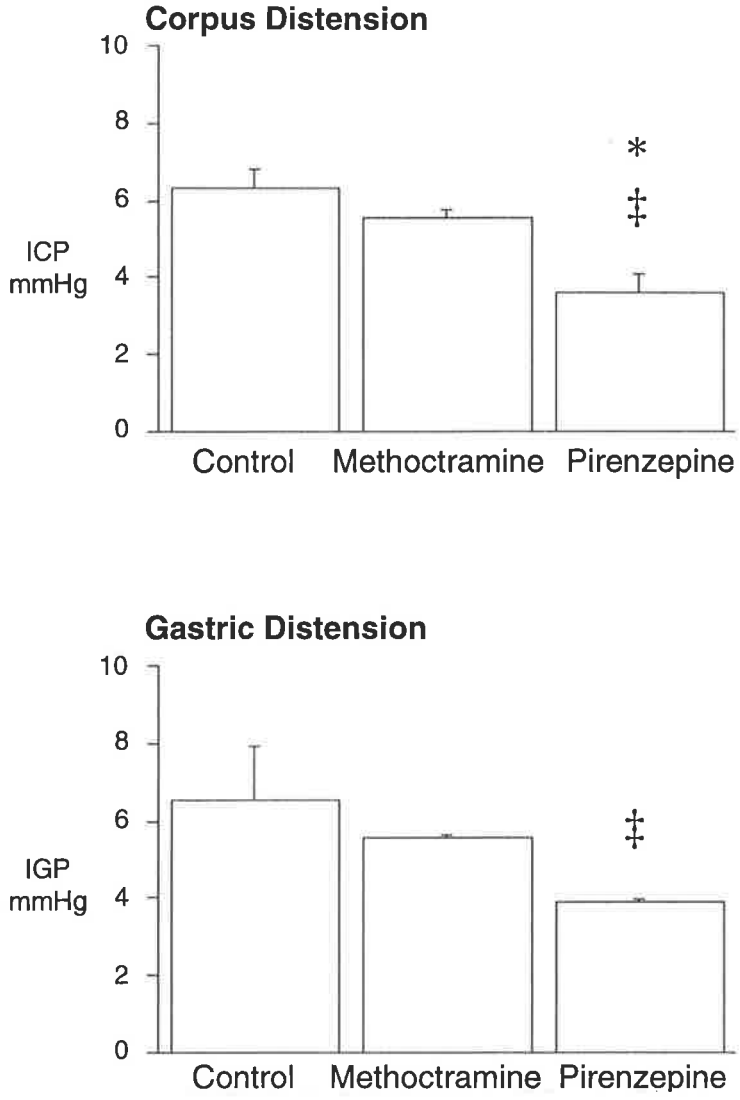


Figure 3-2. Group data representing effect of cholinoceptor antagonism on corpus and gastric pressure during distension.

Both M1 and M2 receptor antagonists, pirenzepine (2.5-5.0μmol/kg iv) and methoctramine (7-14μmol/kg iv) respectively, decreased mean intraluminal pressure during corpus distension (ICP, 20ml saline, n=3) and gastric distension (IGP, 50ml saline, n=2), although it was statistically significant only with pirenzepine.

The effects on intraluminal pressure during distension was specific to the subtype of muscarinic receptor being blocked and was independent of the order of antagonist administration. In the 3 studies where corpus distension was performed, pirenzepine was administered prior to methoctramine in 2/3 studies and the reverse order in the other study. In the 2 studies where whole gastric distension was performed, methoctramine was given before pirenzepine in 1/2 study and the reverse order in the other study.

* $p \leq 0.005$ vs Control using paired t-test
‡ $p \leq 0.05$ vs Methoctramine using paired t-test

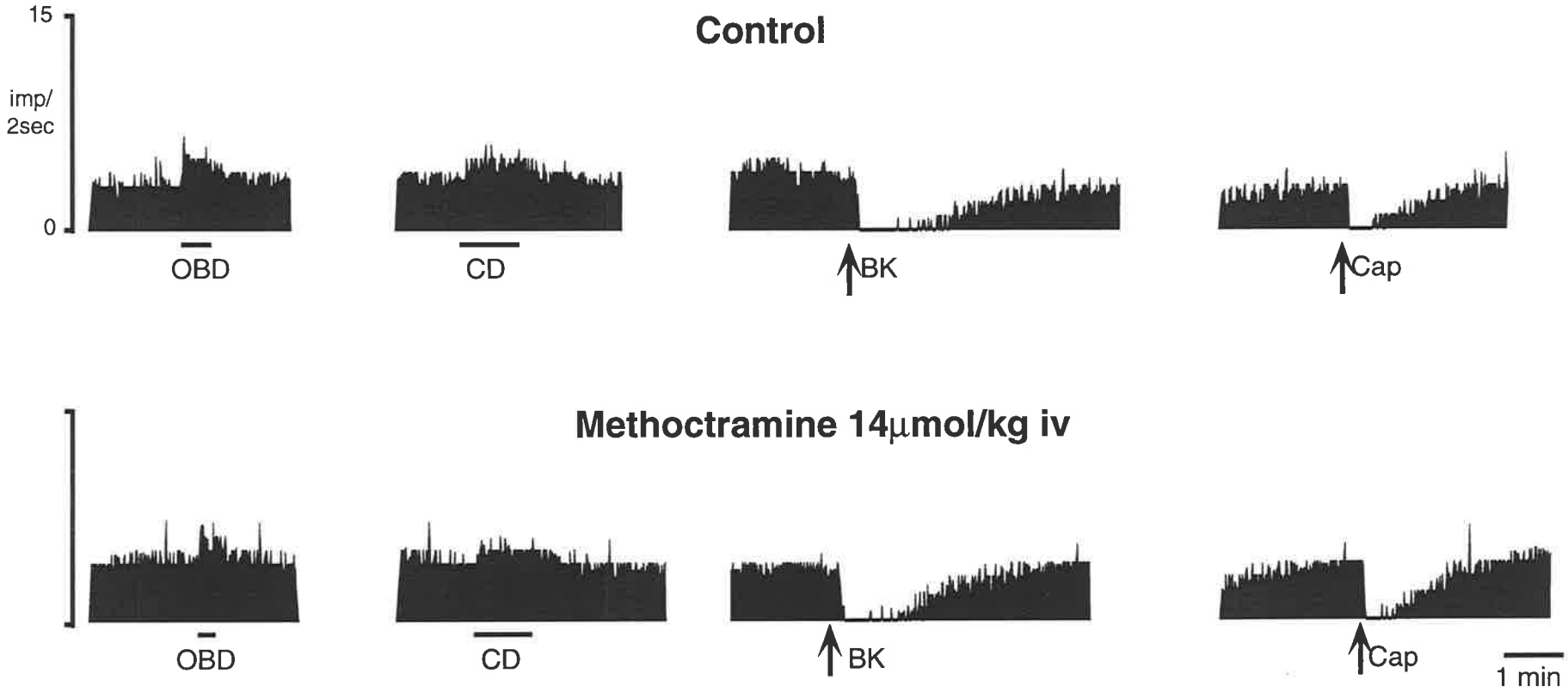


Figure 3-4 Effect of methoctramine on vagal efferent response to peripheral stimuli.
The M2 cholinoceptor antagonist methoctramine was the first treatment administered in this study.
Corpus distension (CD, 20ml saline) and oesophageal balloon distension (OBD, 2ml air) evoked excitation of discharge while bradykinin (BK, 18nmol close ia) and capsaicin (65nmol close ia) evoked inhibition of efferent discharge.

None of the efferent responses to these peripheral stimuli were affected by methoctramine (14μmol/kg iv).

Unit ID	Dose	Pretreat	OBD		GD		CCK		BK		Cap	
				%Cont		%Cont		%Cont		%Cont		%Cont
20/2/97	3.2		+	100	+*	35						
6/3/97	3.2	CNQX	+	100			+	30	++	70		
18/7/97	3.2		+	100	+	100	-	100	-	20		
28/7/97	3.2		-	100	-*	100	+	100	++	60	++	100
29/7/97	3.2		+	35	+*	15	-	100	-	100	-	100
6/8/97	3.2						-	100	-	0	-	100
11/8/97	3.2		+	30			-	0	-	100		
12/8/97	6.4				+*	0	-	100	-	0	-	0
9/10/97	3.2	Baclofen, CGP35348			+	50	-	100	-	100	-	100
16/10/97	3.2	Baclofen,CGP35348					-	100	-	100	-	100
21/10/97	3.2	Bac, CGP35348, CP99994, CNQX, Pirenzepine			+	100			+	100	+	100
Total number of fibres tested			**6**		**7**		**9**		**10**		**7**	
Drug Effect		-None	4		3		7		5		6	
		-Reduced >50%Control	0		0		0		2		0	
		-Reduced ≤50%Control	2		3		1		1		0	
		-Blocked or Reversed	0		1		1		2		1	

Table 3-2. Responses of individual fibres treated with the CGRP receptor antagonist hCGRP$_{8-37}$.
The direction and intensity of response prior to CGRP receptor antagonism is indicated by the symbol in the grey boxes:
+ denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes; rev indicates a reversal in the direction of response after treatment. * indicates that corpus distension (20ml saline) was performed instead of whole stomach distension. Doses are given in nmol/kg.

Oesophageal balloon distension (OBD, 1.5ml air), and gastric distension (GD, 40-60ml saline) elicited mainly excitatory responses whereas close intraarterial administration of cholecystokinin (CCK, 100pmol), bradykinin (BK, 18nmol), and capsaicin (Cap, 65nmol) yielded mainly inhibitory responses.

hCGRP8-37 (3.2-6.4nmol/kg icv) reduced a proportion of efferent responses to all peripheral stimuli tested.

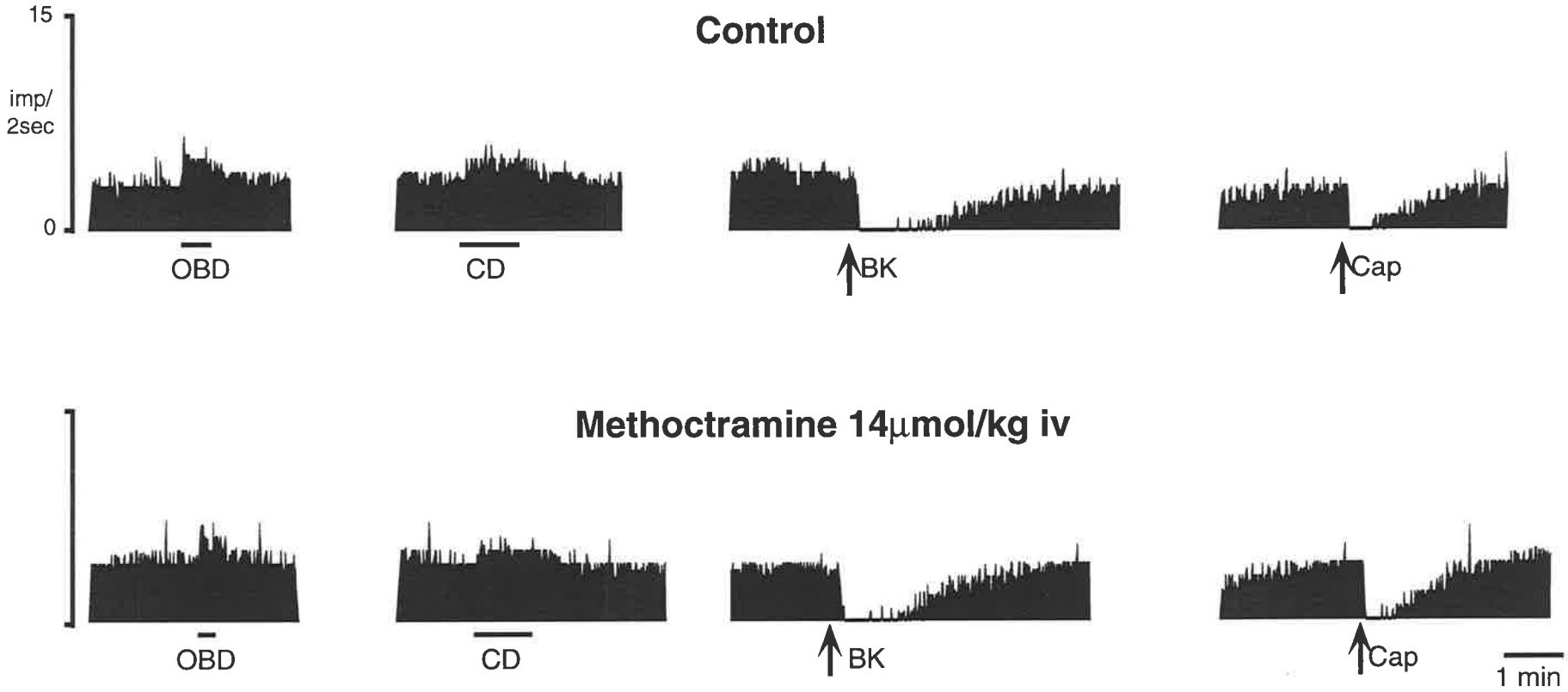


Figure 3-4 Effect of methoctramine on vagal efferent response to peripheral stimuli.
The M2 cholinoceptor antagonist methoctramine was the first treatment administered in this study.
Corpus distension (CD, 20ml saline) and oesophageal balloon distension (OBD, 2ml air) evoked excitation of discharge while bradykinin (BK, 18nmol close ia) and capsaicin (65nmol close ia) evoked inhibition of efferent discharge.

None of the efferent responses to these peripheral stimuli were affected by methoctramine (14μmol/kg iv).

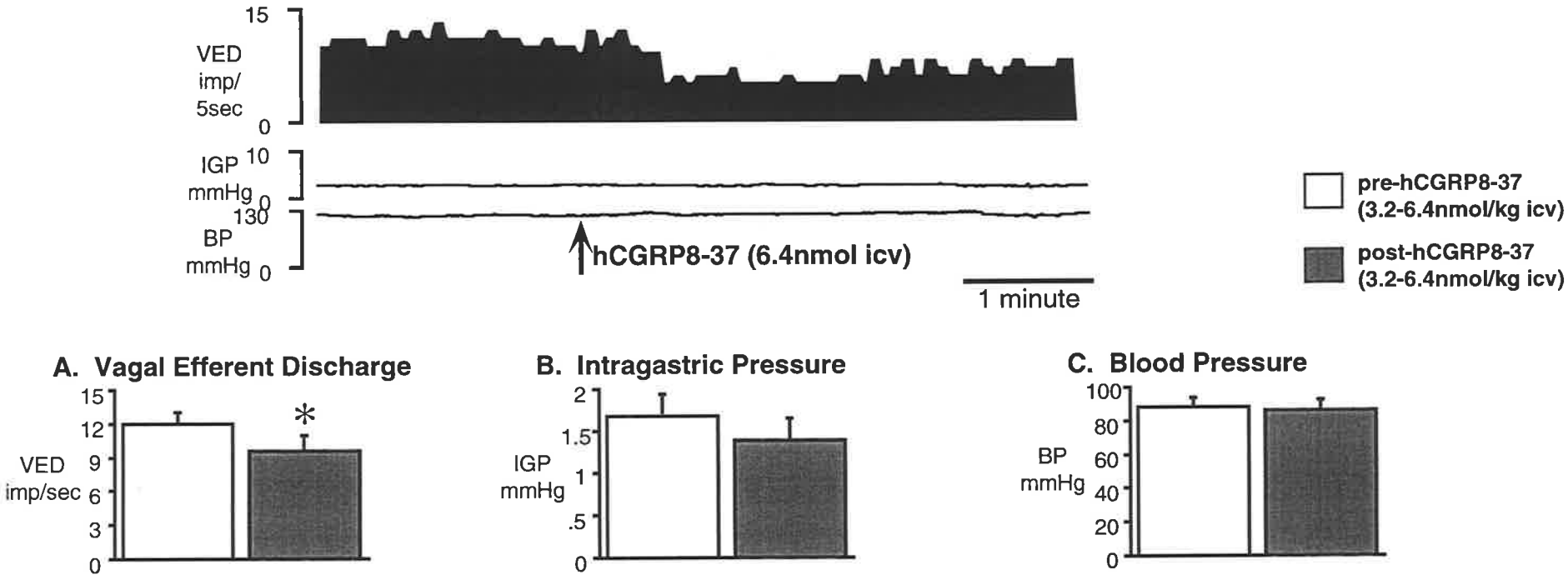


Figure 3-5. Effects of CGRP receptor antagonism on basal efferent discharge, intragastric pressure, and blood pressure.
Original tracing shows that administration of hCGRP8-37 (6.4nmol icv) led to a decrease in basal efferent discharge (top trace) but did not affect either intragastric pressure (middle trace) or blood pressure (bottom trace).

Efferent discharge, intragastric pressure and blood pressure measurements were taken immediately before and after administration of the CGRP receptor antagonist hCGRP8-37 (3.2-6.4nmol icv, n=13). * $p<0.05$ vs before CGRP receptor antagonism.

A. Basal efferent discharge was significantly reduced by administration of hCGRP8-37.
B-C. Blood pressure and gastric pressure were unaffected by administration of hCGRP8-37.

Unit ID	Dose	Pretreat	OBD		GD		ACID		CCK		BK		Cap	
				%Con		%Con		%Con		%Con		%Con		%Con
12/5/94	8				+	100	-	100						
18/5/94	8		+	100	-	60	+	100						
23/6/94	8		-	100	-	100	-	100						
7/6/94	8				++	100								
14/7/94	8		+	100	+	100	+	100						
3/8/94	8		+	100	+	100	+	100			-	100	-	100
24/8/94	8		+	100	+	100	+	100						
14/9/94	8		+	100	+	100	+	100						
21/9/94	8		+	100	+	60					-	100	-	100
23/9/94	8		+	100	-	100					-	100		
7/10/94	8		+	100	+	100					-	100	+	100
31/10/94	8		-	100	-	100	-	100					-	100
8/11/94	8		+	100	+	100					+	100	+	100
15/11/94	8		+	100	+	100	+	100						
3/1/95	8		+	100	+	100	+	100						
6/1/95	8		+	100	+	100	+	100			+	100	+	100
15/8/95	12*				+	100								
23/8/95	12*		+	100	+	100					-	100		
6/8/97	12*	hCGRP8-37							-	20			-	20
18/9/97	12*	CGS19755							-	100	-	100		
21/10/97	12*	Baclofen, CGP35348	+	100	+	100			-	100	-	100	+	100
Total number of fibres tested			**16**		**19**		**11**		**3**		**9**		**8**	
Drug Effect		-None	16		17		11		2		9		7	
		-Reduced >50%Control	0		2		0		0		0		0	
		-Reduced ≤50%Control	0		0		0		0		0		1	
		-Blocked or Reversed	0		0		0		1		0		0	

Table 3-3. Responses of individual fibres treated with the NK-1 receptor antagonists CP-96345 and CP-99,994.
The direction and intensity of response prior to NK-1 receptor antagonism is indicated by the symbol in the grey boxes:
+ denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes. *indicates that CP99994 was used; CP96345 was administered in all other studies. Doses are given in μmol/kg.

Oesophageal balloon distension (OBD, 1-2ml air), gastric distension (GD, 40-60ml saline) and oesophageal acidification (ACID, 2ml 150mM HCl) elicited mainly excitation whereas close intraarterial administration of cholecystokinin (CCK, 100pmol), bradykinin (BK, 18nmol), and capsaicin (Cap, 65 nmol) yielded mainly inhibition of discharge.

Neither CP96345 (8μmol/kg iv) nor CP99994 (12μmol/kg iv) had an effect on the majority of responses to any of the stimuli tested.

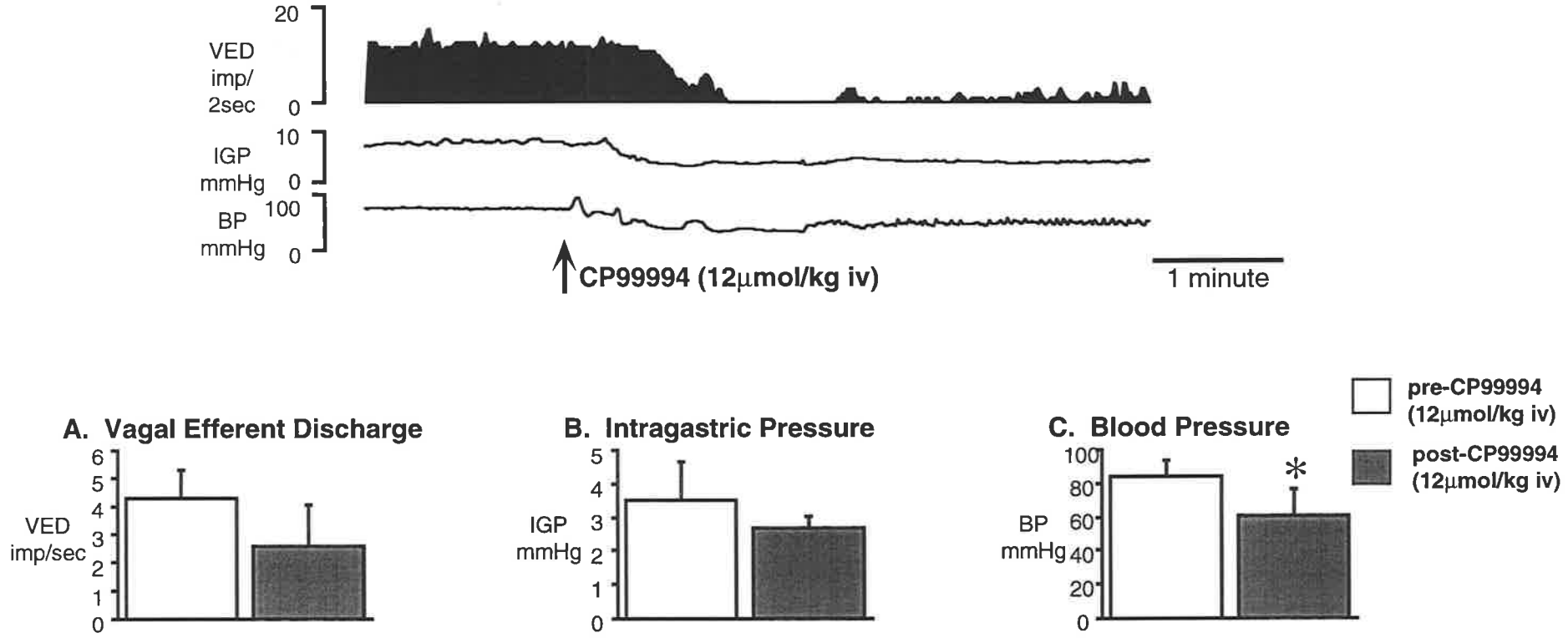


Figure 3-7. Effects of NK-1 receptor antagonism on basal efferent discharge, intragastric pressure, and blood pressure.
In the original tracing, administration of CP99994 (12μmol/kg iv) led to a decrease in basal efferent discharge (upper trace), intragastric pressure (middle trace), and blood pressure (lower trace).

Efferent discharge, intragastric pressure and blood pressure measurements were taken immediately before and after administration of the NK-1 receptor antagonist CP99994 (12μmol/kg iv, n=5). * $p<0.05$ vs before NK-1 receptor antagonism

A-B. Basal efferent discharge and mean gastric pressure were inhibited by CP99994, although neither was significantly reduced.
C. Blood pressure was significantly attenuated by CP99994.

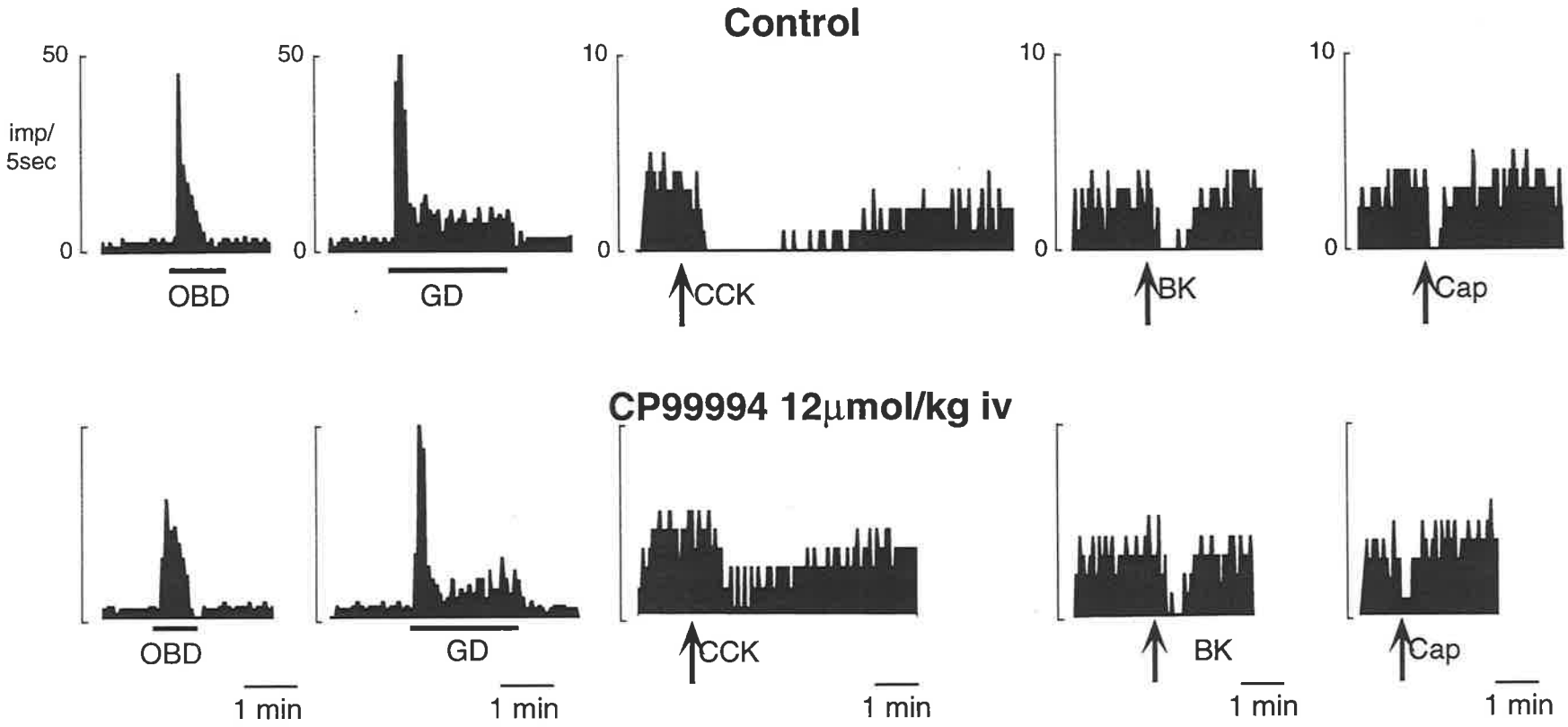


Figure 3-8 Effect of CP99994 on vagal efferent responses to peripheral stimuli.
In this study, baclofen and CGP35348 were administered prior to CP99994.
The inhibition of efferent discharge due to cholecystokinin (CCK, 100pmol, close ia) was attenuated by CP99994 (12μmol/kg iv).

The efferent responses to oesophageal balloon distension (OBD, 2ml air), gastric distension (GD, 60ml saline), bradykinin (BK, 18nmol close ia) and capsaicin (65nmol close ia) were unaffected by CP99994.

Unit ID	Dose	Pretreat	OBD		GD		CCK		BK		Cap	
				%Con		%Con		%Con		%Con		%Con
16/12/96	13						-	100				
17/12/96	13						-	50	-	100	-	100
29/11/96	13		+	100	+*	100	+	100	+	100	+	0
8/7/97	13		+	100			-	80	-	100		
18/9/97	13						-	100	-	100		
4/11/96	13						+	100	+	100	+	100
2/2/96	13		+	100	++	100						
Total number of fibres tested			**3**		**2**		**6**		**5**		**3**	
Drug Effect		-None	3		2		4		5		2	
		-Reduced >50%Control	0		0		1		0		0	
		-Reduced ≤50%Control	0		0		1		0		0	
		-Blocked or Reversed	0		0		0		0		1	

Table 3-4. Responses of individual fibres treated with the NMDA receptor antagonists CGS19755.
The direction and intensity of response prior to NMDA receptor antagonism is indicated by the symbol in the grey boxes:
+ denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes. * denotes corpus distension (20ml saline) was performed instead of whole stomach distension. Doses are given in µmol/kg.

Oesophageal balloon distension (OBD, 1.5ml air), gastric distension (GD, 40-60ml saline) and capsaicin (Cap, 65nmol) elicited mainly excitatory responses whereas close intraarterial administration of cholecystokinin (CCK, 100pmol) and bradykinin (BK, 18nmol) yielded mainly inhibitory responses.

CGS19755 (13µmol/kg iv) had no effect on the responses to OBD, GD, and BK, although it reduced the response to CCK in 2/6 fibres and abolished to Cap in 1/3 fibres tested.

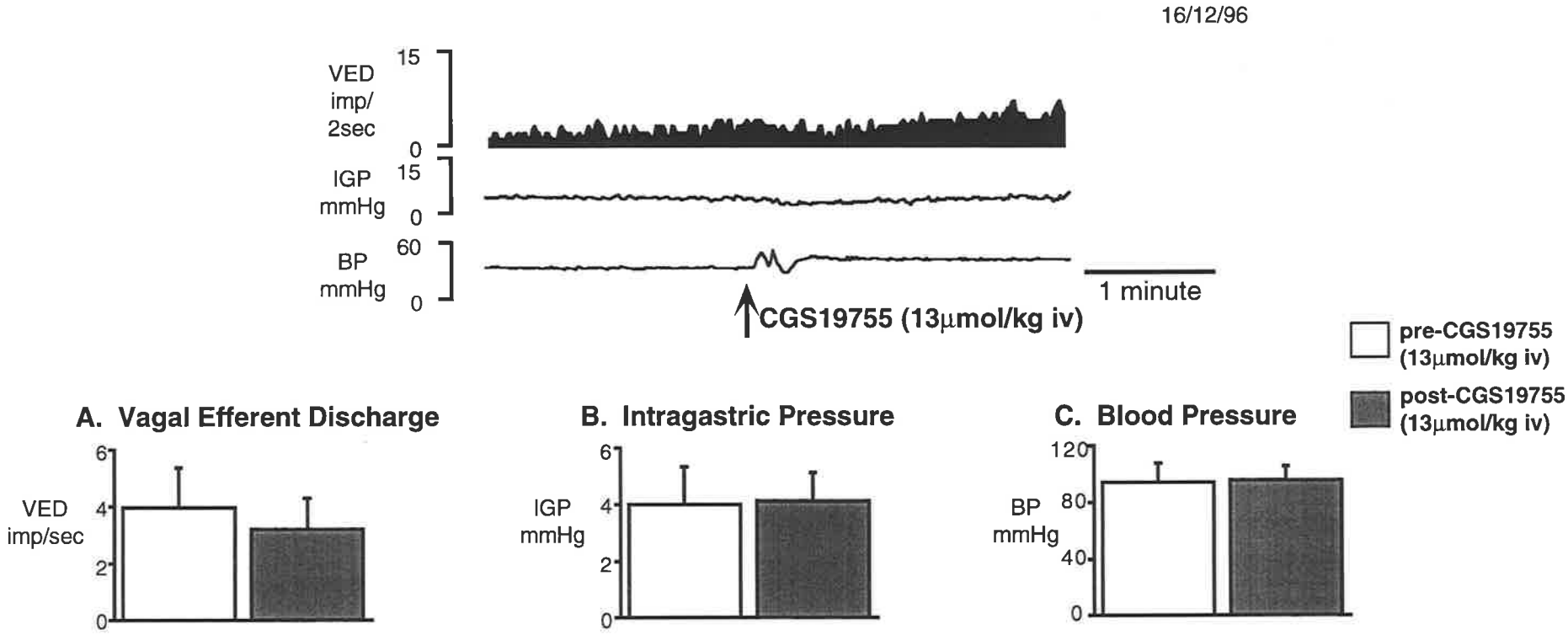


Figure 3-9. Effects of NMDA receptor antagonism on basal efferent discharge, intragastric pressure, and blood pressure. In the original tracing, administration of CGS19755 (13μmol/kg iv) led to an increase in basal efferent discharge (top trace) and blood pressure (bottom trace) and a transient decrease in gastric pressure (middle trace) in this study.

Efferent discharge, intragastric pressure and blood pressure measurements were taken immediately before and after administration of the NMDA receptor antagonist CGS19755 (13μmol/kg iv, n=8).

A-C. Efferent discharge, gastric pressure and blood pressure were unaffected by NMDA receptor antagonism.

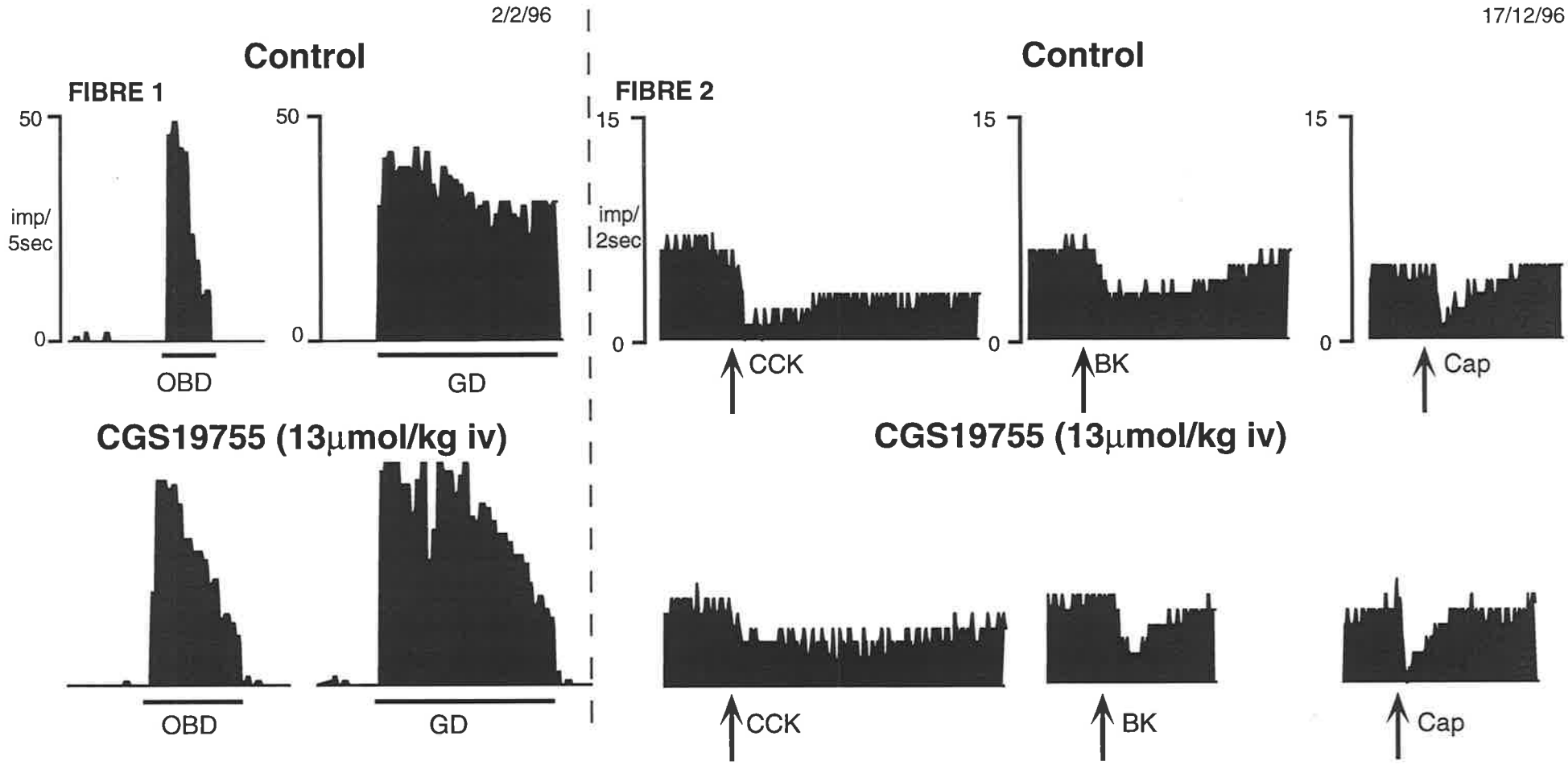


Figure 3-10 Effect of CGS19755 on the response of 2 vagal efferent fibres to different peripheral stimuli.

The first efferent fibre responded with excitation to oesophageal balloon distension (OBD, 2ml air) and gastric distension (GD, 60ml saline). The second efferent fibre responded with inhibition to cholecystokinin (CCK, 100pmol, close ia), bradykinin (BK, 18nmol close ia) and capsaicin (65nmol close ia).

CGS19755 (13μmol/kg iv) was the first treatment administered in these studies. The efferent responses to OBD and GD were enhanced and to CCK was attenuated. The responses to the other stimuli tested were unaffected.

Unit ID	Dose	Pretreat	OBD		GD		CCK		BK		Cap	
				%Cont		%Cont		%Cont		%Con		%Cont
20/6/97	75		+	50	+*	50	++	100	+	100		
6/3/97	75		+	100			++	100	++	100		
16/6/97	75		+	0	+*	20						
14/7/97	75		+	50	+	50						
22/5/97	75		+	0								
18/7/97	155	hCGRP8-37	+	0	+	0	--	rev	-	rev		
28/7/97	75	hCGRP8-37	--	100	--*	100	++	0	+	0	+	0
29/7/97	75	hCGRP8-37	++	100			--	0	--	100	--	0
20/2/97	75	hCGRP8-37	+	100	+*	100						
9/10/97	75	Baclofen, CGP35348, hCGRP8-37			+	0	---	50	--	50	-	20
14/10/97	75	Baclofen, CGP35348	+	50	--	15	--	50	-	0	-	
21/10/97	155	Baclofen, CGP35348, CP99994	+	30	+	100	--	45	+	100	-	100
Total number of fibres tested			**11**		**9**		**8**		**8**		**4**	
Drug Effect		-None	4		3		2		4		1	
		-Reduced >50%Control	0		0		0		0		0	
		-Reduced ≤50%Control	4		4		3		1		1	
		-Blocked or Reversed	3		2		3		3		2	

Table 3-5. Responses of individual fibres treated with the non-NMDA receptor antagonist CNQX.
The direction and intensity of response prior to non-NMDA receptor antagonism is indicated by the symbol in the grey boxes:
+ denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes; --- denotes a complete inhibition lasting >5minutes; rev indicates a reversal in the direction of response after treatment; * denotes that corpus distension (20ml saline) was performed instead of whole stomach distension. Doses are given in nmol/kg.

Oesophageal balloon distension (OBD, 1.5ml air) and gastric distension (GD, 40-60ml saline) elicited mainly excitatory responses whereas close intraarterial administration of cholecystokinin (CCK, 100pmol), and capsaicin (Cap, 65 nmol) yielded mainly inhibitory responses.

CNQX (75-155nmol/kg icv) reduced a proportion of efferent responses to all stimuli tested.

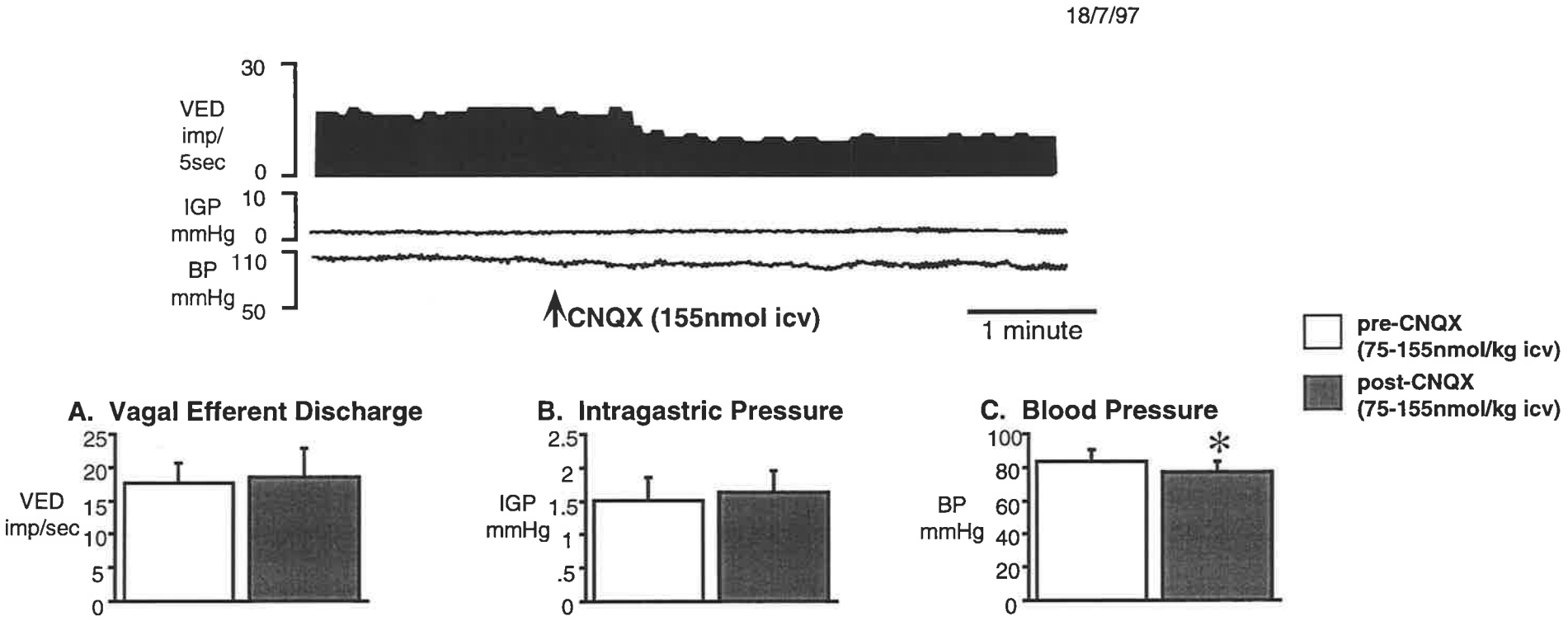


Figure 3-11. Effects of non-NMDA receptor antagonism on basal efferent discharge, intragastric pressure, and blood pressure. In the original tracing above, administration of CNQX (155nmol icv) led to a decrease in basal efferent discharge (top trace) and blood pressure (bottom trace). Gastric pressure (middle trace) was unaffected in this study.

Efferent discharge, intragastric pressure and blood pressure measurements were taken immediately before and after administration of the non-NMDA receptor antagonist CNQX (75-155nmol/kg icv, n=11). * $p \leq 0.01$ vs pre-CNQX using paired t-test.

A-B. Basal efferent discharge and intragastric pressure were unaffected by CNQX.
C. Blood pressure was significantly attenuated by CNQX.

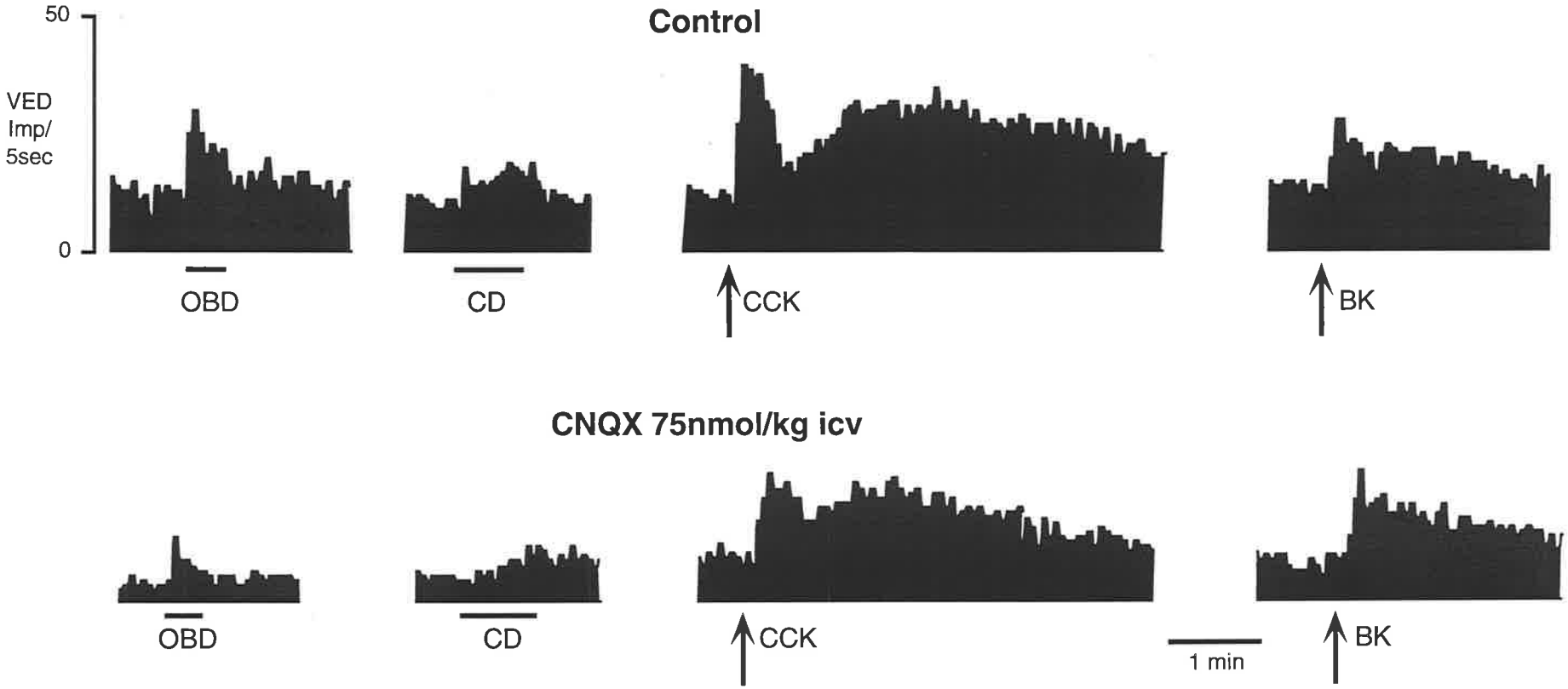


Figure 3-12 Effect of CNQX on vagal efferent response to peripheral stimuli.
CNQX was the first treatment administered in this study.
The increases in efferent discharge due to oesophageal balloon distension (OBD, 2ml air) and corpus distension (CD, 20ml saline) were reduced by CNQX (75nmol/kg icv).

The efferent responses to bradykinin (BK, 18nmol close ia) and cholecystokinin (100pmol close ia) were unaffected by CNQX.

Table 3-6. Effects of hCGRP8-37 and CNQX on vagal efferent responses to peripheral mechanical, chemical, and pharmacological stimuli.
The direction and intensity of efferent response under control conditions is indicated by these symbols: + denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase; -- denotes a total inhibition.

The effect of each drug treatment on the efferent response to each peripheral stimulus is indicated by the symbols: ↔ no change in efferent response, ↓ attenuation in efferent response, ⇊ total abolition of the efferent response, ℜ reversal of the direction of the efferent response, **n/t** response was not tested.

The responses of 5 vagal efferent fibres to oesophageal balloon distension (OBD, 1.5-2ml air), gastric distension (GD, 40-60ml saline, * indicates that corpus distension with 20ml saline was performed), cholecystokinin (CCK, 100pmol close ia), bradykinin (BK, 18nmol close ia) and capsaicin (Cap, 65 nmol close ia) were tested under control conditions and after each drug treatment.

In Study 1, CNQX (non-NMDA receptor antagonist) was administered prior to hCGRP8-37 (CGRP receptor antagonist). The order of receptor antagonism was reversed in the other 4 studies.

The efferent responses in Study 1 were unaffected by CNQX. Subsequent hCGRP8-37 administration reduced the responses to CK and CCK but did not change responses to OBD and GD.

The responses of 4/5 fibres to mechanical stimuli were unchanged by CNQX. In the remaining fibre, responses to OBD and GD were abolished. In this fibre, the direction of response to CCK and BK were reversed after the addition of CNQX. The responses of this fibre are illustrated in Figure 3-13.

STUDY 1 (6/3/97)

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	+	0	++	++	0
CNQX (75nmol/kg icv)	↔		↔	↔	
hCGRP8-37 (3.2nmol/kg icv)	↔		↓	↓	

STUDY 2 (18/7/97)

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	+	+*	- -	- -	
hCGRP8-37 (3.2nmol/kg icv)	↔	↔	↔	↓	
CNQX (155nmol/kg icv)	⇊	⇊	ℜ	ℜ	

STUDY 3 (28/7/97)

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	- -	- -*	+	++	++
hCGRP8-37 (3.2nmol/kg icv)	↔	↔	↔	↓	↔
CNQX (75nmol/kg icv)	↔	↔	⇊	⇊	⇊

STUDY 4 (29/7/97)

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	+	+	- -	- -	- -
hCGRP8-37 (3.2nmol/kg icv)	↓	↓	↔	↔	↔
CNQX (75nmol/kg icv)	↔	n/t	⇊	↔	⇊

STUDY 5 (20/2/97)

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	+	+*	n/t	0	n/t
hCGRP8-37 (3.2nmol/kg icv)	↔	↓			
CNQX (75nmol/kg icv)	↔	↔			

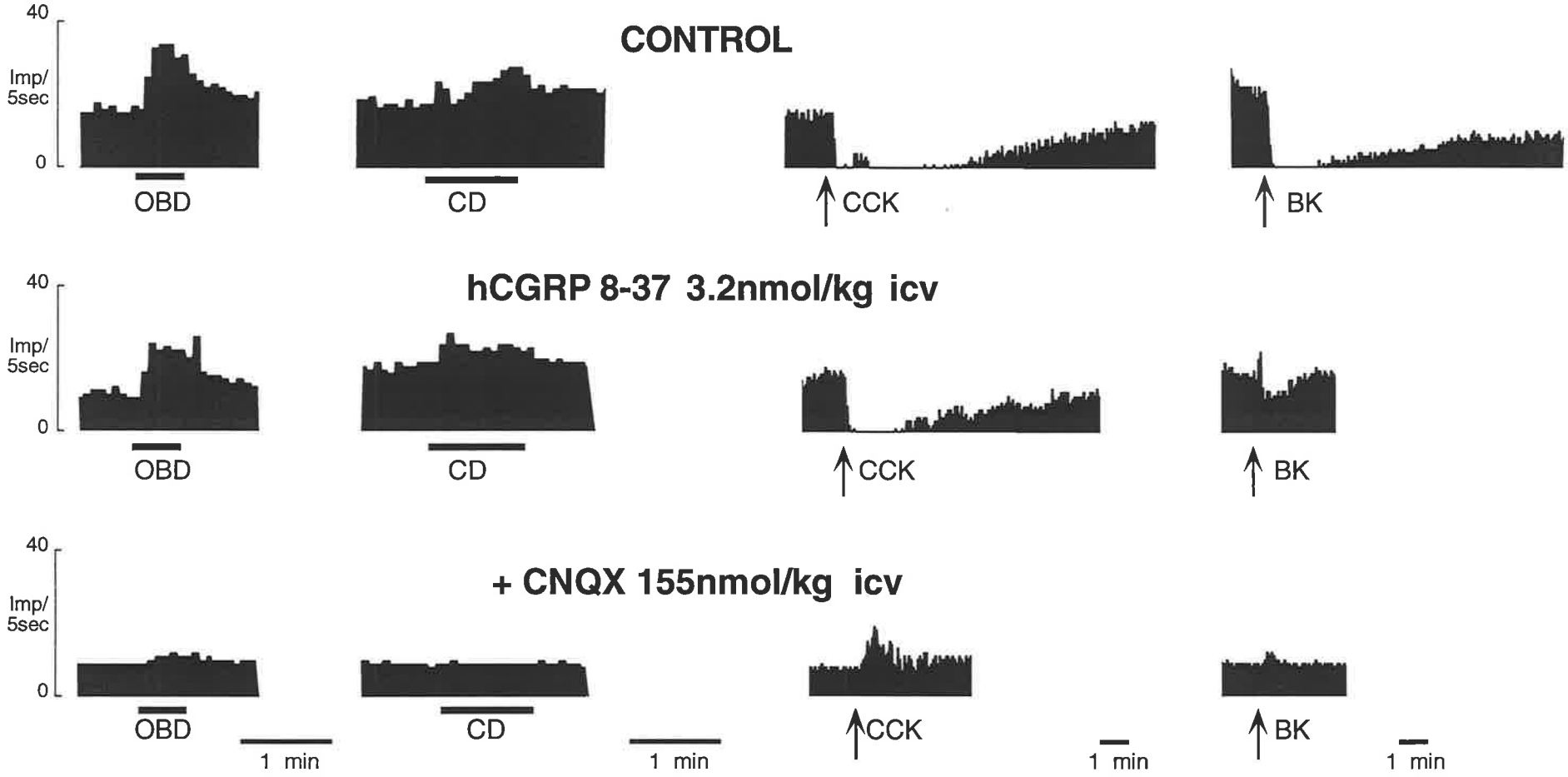


Figure 3-13. Effects of consecutive administration of hCGRP8-37 and CNQX on a vagal efferent response to peripheral stimuli. CGRP receptor antagonism with hCGRP8-37 reduced the inhibitory response to bradykinin (BK, 18nmol close ia) and shortened the period of complete inhibition in response to cholecystokinin (CCK, 100pmol, close ia) but did not affect the efferent response to any other stimuli. Subsequent non-NMDA receptor antagonism with CNQX attenuated the oesophageal balloon distension (OBD, 2ml air) response, abolished the corpus distension (CD, 20ml saline) response and reversed the direction of response to CCK and BK.

CHAPTER 4 Effects of $GABA_B$ receptor ligands on vagal afferent responses to gastro-oesophageal stimuli

1 SUMMARY

1. The potential involvement of the peripheral GABAb receptors was investigated in single unit recordings of vagal tension and mucosal receptors arising from the gastro-oesophageal region of the urethane anaesthetized ferret.

2. The endings of the tension receptors were in the oesophagus (n=2), corpus, (n=7), and the antrum (n=1). These responded with a sustained increase in discharge to distension. Gastric tension receptors were classified according to their pattern of discharge during distension as being either phasic (n=3) or tonic (n=4) in nature. The discharge pattern of remaining gastric tension receptor during distension had both phasic and tonic components to its rhythm. Baclofen (14μmol/kg iv) left the responses of the oesophageal and phasic gastric tension receptors to distension unaffected but abolished the response of 2/4 tonic gastric tension receptors. This abolition was reversed by CGP35348 (100μmol/kg iv) in both studies. Baclofen also abolished the tonic component of the gastric tension receptor response which had both phasic and tonic components to its rhythm.

3. Intraluminal pressure was recorded during gastric distension. Baclofen significantly decreased gastric compliance, resulting in a higher gastric pressure during distension. This was reversed with CGP35348. The corpus tension receptor discharge/ intraluminal pressure relationship during distension was significantly enhanced after baclofen and partially reversed after CGP35348.

4. The ending of 1 mucosal afferent was in the corpus while the receptive field of the second mucosal fibre was unidentified. These mucosal receptors responded to mucosal stroking (n=1) and cholecystokinin (CCK, n=2). Baclofen (14μmol/kg iv) potentiated the mucosal receptor responses to CCK in both fibres.

5. In conclusion, baclofen potentiates mucosal receptor sensitivity to CCK and selectively modulates the sensitivity of those corpus tension receptors with a tonic pattern of response to distension. It does not affect oesophageal tension receptors or gastric tension

receptors which have a predominantly phasic response to distension. The effect of GABAb ligands on afferent sensitivity is independent of the effects on gastric compliance.

2 INTRODUCTION

γ-amino butyric acid (GABA), the main inhibitory amino acid in the CNS, is also found peripherally in the gut. It has been found located within both mucosal and muscle layers of the gastrointestinal tract. Immunoreactive fibres have been identified in the circular muscle: these are thought to originate from neurones in the myenteric plexus as immunopositive fibres were seen to run from the plexus into the circular muscle layer. GABAergic neurones have also been identified in the myenteric ganglia of the rat and guinea pig stomach, duodenum, ileum and colon where GABA acts as a neurotransmitter in the enteric nervous system [140]. In the rat gastric mucosa, GABA is colocalised with gastrin in G-type endocrine cells[89]. It is also found in somatostatin producing D-type endocrine cells[161] and in serotonin producing enterochromaffin cells[89].

Sensory neurones in the nodose ganglion, spinal dorsal root ganglion and the trigeminal ganglion of the rat also displayed GABA-immunoreactivity[252]. Receptors for GABA are also found within the nodose ganglion: these are presumably transported along the axons[139]. Ligation of the vagus nerve distal to the nodose ganglion resulted in an increase in GABA immunoreactivity in the neuronal somata of the ganglion as well as in the nerve fibres on the ganglionic side of the ligature. This increase in GABA immunoreactivity is paralleled by an increase of 2 other neurotransmitters, CGRP and substance P, which were accumulated at the same rate as GABA. This implies that GABA, most probably synthetised within the nodose ganglion, is transported peripherally along the vagus to the peripheral terminals of sensory neurones.

High affinity uptake sites for GABA have been identified in GABA immunoreactive cells in the rat gastric and small intestinal mucosa using autoradiography although these appeared to be associated with endocrine cells, and not with nerve fibres[149]. GABA

receptors sites have been identified in the cell soma of the nodose ganglion[139] and in the central terminals of vagal sensory afferents[244]. However, they have not yet been identified in the peripheral terminals of vagal sensory neurones.

There have been functional studies which have investigated the peripheral involvement of the GABAb receptor. In one of these studies[25], the GABAb receptor was thought to modulate the non-adrenergic, non-cholinergic (NANC) bronchoconstriction evoked by peripheral vagal stimulation, possibly through the inhibition of peptide release. Both GABA and baclofen, administered systemically, dose dependently inhibited the neurally-evoked NANC bronchoconstriction but not the bronchoconstriction generated by exogenous substance P. The GABAa antagonist bicuculline did not affect the inhibitory effect produced by GABA. This suggects that the GABAb receptor is involved in the NANC bronchoconstriction response evoked by vagal stimulation. In other studies, the involvement of GABAb receptors in the cough reflex was investigated[49, 50]. Here, the cough reflex was generated either by exposing guinea pigs to capsaicin aerosal or probing the intrathoracic trachea of cats with a thin cannula. Systemic administration of the 2 GABAb receptor agonists used, baclofen and APPi, inhibited the cough reflex in both models. The effects of baclofen was presumed to be centrally mediated was APPi was presumed to be peripherally mediated as central APPi did not modulate the cough reflex. However, baclofen was effective administered both centrally and systemically. Thus, it was proposed that both peripheral and central GABAb receptors were thought to be involved in antitussive effects, ie inhibition of the cough reflex.

However, to date no work has been performed to investigate the involvement of peripheral GABAB receptors within the gastrointestinal tract. In this chapter, the role of the peripheral GABAb receptors in modulating the sensitivity of vagal gastrointestinal tension and mucosal afferents in the *in vivo* urethane anaesthetised ferret was investigated in detail.

3 RESULTS

3.1 Patterns of spontaneous activity

Recordings were made from 10 tension receptors and 2 mucosal receptors. Tension receptors responded with a sustained increase in discharge upon distension of the region where the receptive field of the fibre was located. The 2 mucosal receptors were silent in the absence of any intentional stimuli, did not show a sustained response with gastric distension, and responded to cholecystokinin. The receptive field of one mucosal fibre was identified mechanically whereas the identity of the second fibre as a mucosal receptor could only be inferred from its powerful response to CCK.

The basal discharge of the 2 oesophageal tension receptors was modulated by the respiratory rhythms of the animal. These respiratory patterns were not evident when a higher level of discharge was elicited during oesophageal balloon distension. The 7 corpus tension receptors had an irregular low level spontaneous discharge (4.21imp/sec, range 0.45-9.32 imp/sec). Administration of the $GABA_b$ receptor agonist baclofen (7-14µmol/kg iv) led to a significant decrease in the basal level of discharge ($p<0.05$) (Figure 4-1). Subsequent administration of either GABAb receptor antagonist CGP36742 or CGP35348 (100µmol/kg iv) reversed the level of basal discharge to one similar to control levels. The discharge of the single antral tension receptor in the absence of any stimulus was phasic in nature and was unaffected in the presence of baclofen (14µmol/kg iv).

3.2 Oesophageal tension receptor sensitivity

The receptive fields of the 2 oesophageal tension receptors were located in the distal 5 cm of the oesophagus, as seen by the change in discharge when the oesophageal assembly was moved up and down the oesophageal body.

Oesophageal balloon distension (OBD, 1.5ml air) caused a sustained increase in discharge levels in the fibre illustrated in Figure 4-2. The pattern of response has been described in detail in Chapter 1, section 4.2.1. Baclofen administration (14µmol/kg iv) did

not affect the OBD response of this fibre, even after the balloon was inflated with an additional 0.5ml air. The response of the second oesophageal tension receptor to OBD before and after baclofen was similar to the responses described above.

Thus, administration of the $GABA_b$ receptor agonist baclofen (14μmol/kg iv) did not affect the responses of 2/2 oesophageal tension receptors to OBD (Table 4-1a).

3.3 Antral tension receptor sensitivity

The receptive field of one vagal tension receptor was located in the antrum. In Figure 4-3, changes in vagal afferent activity and intraluminal pressure were recorded when the antrum was distended with saline in 1ml increments to a total of 5mls. Distension led to an increase in discharge which was phasic in nature and could be correlated roughly with the pattern of antral motility as evidenced by intraluminal pressure recordings. When baclofen (14μmol/kg iv) was administered and the distension repeated, no discernible change in the intensity and pattern of afferent discharge could be seen, although the intraluminal pressure for a given volume of distension was increased.

3.4 Corpus tension receptor sensitivity

The responses of 7 corpus tension receptors to distension were studied. 6 of these afferents had endings located in the proximal part of the corpus, while the remaining 1 was located within the incisura angularis of the corpus.

Corpus distension (CD) was administered either as a single bolus (15-20ml saline) or in 5ml increments for a maximum of 20ml. CD in the fibre illustrated on Figure 4-4 led to a sharp increase in afferent discharge during the dynamic phase of inflation. Discharge then decreased to a lower level during the static component of the distension period, although this second level was still above the resting level. The sustained response was roughly correlated with pattern of corpus motility as evidenced by the intraluminal pressure

recordings. Aspiration of saline from the corpus led to a brief inhibition of discharge before it returned to resting levels.

Gastric tension receptor responses to distension were classified according to their pattern of discharge during the maintained (or static) phase of the response. When the discharge rate at the nadir of the response to distension was >50% of the mean discharge rate during the peak of the afferent response, the gastric tension receptor was classified as having a tonic rhythm of discharge. When the discharge rate at the nadir of the response to distension was <10% of the mean discharge rate during the peak of the afferent response to distension, the gastric tension receptor was classified as having a phasic discharge rhythm. 4/7 afferents exhibited a tonic pattern of discharge, 2/7 fibres a predominantly phasic rhythm, while the remaining fibre had components of both phasic and tonic rhythms in its response to distension (Table 4-1b).

The 2 fibres that exhibited a predominantly phasic pattern of discharge in response to CD were unaffected by baclofen (14μmol/kg iv). Of the 4 corpus tension receptors which showed a tonic response to CD, the responses of 2 afferents to CD were attenuated by baclofen and reversed with subsequent CGP35348. With the fibre which had both tonic and phasic components in its response to CD under control conditions, baclofen removed the tonic component of the discharge while leaving the phasic component relatively intact. More detail is given below of the 3 fibres that were affected by baclofen.

The response of the fibre in Figure 4-4 under control conditions has already been described above. Baclofen (14μmol/kg iv) abolished the tension receptor response to CD, even though the increase in intraluminal pressure was potentiated. Subsequent CGP35348 (100μmol/kg iv) administration reversed the effects of baclofen on both the tension receptor and intraluminal pressure responses to distension.

The response of the second corpus tension receptor, illustrated in Figure 4-5, to CD (20ml saline) was essentially tonic in nature with a phasic rhythm incorporated into its

pattern of discharge. Administration of baclofen (7μmol/kg iv) did not noticeably change the afferent response to distension. However, an additional 7μmol/kg administered during CD led to the abolition of the tonic component of the afferent response while leaving the phasic portion of the response intact. Subsequent distension 5 minutes later resulted in a dramatically attenuated response, showing only phasic characteristics.

The response of the third corpus tension receptor that was affected by baclofen (14μmol/kg iv) is shown in Figure 4-6 (bottom trace). The nerve strand with this afferent unit also had a mucosal receptor contained within it. The response of the mucosal receptor is described later in section 3.6. The corpus was distended with 15ml saline during the first half of the recording. Under control conditions, CD evoked an increase in tension receptor discharge which was tonic in nature. Subsequent administration of cholecystokinin (100pmol close ia) inhibited discharge, secondary to a drop in intraluminal pressure (not shown). Baclofen (14μmol/kg iv) administered during CD abruptly attenuated the tension receptor activity while leaving the effect of CCK unchanged. CD 15 minutes after baclofen failed to elicit a response. Administration of CGP35348 (100μmol/kg iv) during distension did not obviously reverse the effect of baclofen on afferent discharge immediately. However, when distension was repeated 10 minutes after $GABA_b$ receptor antagonism, the pattern of the tension receptor response was reversed to one similar to that obtained under control conditions.

3.5 Afferent discharge-intraluminal pressure relationship during corpus distension

Intraluminal pressure recordings were obtained throughout the studies. The change in pressure during CD was measured under control conditions, after baclofen administration and again after CGP35348 or CGP36742 administration (Figure 4-7). Baclofen significantly increased intraluminal pressure ($p<0.01$ vs Control) and the amplitude of the rhythmic pressure waves during CD (example on Figure 4-4). The antagonists reversed both effects of baclofen, although this did not reach significance levels.

The relationship between the change in vagal tension receptor discharge and intraluminal pressure in response to corpus distension is examined in Figure 4-8. Only data from the 3 studies where a decrease in afferent response to distension was seen after baclofen were included. After baclofen administration, the reduction in discharge-pressure relationship during corpus distension is statistically significant ($p<0.05$ vs Control). This trend was reversed following the administration of CGP35348.

3.6 Mucosal receptor sensitivity

The responses of 2 mucosal receptors to mechanical and chemical stimuli were studied. The receptive field of 1 afferent was located in the corpus by mucosal stroking. This was achieved by probing the serosal surface with a blunt probe in order to generate a shear force as folds of mucosa were rubbed against each other. When the shear force was directed against the receptive field of the afferent, this resulted in a sharp burst of activity. The receptive field of the other afferent was unidentified. Although the receptive field of this second mucosal afferent was unidentified, it is likely to lie within the mucosa of the upper gastrointestinal tract as its response to CCK was similar in duration and latency to that evoked by the corpus mucosal receptor reported above and in other studies, where no other category of afferents were seen to respond directly to CCK^{38}. Both of these afferents showed no resting activity in the absence of any intentional stimulus.

The responses of the corpus mucosal receptor to mucosal stroking of the receptive field, and to close intraarterial administration of cholecystokinin (CCK, 100pmol) are described in detail in Chapter 1. Briefly, the response to mucosal stroking was rapidly evoked and short lasting, occurring only when the probe was on the receptive field. Responses to cholecystokinin were evoked within 1-5 seconds, not associated with any corpus contractile activity and lasted well in excess of 7 minutes before discharge levels slowly returned to prestimulus levels. After baclofen (Figure 4-9 and Table 4-1b), the mucosal receptor response to CCK was enhanced.

The recording from the second mucosal receptor was performed simultaneously with the recording from a corpus tension receptor contained within the same strand (described above, see Figure 4-7, top trace). CD evoked a typical excitatory response from the tension receptor but no response from the mucosal receptor. CCK, on the other hand, elicited an large increase in discharge of the mucosal receptor. Baclofen increased the effect of CCK on the mucosal receptor. The response to CCK was not tested after subsequent CGP35348 administration.

Thus, baclofen enhanced the sensitivity of both mucosal afferents to CCK. The ability of GABAb receptor antagonism in reversing these effects was not tested.

4 DISCUSSION

In this study, GABAb receptor ligands were found to selectively modulate the sensitivity of mucosal and corpus tension receptors while leaving that of oesophageal and antral tension receptors unchanged. The corpus tension receptors that were affected had a tonic discharge pattern in response to distension, whereas those with a phasic rhythm in its distension response were unaffected. This effect of the GABAb receptor ligands is peripheral and independent to that on gastric compliance and may prove to be useful in designing drugs which may be of potential therapeutic use in patients with upper gastrointestinal complaints.

4.1 Involvement of peripheral GABAb receptors in gastric tension receptor sensitivity

Afferent recordings were obtained from fine filaments dissected from the main cervical vagal trunk. As this location is aboral to the nodose ganglion, the only site of action that systemically administered drugs can act upon is on receptors, presumably presynaptic ones, located on the peripheral terminals of these afferents. Although there is abundant

literature citing the presence of presynaptic receptors at the central terminations of afferents and on cell bodies[139, 244], to date there is very little evidence on the presence of receptors located presynaptically on the peripheral terminals of these same nerve fibres.

In this chapter, we have shown that GABAb receptors are located on the peripheral terminals of some vagal afferent fibres. These peripheral GABAb receptors appear to be selectively involved in mediating inputs from tension receptors located within the corpus. Of the 7 corpus tension receptors that were recorded, the response of only 3 of these were affected by baclofen. These 3 all had a tonic pattern of discharge to their control response to distension. The distension response of neither of the 2 corpus mechanoreceptors with a phasic rhythm were affected by the GABAb receptor agonist.

In my study, baclofen decreased the basal discharge rate of vagal corpus tension receptors. This effect is reversed by GABAb receptor antagonism with either CGP35348 or CGP36742. This affect may be secondary to the influence on tonic mechanosensitivity. As well as influencing the resting discharge of corpus tension receptors, systemically administered baclofen also increased intraluminal pressure during corpus distension as well as increasing the rate and amplitude of the gastric pressure waves seen during the distension period. These effects have been documented elsewhere[7, 15, 272] where baclofen was administered via the same route, subcutaneously, or into the ventromedial hypothalamus. The action is proposed to be centrally mediated as baclofen does penetrate the blood brain barrier[106]. The action of baclofen on increasing gastric rhythmic activity is likely to be through an increase in vagal drive to intramural cholinergic neurones[15] as either bilateral vagotomy or atropine reduced both gastric tone and amplitude of contractions. The increase in basal gastric pressure, both seen in the intact stomach and in the corpus alone, is primarily through a reduction in the tonic vagal drive to the intramural non-adrenergic, non-cholinergic inhibitory neurones in the corpus region[7]. This effect of baclofen is partially reversed in this study by CGP35348, a brain penetrating GABAb receptor blocker[204], indicating that the effects are specific to the GABAb receptor.

The changes in gastric compliance and corpus tension receptor discharge caused by baclofen were opposite in direction. This indicates that the influence of baclofen on afferent activity is independent of that on intragastric pressure as an increase in intraluminal pressure would normally lead to an increase in tension receptor activity[91]. As recordings were made at the cervical level distal to the nodose ganglion, the only possible location of the GABAb receptors that were activated and blocked in this study would be at the erpiheral terminlas of the vagal afferents. Thus, this signifies that are GABAb receptors located peripherally on vagal afferent nerves.

4.2 Involvement of peripheral GABAb receptors in oesophageal and antral tension receptor sensitivity

When examining the effects of GABAb receptor ligands on the sensitivity of vagal tension receptors, from this study, peripheral GABAb receptors do not appear to play a role in modulating the sensitivity of either oesophageal or antral mechanoreceptors. This is accentuated in one study where inflating the oesophageal balloon with an additional 33% volume of air, ie from 1.5ml to 2.0ml air, after baclofen was administered at 14μmol/kg iv did not result in a dimunition or an enhancement in the afferent discharge. In the single recording of an antral mechanoreceptor, intraluminal pressure during antral distension increased after baclofen without affecting the efferent response to the mechanical stimulus. This implies that baclofen can affect gastric compliance without modulating the sensitivity of the tension receptor ending. This change in gastric wall tension for given amount of volume with GABAb receptor activation was also seen in the corpus.

4.3 Involvement of peripheral GABAb receptors in mucosal receptor sensitivity

Baclofen has been shown to modulate the effect of exogenously administered cholecystokinin in other systems. In one study, baclofen attenuated the appetite suppressant effect of systemic cholecystokinin in both nondeprived rats and rats which had been

deprived of food for 22 hours[99]. Baclofen did not affect food intake when administered in the absence of exogenous cholecystokinin. This suggests that the inhibitory effect of CCK may involve interaction with a GABAb receptor mediated mechanism. In my study, baclofen potentiated the response of the mucosal fibres to cholecystokinin. This may be direct through presynaptic GABAb receptors located on the peripheral terminals of the vagal mucosal afferents, or indirectly via another mechanism.

The effect of baclofen on the cholecystokinin response of mucosal fibres is unlikely to be direct even though the presence of high affinity GABA uptake sites within the mucosal layers of the rat stomach has been identified. The effects of baclofen are thought to be indirect as these uptake sites have been associated with a subpopulation of mucosal endocrine cells, not with nerve fibres which extend to the mucosal layers[149]. Also, if presynaptic GABAb receptors were present on the peripheral terminals of vagal mucosal fibres, the effect would presumably be inhibitory. Activation of the receptors would therefore attenuate, not potentiate, the responses of mucosal afferents to cholecystokinin, similar to the effects of GABAb receptor activation on the sensitivity of corpus tension receptors to distension. It may therefore be unlikely that baclofen acts directly through presynaptic GABAb receptors located on peripheral vagal endings which also contain CCK receptors. However, a more thorough investigation of GABA uptake sites or receptors within the mucosal layer will need to be performed as the terminations of extrinsic nerve fibres within this layer tend to be difficult to identify.

Another possible way that baclofen could influence the cholecystokinin response is indirectly through the effects of the GABAb receptor agonist on somatostatin. Urethane, the anaesthetic used in all experiments, has been shown to cause a marked increase in somatostatin secretion into the hypophyseal portal blood[69], and an increase in the synthesis and local release of antral somatostatin[274] where the peptide is found in the endocrine D cells in the mucosal layer and in neurones in the muscle layer of the stomach[153, 154]. The increase in antral somatostatin mRNA concentration is linked to a decrease in basal gastric acid secretion through the inhibition of gastrin secretion[274]. Indeed, basal acid secretion in

urethane anaesthetised rats is markedly lower when compared to with either conscious or pentobarbital- or alpha-chloralose-anaesthetised rats[172].

The involvement of the GABAb receptor in the somatostatin-gastric acid secretory process may be inferred from several pieces of evidence. Firstly, baclofen stimulated acid secretion in urethane anaesthetised rats[15, 172], but decreased acid secretion in both conscious rats and those anesthetised with either pentobarbital or alpha-chloralose[172]. Secondly, intravenous GABA, a GABAa and GABAb receptor agonist, can reduce plasma somatostatin levels and increase basal gastric acid output and serum gastrin levels in the dog[256]. GABA can also inhibit antral somatostatin release and stimulate gastrin release in incubated antral mucosal fragments, although this effect of GABA is inhibited by bicucullline, a specific GABAa receptor antagonist[133]. In another study using the rat isolated stomach, GABA was found to inhibit the bombesin stimulated release of somatostatin while leaving basal somatostatin and gastrin levels unchanged[130]. The effects of GABA on gastric acid secretion is mimicked by intravenous baclofen. Taken together, baclofen may increase gastric acid secretion in urethane-anaesthetised rats indirectly through the reduction of urethane stimulated-somatostatin release.

The effect of baclofen on somatostatin release does not, however, extend to somatostatin secretion in rat and mouse pancreatic islets[120], indicating that the GABAb receptors are selectively distributed along the gut. This effect of baclofen on somatostatin release is also seen centrally where the GABAb receptor agonist caused a decrease in the calcium dependent release of somatostatin from rat cerebrocortex [51] and human neocortex nerve terminals[52].

Somatostatin has been inferred to have a direct inhibitory effect on afferent sensitivity. Octreotide, a somatostatin analogue, could increase the pressure and volume threshold required for non-noxious and noxious sensation in response to slow tonic rectal distension in healthy volunteers. This effect was blocked with intrarectal lidocaine and was seen to be a direct inhibitory effect of the somatostatin analogue on extrinsic primary

afferents which was presumed to have their receptive fields in the mucosa[214]. In patients with irritable bowel syndrome, octreotide also reduced perception in response to rectal distension[68, 134, 206], an effect which is attributed to inhibition of visceral afferent pathways. Octreotide was also thought to directly inhibit gastric afferents responsible for mediating innocuous sensations as it reduced the sensation of fullness with gastric distension[193].

Thus, the endogenous somatostatin released by urethane may have an inhibitory effect of mucosal receptor responses to CCK under control conditions. When baclofen is administered systemically, the amount of somatostatin released is decreased by the GABAb receptor agonist. This means that the inhibitory effect of somatostatin on the mucosal receptor response to CCK is diminished, thus allowing the mucosal receptor to be more sensitive in its response to appropriate stimuli.

4.4 Conclusions

GABAb receptors located on the peripheral terminals of vagal afferents are selectively located on tension receptors with endings in the corpus, but not those with endings in the distal oesophagus or antrum. Those corpus tension receptors with GABAb receptors responded to mechanical stimuli with a tonic discharge pattern. Mucosal receptor sensitivity was also affected by GABAb receptor activation. However, this was thought to be an indirect effect through fluctuations in the release of endogenous somatostatin.

a) Oesophageal tension receptors

Unit no	Location of Ending	Distension Stimulus	Baclofen	
				%Con
20/9/96	Oesophagus	1ml air	++	100
30/10/96	Oesophagus	1.5-2.0ml air	++	100*
Total no of responses studied				**2**
Drug Effect		- None		2
		- ≤ 50%Control		0
		- Blocked		0

b) Gastric tension receptors

Unit no	Location of Ending	Distension Volume (ml saline)	Contractile Rhythm	Baclofen		CGP35348	
					%Cont		%Cont
26/11/96	Corpus	15ml	Tonic	+	0	0	∅
19/9/96	Corpus	5-15ml	Tonic	+	0	0	∅
29/8/97	Corpus	5-20ml	Tonic	+	100		
5/9/97	Corpus	5-20ml	Tonic	+	100*		
21/10/96	Corpus	20ml	Tonic/Phasic	++	20%-Tonicity lost		
28/10/96	Corpus	5-15ml	Phasic	+	100		
21/2/97	Corpus inc.	5-20ml	Phasic	+	100		
14/8/97	Antrum	1-5ml	Phasic	+	100		
Total no of responses studied					**8**		**2**
Drug Effect			- None		5		0
			- ≤ 50%Control		1		0
			- Abolished or reestablished		2		2

c) Mucosal receptors

Unit no	Location of Ending	Baclofen CCK	
			%Cont
21/11/96	Corpus	++	200
26/11/96	?	++	150

Table 4-1. $GABA_b$ receptor influences on gastrooesophageal vagal afferents. + denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes; * denotes that baclofen was administered at a dose of 7μmol/kg iv instead of 14μmol/kg iv; ∅ reversal of afferent response to original response.

a) 2/2 afferent responses to oesophageal balloon distension were unaffected by baclofen.

b) Tonic denotes that the mean discharge rate between bursts ≥50% peak mean; Phasic denotes that the mean discharge rate between bursts <10% peak mean. Baclofen abolished the response of 2/4 tonic corpus tension receptors to distension. In 1/7 fibre where the contractile pattern was a mixture of tonic and phasic activity, baclofen administration during corpus distension abolished the tonic component of the activity and greatly reduced the remaining response. Baclofen did not affect the antral or the phasic corpus tension receptor response to distension. CGP35348 reversed the response to corpus distension to one similar in profile obtained under control conditions.

c) Baclofen potentiated the mucosal receptor responses to close intraarterial application of cholecystokinin (CCK, 100pmol).

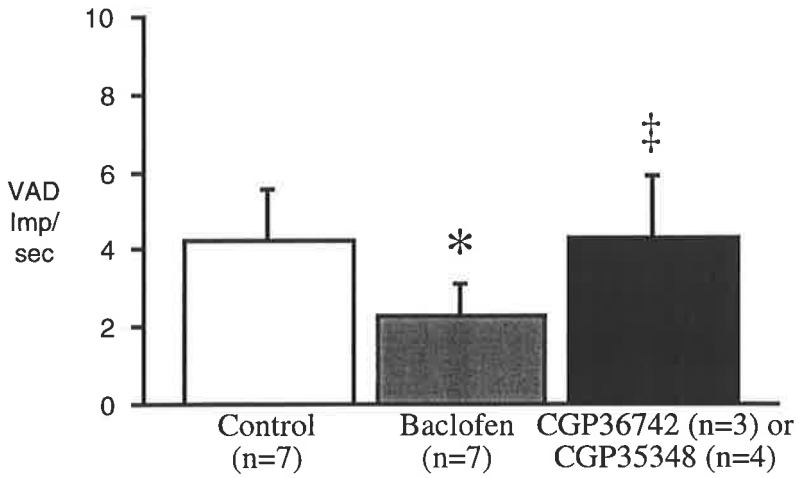


Figure 4-1. GABAb receptor influences on basal discharge rates of corpus mechanoreceptors.

The GABAb receptor agonist baclofen (7-14μmol/kg iv, n=7) significantly reduced the basal discharge rate of corpus mechanoreceptors. This effect was reversed with subsequent GABAb receptor antagonism with either CGP36742 (100μmol/kg iv, n=3) or CGP35348 (100μmol/kg iv, n=4).

* $p<0.05$ vs Control
‡ $p<0.05$ vs Baclofen

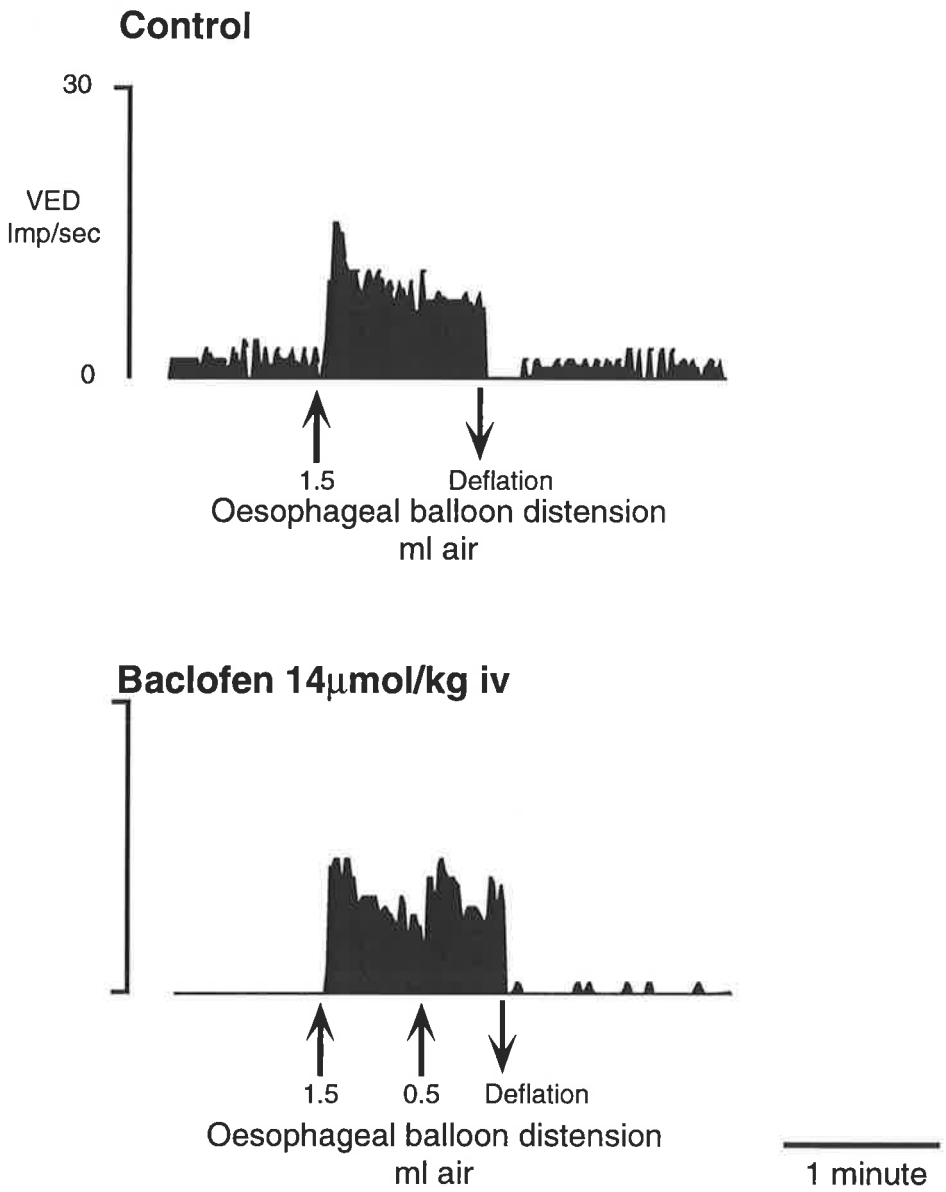


Figure 4-2. Integrated record of the effect of baclofen on an oesophageal tension receptor response to distension.
Oesophageal balloon distension (OBD, 1.5ml air) led to a sharp increase in discharge during the dynamic phase of inflation in this fibre. This increase adapted during the static period of sustained OBD to a level that remained above the resting level of discharge. Balloon deflation led to a silent period lasting for <5seconds before the discharge levels returned to prestimulus levels.

After baclofen administration (14μmol/kg iv), spontaneous afferent discharge decreased. However, the OBD response was unchanged, even after the balloon was inflated with an additional 0.5ml air.

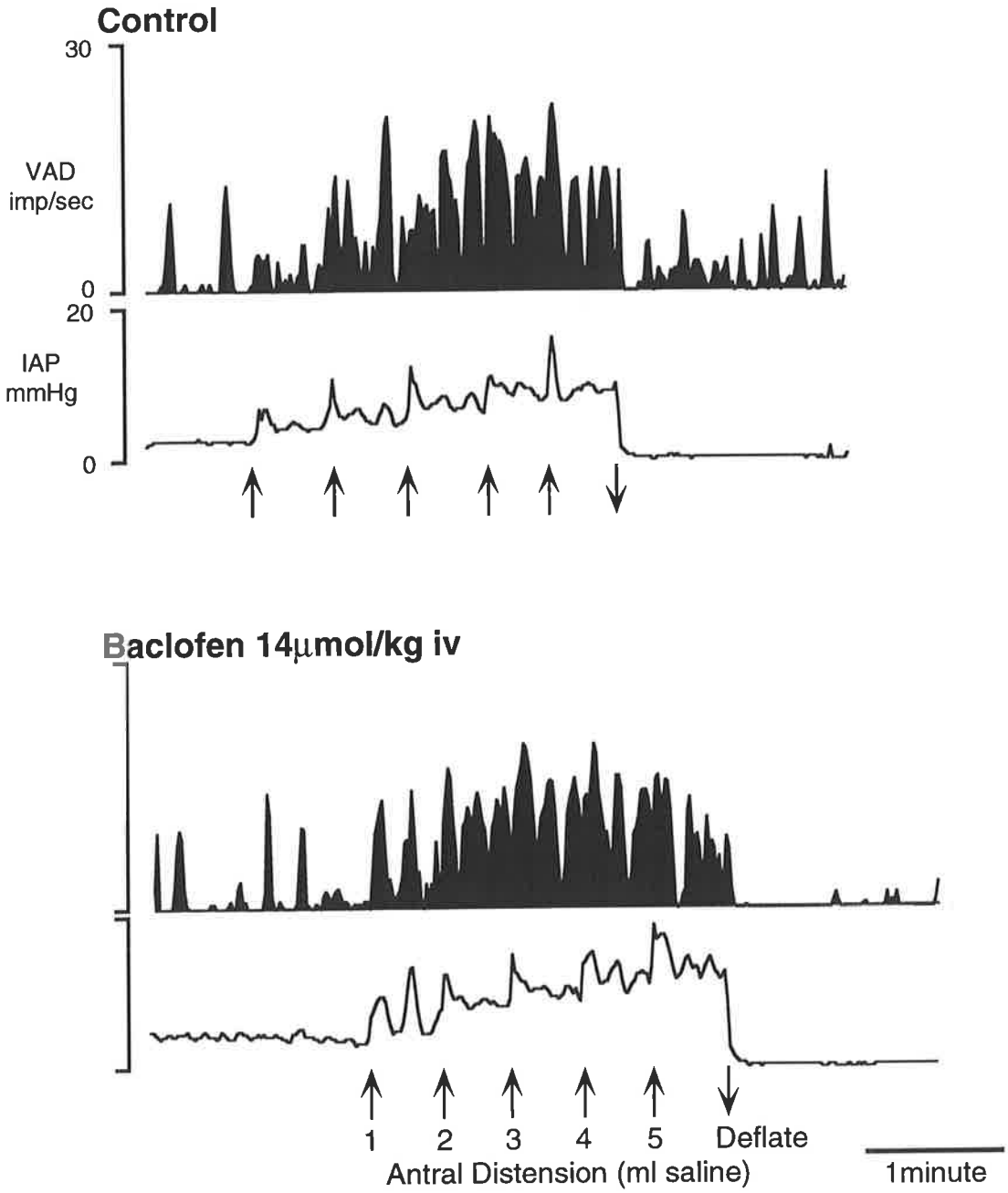


Figure 4-3. Effects of graded distension on vagal afferent discharge of an antral tension receptor and intraluminal pressure.
Top trace: integrated record of antral tension receptor discharge
Bottom trace: intraluminal pressure

Antral distension in 1ml increments led to an increase in afferent discharge and intraluminal pressure. This phasic increase in afferent disharge was roughly correlated to fluctuations in antral pressure. Baclofen did not affect the spontaneous discharge of the fibre or the afferent response to antral distension while intraluminal pressure during this stimulus was increased.

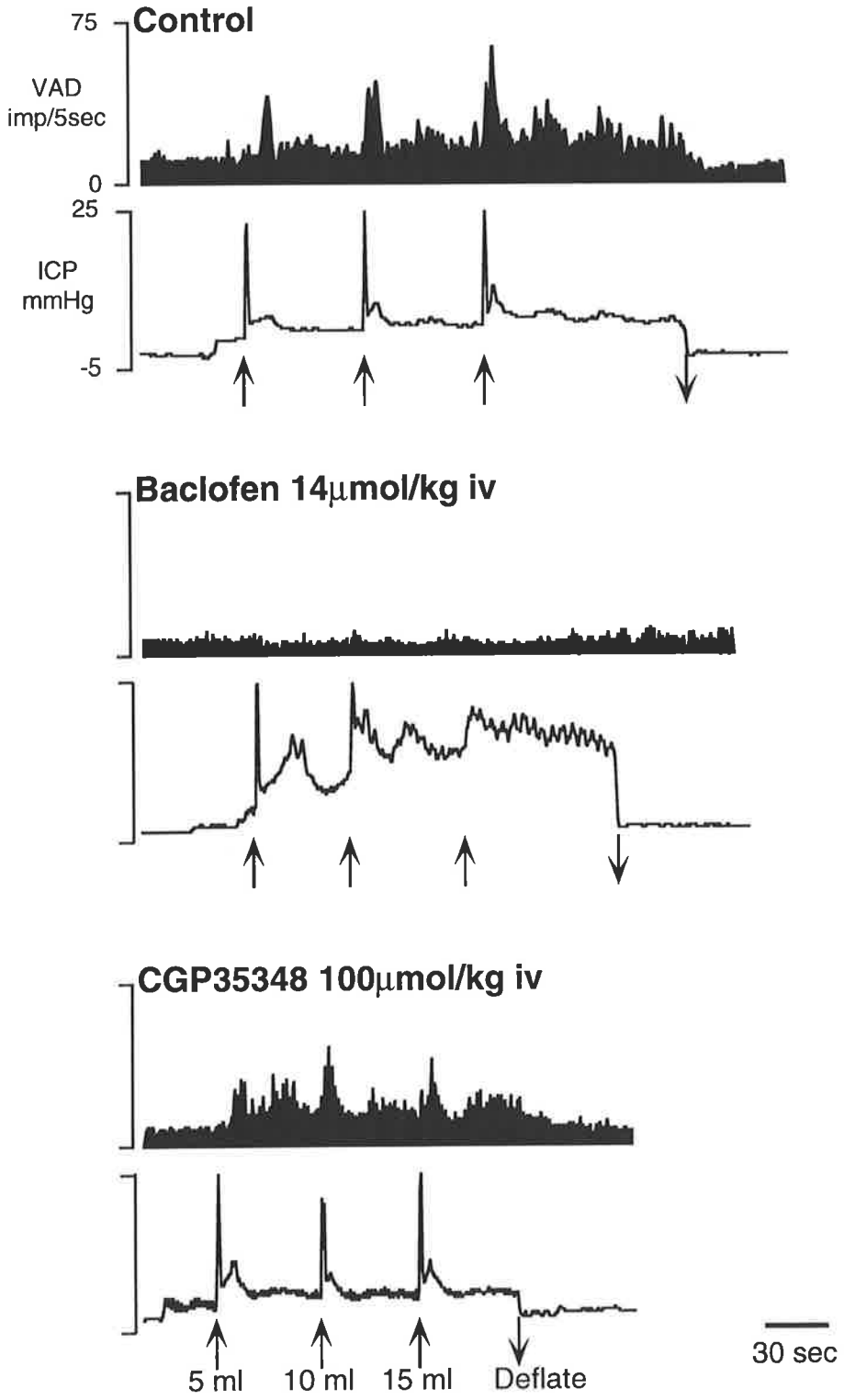


Figure 4-4. Effects of graded distension on vagal afferent discharge of a corpus tension receptor and intraluminal pressure.
Top trace: integrated record of vagal corpus tension receptor discharge
Bottom trace: intraluminal pressure

Under control conditions, corpus distension in 5ml increments led to an increase in afferent discharge and intraluminal pressure. Baclofen decreased basal afferent discharge. The afferent response to distension was abolished while increasing the intraluminal pressure response to corpus distension. Subsequent CGP35348 administration reestablished the afferent and intraluminal pressure responses to step distension to those similar in profile obtained under control conditions. The spontaneous discharge returned to a level similar to that prior to baclofen administration.

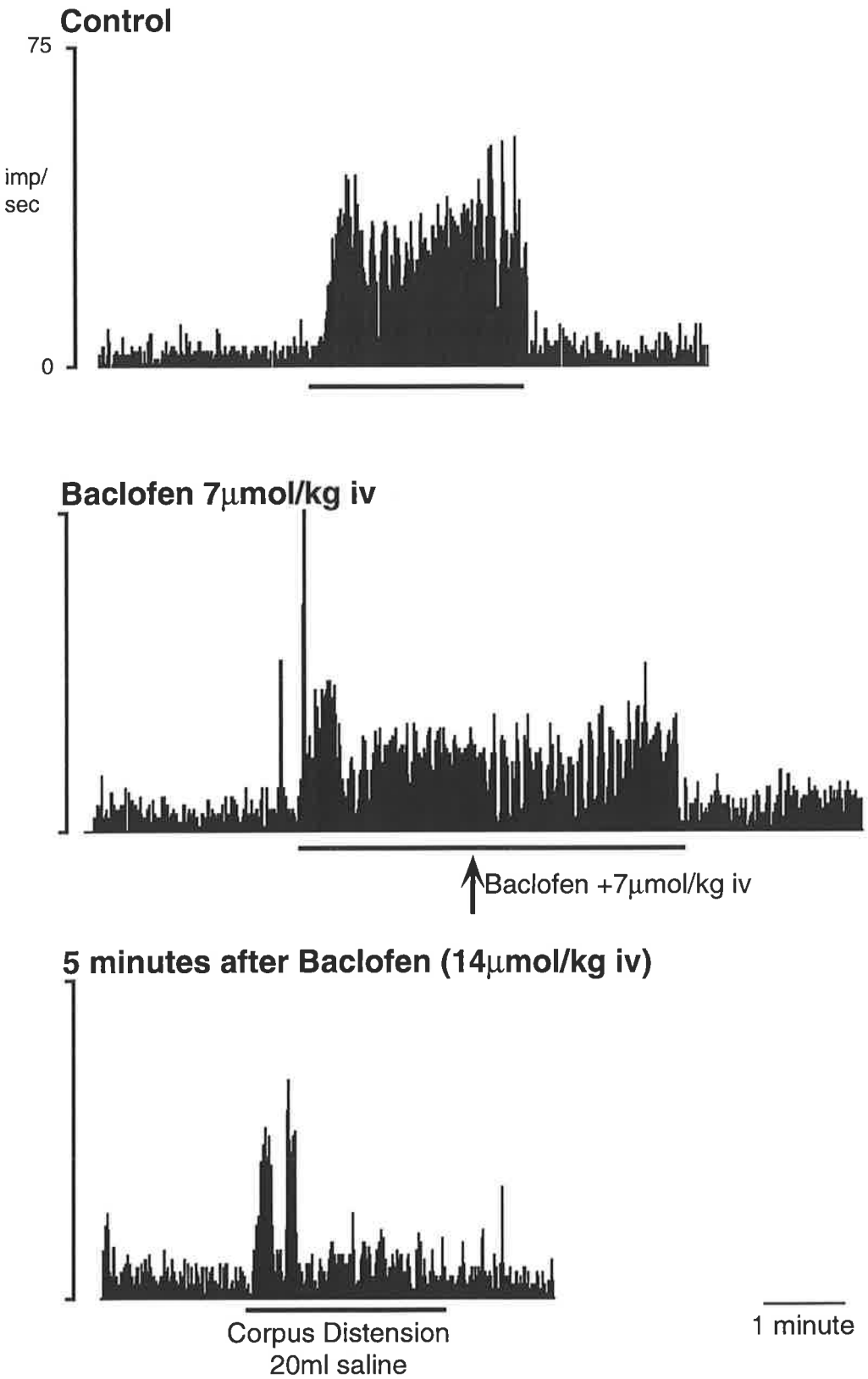


Figure 4-5 Response of a vagal corpus tension receptor to distension. This excitation evoked by corpus distension (20ml saline) contained both phasic and tonic components. The first dose of baclofen did not significantly alter this response to distension. The second dose of baclofen, administered during distension, reduced the tonic component of the afferent response. Distension 5 minutes after that revealed an attenuated tension receptor response.

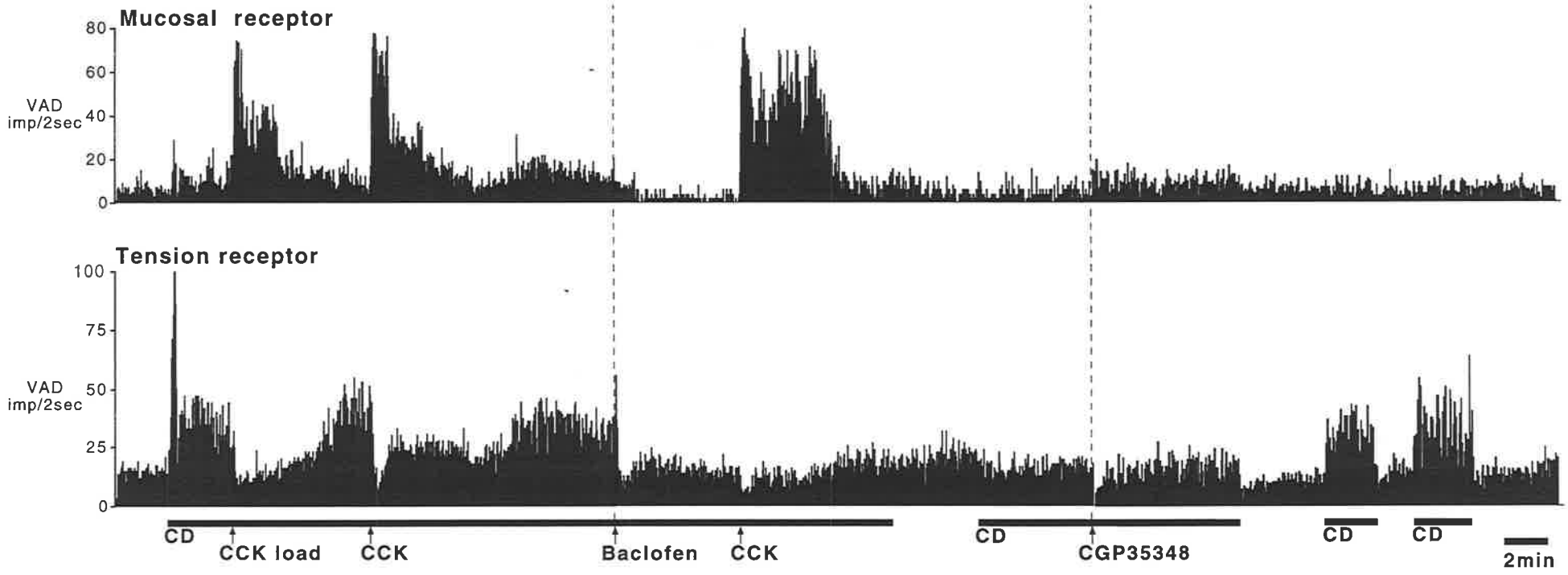


Figure 4-6 Double recording of Corpus Afferent responses to Mechanical and Chemical Stimuli
Upper trace: integrated record of a vagal mucosal receptor response to corpus distension and cholecystokinin.
Lower trace: integrated record of a vagal tension receptor response to corpus distension and cholecystokinin.

Corpus distension (CD, 15ml saline) increased the discharge rate of the tension receptor while having no effect on the discharge of the mucosal receptor. The reverse was true when close intraarterial cholecystokinin (CCK, 100pmol) was administered. Baclofen (14µmol/kg iv) abolished the tension receptor response to corpus distension and potentiated the mucosal response to CCK. The antagonist CGP35348 (100µmol/kg iv) reversed the tension receptor response.

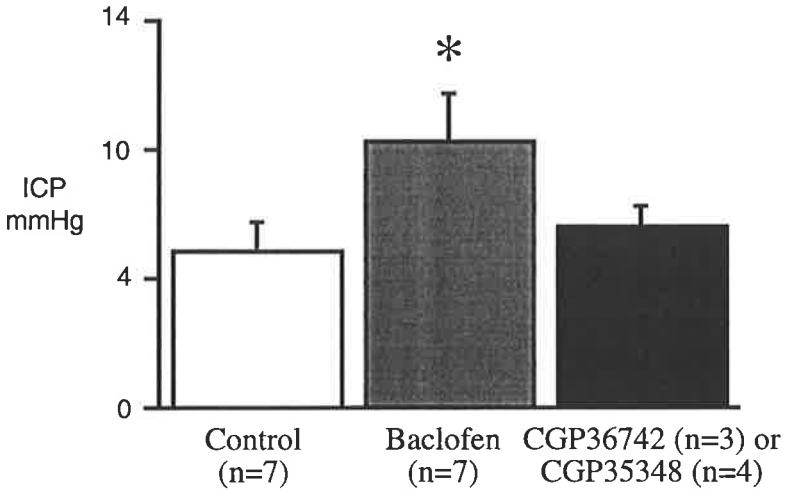


Figure 4-7. GABAb receptor influences on intraluminal pressure during corpus distension (20ml saline).

Intracorpus pressure during distension was significantly increased by baclofen (7-14μmol/kg iv, n=7) and reversed with subsequent antagonism with either CGP38742 (100μmol/kg iv, n=3) or CGP35348 (100μmol/kg iv, n=3) administration.

* $p \leq 0.01$ vs Control using paired t-test.

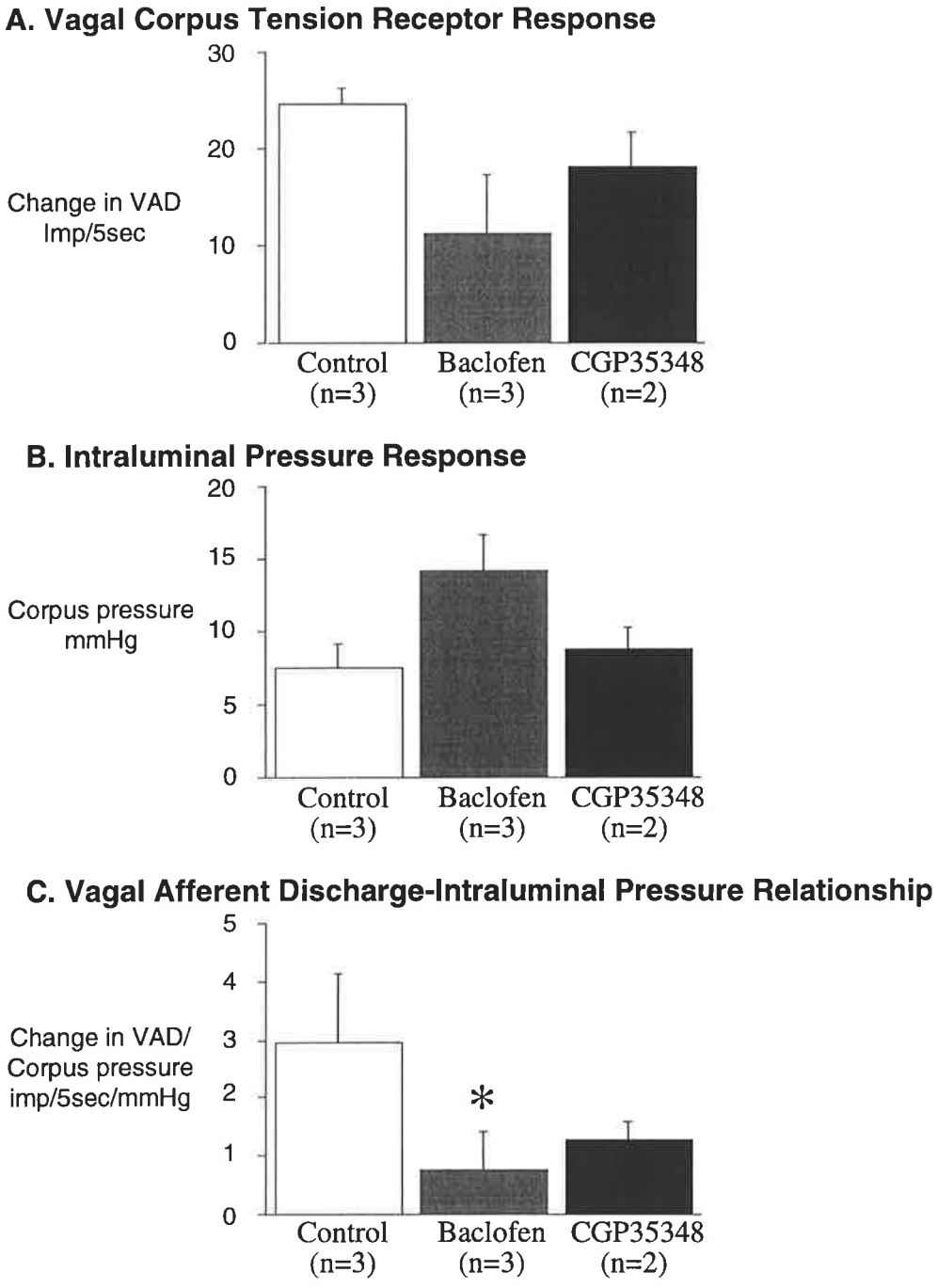


Figure 4-8. GABAb receptor influences on gastric mechanics and tension receptor sensitivity during corpus distension (15-20ml saline).

A. Baclofen (7μmol/kg iv, n=3) attenuated the vagal afferent response to corpus distension. Subsequent CGP35348 administration (100μmol/kg iv, n=2) reversed this trend.
B. Intraluminal pressure during corpus distension was increased after baclofen administration compared with control. CGP35348 also reversed this trend.
C. The relationship between afferent discharge and intraluminal pressure was reduced after baclofen administration. Subsequent CGP35348 administration had a negligible effect on this ratio.

* $p \leq 0.05$ vs Control using paired t-test.

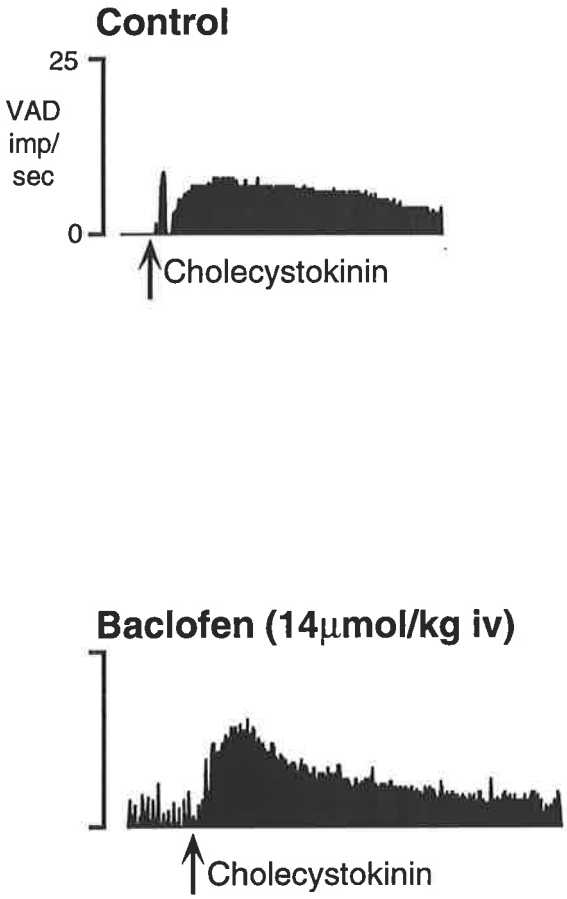


Figure 4-9. Integrated response of the vagal afferent discharge of a corpus mucosal receptor to intraarterial drugs.

The receptor was silent in the absence of stimulus. Close intraarterial administration of cholecystokinin (CCK, 100pmol) evoked a long lasting excitatory response in the mucosal receptor.

Baclofen administration potentiated the afferent response to CCK.

CHAPTER 5.
Effects of
$GABA_b$ receptor ligands
on
vagal reflex pathways

1 SUMMARY

1. The potential involvement of central and peripheral GABAb receptors in modulating responses of vagal efferent fibres to peripheral stimuli from the upper gastrointestinal tract was investigated in 20 ferrets.

2. The effects of central and systemic GABAb receptor activation and antagonism on vagal efferent responses to distension of the intact stomach (GD, n=15), the isolated corpus (CorpD, n=3), the isolated antrum (AD, n=3), the oesophagus (OBD, n=6), and the colon (ColD, n=2), and to close intraarterial cholecystokinin (CCK, n=9), bradykinin (BK, n=10) and capsaicin (Cap, n=9) were investigated.

3. When GABAb receptor ligands were only administered systemically, baclofen (7-21μmol/kg iv) attenuated efferent responses to CorpD in 2/3 fibres and to GD in 8/10 fibres. Subsequent systemic CGP35348 partially reversed the effects of baclofen in 2/2 studies. The effects of baclofen on the sensitivity of efferent responses to GD showed no dose dependence: in 7/8 fibres, baclofen at a dose of 7μmol/kg was sufficient to abolish the responses whereas in one fibre, baclofen at 21μmol/kg only slightly reduced the response. When GABAb receptor ligands were administered both centrally and systemically, initial treatment with central baclofen reduced or abolished the responses to GD in 3/5 fibres. In these 3 studies, the effect of central baclofen on GD responses was reversed by central CGP35348. The response to GD was unchanged by subsequent systemic baclofen and CGP35348 but was again abolished by central CNQX.

4. When GABAb receptor ligands were only systemically administered, baclofen reduced responses to OBD in 2/4 fibres and to ColD in 2/2 fibres. Subsequent CGP35348 reversed the effects of baclofen to ColD in only one of 2 studies. When baclofen was administered centrally and systemically, central baclofen had no effect in 2/2 responses to OBD. However, subsequent systemic baclofen abolished the OBD response in 1/2 fibres.

5. When GABAb receptor ligands were only systemically administered, baclofen potentiated 1/2 excitatory efferent responses to CCK and attenuated 1/2 inhibitory efferent responses to CCK. Subsequent CGP35348 had no effect in the study where baclofen attenuated the inhibitory response. When GABAb receptor ligands were systemically and centrally administered, central baclofen affected 4/4 inhibitory responses to CCK. Subsequent central CGP35348 reversed the effect of baclofen in only 1/4 studies. Subsequent central non-NMDA receptor antagonism with CNQX attenuated the depth of inhibition in 3/3 fibres tested. The single excitatory efferent response to CCK was unaffected by any drug treatment.

6. When baclofen was only systemically administered, baclofen abolished the efferent response to BK and to Cap in the same 1/5 fibres. When GABAb receptor ligands were administered both centrally and systemically, neither baclofen nor CGP35348, administered via either route, affected the responses to BK and Cap in 4/5 fibres tested. In the remaining fibre, the direction of response to both BK and Cap was changed first by central baclofen, then again by systemic baclofen. Subsequent central administration of CGP35348 changed the direction of the Cap response a third time while leaving that of the BK response unchanged. The fourth treatment, systemic CGP35348, changed the direction of the BK response but did not affect the Cap response.

7. These results indicate that 1) central presynaptic GABAb heteroreceptors modulate inputs from gastric mechanoreceptors, with glutamate being the neurotransmitter released by the neurones to act via the non-NMDA receptors, 2) GABAb receptors are likely to be present solely on the peripheral terminations of some oesophageal distension-sensitive afferents, 3) CCK-sensitive afferents possess presynaptic GABAb autoreceptors on their central terminations. Glutamate is also released along the pathway to act via non-NMDA receptors, 4) presynaptic GABAb autoreceptors may also be involved in modulating inputs from colonic mechanoreceptors and 5) GABAb receptors play only a minor role in modulating inputs from bradykinin- and capsaicin-sensitive.

2 INTRODUCTION

GABA, the main central inhibitory amino acid, is also found extensively within the CNS. All 3 receptor subtypes, GABAa, GABAb and and GABAc receptors, are abundant in rat CNS[57]. GABA is also thought to play a role centrally in some gastrointestinal functions. For example, activation of central GABAb or GABAa receptors can increase gastric acid secretion in urethane anaesthetized rats via a vagally mediated cholinergic pathway[121]. Baclofen can also increase the basal levels of multiunit vagal efferent discharge[121, 273]. In brainstem slice recordings in the rat[60, 61], both pre- and post-synaptic GABAb receptors were found to be involved in the synaptic transmission in the NTS. The postsynaptic action of GABAb receptors is seen when baclofen caused a direct hyperpolarisation action in voltage clamp mode. The presynaptic component was apparent when both evoked nad spontaneous synaptic transmission were reduced by baclofen at a dose without any direct postsynaptic hyperpolarisation effects.

More specifically, GABAb receptors has been demonstrated to influence inputs from baroreceptors and respiratory afferents in the DVC. The involvement of GABAb receptors in respiration was demonstrated when baclofen, administered subcutaneously, led to an inhibition of minute ventilation and respiratory rate due to room air or carbon dioxide-enriched gas inhalation[138]. The central component was determined when the dose dependent inhibition was reversible by central administration of CGP35348. GABAb receptors are also involved in the Hering-Breur reflex[237, 259] as NTS injections of baclofen abolished the expiratory-promoting reflex, and attenuated the deflation and the inspiratory-inhibitory reflexes. The effects of baclofen were reversible by NTS injections of the antagonist CGP35348. GABAb receptors are also thought to be present on NTS baroreceptor neurones as baclofen, applied microiontopheretically into the NTS, reduced the spontaneous discharge rate of baroreceptive NTS neurones[227]. This effect was abolished by CGP35348 and was attributed to presynaptic as only the pulse-synchronous peaks of discharge were inhibited without affecting the intra-peak activity.

On the other hand, there is little evidence to suggest that GABAb receptors in the NTS may be involved in modulating inputs from gastrointestinal afferents. One such piece of data is of a neonatal gastric-brainstem preparation[275], where recordings made in the NTS were identified as arising from the stomach as stimulation of the gastric branch of the subdiaphragmatic vagus led to activation of the neurone. In this study, the activity of less than half the neurones were reduced by baclofen and the GABAa receptor agonist muscimol. These effects were relatively modest. Also, no attempt was made to determine the site of termination of the peripheral ending as it may well have been in a region distal to the stomach. In addition, electrical stimulation was the only stimuli used. Again, this made it difficult to ascertain whether or not the inputs were from mechano- or chemo-sensitive afferents.

In the previous chapter, the presence of GABAb receptors on the peripheral terminals of vagal afferent fibres was investigated. They were found to selectively affect tonically active corpus tension receptors, but not antral or oesophageal receptors. GABAb receptors also modulated mucosal afferent responses to cholecystokinin, although this might be indirect through the modulation of urethane-stimulated somatostatin levels. In this chapter, the involvement of GABAb receptors is investigated in the central processing of afferent information from gastric mechanoreceptors. The relative contributions of central and peripheral GABAb receptors in modulating efferent discharge in response to other peripheral stimuli is also analysed.

3 PROTOCOL

The primary aim of this series of experiments was to determine the role of central GABAb receptors in the vagal efferent responses to distension of the stomach and to further elucidate the role of peripheral GABAb receptors in the same system. The selective brain-penetrating GABAb receptor agonist and antagonist, baclofen and CGP35348 respectively[106, 204], were administered both systemically and centrally to investigate the relative contribution of peripheral and central GABAb receptors. The roles

of other neurotransmitters in mediating various inputs onto vagal central neurones were also investigated by performing cumulative antagonism of the CGRP, NK-1, non-NMDA, and M1 receptors subsequent to central and systemic activation and blockade of the GABAb receptors.

In 13 studies, baclofen, and subsequently CGP35348, were only administered systemically. In 7 other studies, both GABAb receptor ligands were administered centrally and systemically. CP99994 (12μmol/kg iv), pirenzepine (5μmol/kg iv), CNQX (75-155nmol/kg icv) and hCGRP8-37 (3.2-6.4nmol/kg icv) were also administered to determine the effects of NK-1, M1, non-NMDA, and CGRP receptor antagonism respectively. The efferent responses to different peripheral stimuli were determined under control conditions and after each treatment.

The involvement of central GABAb receptors in vagal reflexes were studied in isolation by administering baclofen centrally into the fourth ventricle as the initial treatment in 7 studies. If the vagal efferent response to whole gastric distension (GD) was abolished or attenuated by central baclofen (3nmol/kg icv), an excess of the antagonist CGP35348 (100nmol/kg icv) was administered centrally to determine whether the central effects of baclofen could be reversed. The peripheral effects of systemic baclofen (14μmol/kg iv) and, subsequently, CGP35348 (100μmol/kg iv) on vagal efferent responses could then be examined in relative isolation from the central effects. If, on the other hand, the vagal efferent response to GD remained unchanged by central baclofen, an additional dose of the GABAb receptor agonist (3nmol/kg icv) was administered centrally to confirm that the an excess of the drug had no effect on the efferent response to GD. This was then followed by systemic baclofen (7-14μmol/kg iv), central CGP35348 (100nmol/kg icv) and systemic CGP35348 (100μmol/kg iv) administration to investigate only the peripherally mediated effects. The effects of the various drugs on the efferent responses to each stimuli tested in the 5 studies are represented in Table 5-2. Thus, the protocol for each study was dictated by the changes in efferent response to GD evoked by central and/or systemic application of GABAb receptor ligands.

The vagal efferent responses to oesophageal balloon distension (n=6) and gastric distension, either as an intact organ (n=15) or as its separate corpus and antral compartments (n=3), were evaluated in 18 studies. Responses to close intraarterial administration of cholecystokinin (n=9), bradykinin (n=10) and capsaicin (n=9) under control conditions and after each drug administration were also evaluated. The efferent responses in 2 other studies have not been included. This was because the sensitivity of the efferent responses to various stimuli in one of these studies was substantially diminished within the first part of the experiment prior to the first treatment being administered. Off line analysis of the other study revealed that the recording made from this experiment was from more than 1 efferent unit, and these were too similar in shape and amplitude to be distinguished clearly. The responses to stimuli within each treatment group were therefore inconsistent as the discharge frequency of more than one efferent was often being counted.

4 RESULTS

In 13 studies, baclofen and CGP35348 were only administered systemically. A summary of the effects of GABAb receptor ligands on the responses of individual vagal efferent fibres to peripheral stimuli are presented in Table 5-1. In 5 other studies, both GABAb receptor ligands were administered centrally and sytemically. The effect of these and other drugs on responses of efferent fibres to peripheral stimuli is detailed in Table 5-2. A brief description of each of these 5 studies is attached as an Appendix to this chapter as in 4 of 5 of these studies, additional drugs were also given after the effects of central and systemic baclofen and CGP35348 on vagal efferent responses were ascertained.

4.1 Patterns of spontaneous activity

The effects of both central and systemic GABAb receptor activation and blockade on basal vagal efferent discharge, mean gastric pressure and mean arterial pressure were examined in 7 studies.

Although baclofen and CGP35348 were not administered in the same order peripherally and centrally in all experiments, statistical analysis for this section was performed on the combined data from 7 studies. The effects of the GABAb receptor ligands on the following basal parameters were obtained by measuring the mean efferent fibre discharge, arterial pressure and gastric pressure immediately before and after each administration of either baclofen or CGP35348. The effect of each compound on these parameters were not influenced by prior treatments as, when any change in measurements occurred, the direction of response was consistent regardles of the order of drug administration.

All fibres evaluated showed either no basal discharge or a low frequency, irregular pattern of basal discharge which bore no obvious relationship to respiratory, cardiovascular or gastrointestinal contraction rhythms. This remained constant throughout the study prior to any drug administration. Central administration of neither baclofen nor CGP35348 affected the basal level discharge of the efferent fibres tested. Systemic baclofen also had no effect on basal discharge. However, systemic CGP35348 led to a small but significant decrease in vagal efferent discharge (Figure 5-1).

Mean arterial pressure was monitored continuously throughout these studies. Under control conditions, blood pressure was maintained at 101+5mmHg (range 76-116mmHg). Both central and systemic baclofen administration lowered blood pressure, although this reached statistical significance only with latter treatment. CGP35348, administered either centrally or systemicallly, significantly increased blood pressure (Figure 5-2), thus reversing the effects of baclofen on blood pressure.

Gastric pressure was also monitored throughout these studies. A significant increase in basal intragastric pressure was evoked by central baclofen. However, systemic baclofen significantly decreased basal pressure regardless of the order of administration. CGP35348 via both routes of administration led to a slight decrease in basal pressure. Group data for this is presented on Figure 5-3.

4.2 Vagal efferent responses to oesophageal balloon distension

Vagal efferent responses to oesophageal balloon distension (OBD, 2ml air) were obtained in 6 fibres, with all fibres showing excitatory responses under control conditions (Table 5-1 & 5-2). These responses were rapidly evoked, maintained above resting levels for the duration of the stimuli, and returned to resting levels upon cessation of the stimuli (example on Figure 5-4).

In studies where the GABAb receptor ligands were only administered systemically, baclofen (7-14μmol/kg iv) did not affect vagal efferent responses to OBD in 2/4 fibres tested. The efferent response was reduced in 1/4 fibre (Figure 5-4) tested and abolished in the remaining fibre (Table 5-1). The responses to OBD after subsequent CGP35348 administration were not tested.

In studies where the GABAb receptor ligands were administered both systemically and centrally, the response of 1/2 fibres tested to OBD was unchanged by central baclofen (6nmol/kg icv) but was abolished by additional systemic baclofen (14μmol/kg iv). The effects of other drugs on the response to OBD were not tested in this fibre. In the other study, central administration of neither baclofen nor CGP35348 affected the efferent response to OBD. However, CNQX (155nmol/kg icv), administered after baclofen, CGP35348 and CP99994 (12μmol/kg iv), reduced the response to OBD. Pirenzepine (5μmol/kg iv) and hCGRP8-37 (3.2nmol/kg icv) were given after CNQX but neither treatment modulated the efferent response to OBD.

4.3 Vagal efferent responses to gastric distension

Gastric distension was performed in 2 ways. In 15/18 animals, the intact stomach was distended with 40-60 ml saline. In the remaining 3 animals, the stomach was divided into its separate corpus and antral compartments and distended with 15-20ml and 10ml saline respectively. Baclofen and CGP35348 were administered only systemically in the 3 studies with the divided stomach and in 10 studies with the intact stomach. In the other 5

studies, GABAb receptor ligands were administered both centrally and systemically. The effects of baclofen and CGP35348 on the responses of individual efferent fibres to GD is described in Table 5-1 and 5-2.

4.3.1 Efferent responses to distension of the divided stomach

Corpus distension (CorpD, 20ml saline) elicited excitation of discharge in 3/3 fibres under control conditions (Table 5-1). In the example on Figure 5-4, the efferent discharge increased rapidly in response to CorpD under control conditions and was maintained above predistension levels until the corpus was deflated. Baclofen (7μmol/kg iv) in this study abolished the efferent response to CorpD. In the other two studies where responses to CorpD were observed, baclofen (7-14μmol/kg iv) did not affect the efferent response to 1 fibre, while reducing the excitation in the remaining fibre. The effect of CGP35348 on the vagal efferent responses to CorpD was not tested.

Antral distension (AD, 10ml saline) yielded excitatory responses in 3/3 studies under control conditions (Table 5-1). In the example on Figure 5-4, the response was maximal during the dynamic phase of distension, after which discharge adapted but was maintained above basal levels until deflation of the antrum occured. Baclofen (7μmol/kg iv) left the efferent response to AD largely intact in this study. The responses to antral distension in 2 other studies were also unaffected by baclofen (Table 5-1). The effect of subsequent CGP35348 on the vagal efferent responses to AD was not tested.

4.3.2 Efferent responses to distension of the intact stomach

In 10 studies where GABAb receptor ligands were only administered systemically, distension of the intact stomach (GD, 40-60ml saline) yielded excitation in 5 fibres and inhibition of discharge in 5 other fibres under control conditions (Table 5-1). In the example on Figure 5-5, vagal efferent discharge was rapidly inhibited by GD under control conditions. This total inhibition of discharge was maintained throughout the distension

period. Baclofen (7μmol/kg iv) abolished the efferent response to GD. The response to GD was later reinstated by CGP35348 (100μmol/kg iv). Overall, baclofen (7-14μmol/kg iv) completely abolished the efferent response to gastric distension in 8/10 fibres and left the remaining 2/10 responses unaffected. The modulation in efferent response was seen both in fibres that were inhibited and those that were excited by GD under control conditions (Table 5-1). CGP35348 (100μmol/kg iv) was administered subsequently in 2 studies where the initial inhibitory response to GD had been heavily reduced by baclofen. In both fibres, the effects of baclofen were partially reversed by GABAb receptor antagonism. In one of these 2 studies, bilateral vagotomy was performed as the last treatment. The efferent response to GD was abolished after vagal section.

In 5 studies where the GABAb receptor ligands were administered both systemically and centrally, 3 fibres responded with excitation of discharge to GD and 2 fibres with inhibition under control conditions. When baclofen (3nmol/kg icv) was administered centrally as the first treatment in all studies, 2 inhibitory responses and 1 excitatory response to GD were markedly attenuated (see example on Figure 5-6). Subsequent central administration of CGP35348 (100nmol/kg icv) reversed the effects of central baclofen. In the fibre that was excited by GD under control conditions, the response to GD after central CGP35348 was larger in amplitude when compared to the control response. In 2 of the 3 fibres influenced by central GABAb receptors ligands, the response to GD after subsequent treatment with systemic baclofen (14μmol/kg iv) and CGP35348 (100μmol/kg iv) remained unchanged (not shown). Central CNQX (75nmol/kg icv), administered as the last treatment in each of the 2 studies, abolished the response of both fibres to GD (example on Figure 5-6 and Table 5-2). The response to GD in the other fibre influenced by central GABAb receptors ligands was not tested after any other treatment.

The responses to GD of the other 2 of 5 fibres tested with central baclofen were unchanged, even with a further bolus of the agonist, making the total dosage 6nmol/kg icv. The responses of these fibres were also unchanged by CGP35348, administered both

centrally (100nmol/kg icv) and systemically (100μmol/kg iv), and by systemic baclofen (7-14μmol/kg iv). The response to GD of one of those fibres was tested after the cumulative administration of each of the following drugs: CP99994 (12μmol/kg iv), CNQX (155mol/kg iv), pirenzepine (5μmol/kg iv), and hCGRP8-37 (3.2nmol/kg icv). No change in the pattern of response of this fibre to GD was discernible after treatment with any antagonist.

4.3.3 The dose dependent effects of baclofen

In 13 studies where GABAb receptor ligands were only administered systemically, baclofen was administered intravenously at doses of 7, 14 and 21 μmol/kg. In 7/13 studies, a dose of 7μmol/kg was sufficient to reduce or block the efferent responses to either GD or CorpD. In 3/13 studies, 14μmol/kg was required to modulate efferent responses to GD or CorpD. However, in 1 study, a cumulative dose of 21μmol/kg of baclofen only slightly attenuated the efferent response to GD. The effect of baclofen in this study can be seen in Figures 5-7 and 5-8. Blood pressure and intragastric pressure were monitored continuously. Baclofen was administered cumulatively in boli of 7μmol/kg and the efferent response to GD (40ml saline) obtained after each bolus. Mean arterial pressure decreased with each successive administration of baclofen. The rise in intragastric pressure with distension increased with increasing doses of baclofen, indicating a reduction in gastric compliance. However, the efferent response to GD was similar in intensity throughout the study, although a reduction in response was seen at a cumulative dose of 21μmol/kg.

Overall, baclofen attenuated the efferent responses to gastric or corpus distension in 10 of 13 fibres. This reduction in the efferent response was not dose dependent as a decrease in the response was seen in 7/8 fibres treated with a dose of 7μmol/kg, in 3/4 fibres treated with a dose of 14μmol/kg, and in 0/1 fibre treated a dose of 21μmol/kg.

Thus, although baclofen exerted a dose dependent effect on mean arterial pressure and mean gastric pressure during gastric distension, it did not have a similar effect on efferent discharge. In fact, the GABAb receptor agonist was either effective at low doses or ineffective, even at high doses, at reducing efferent responses to gastric distension.

4.3.4 Efferent discharge-intraluminal pressure relationship during gastric distension

In studies where the GABAb receptor ligands were only administered systemically, data from 7 studies in which baclofen reduced or blocked the efferent responses to distension of the whole stomach (GD) were divided into 2 groups: the first group consisted of 4/7 fibres that were excited by GD under control conditions and the second group contained the remaining 3 fibres that were inhibited by GD under control conditions. Baclofen attenuated the excitatory responses and reversed the inhibitory responses (Figure 5-9A). Intraluminal pressure was relatively unaffected by baclofen administration (Figure 5-9B). The relationship between vagal efferent discharge and intraluminal pressure was reduced for the excitatory responses after baclofen administration and reversed for the inhibitory responses, although changes in neither ratio were not statistically significant (Figure 5-9C).

4.4 Vagal efferent responses to colonic distension

The vagal efferent responses to colonic distension (ColD, 3ml saline) were tested in 2 fibres, with both showing inhibitory responses to this stimulus. These were rapidly evoked and maintained only for the duration of the stimuli (example on Figure 5-5).

Efferent responses in both fibres to ColD were abolished in the presence of baclofen (7μmol/kg iv) (example on Figure 5-5). However, CGP35348 (100μmol/kg iv) reversed the effects of baclofen only in one of 2 fibres. In the other fibre (Figure 5-5), even an additional 33% increase in the volume used for ColD failed to evoke a further change in efferent discharge.

Bilateral cervical vagotomy was performed in the study where CGP35348 reversed the effects of baclofen on the efferent response to colonic distension. The efferent response to colonic distension was abolished after vagal section.

4.5 Vagal efferent responses to cholecystokinin

In 9 studies, the vagal efferent responses to close intraarterial administration of cholecystokinin (CCK, 100pmol) were examined (Table 5-1 and 5-2). In 2 of 9 of these fibres, systemic baclofen was administered as the only treatment. In another 2 of 9 fibres, both baclofen and CGP35348 were administered systemically. In the remaining 5 studies, both GABAb receptor ligands were administered centrally and systemically.

In studies where the GABAb receptor ligands were only administered systemically, CCK inhibited efferent discharge in 2 of 4 fibres and caused excitation in the other 2 fibres under control conditions. These responses were evoked within 5seconds and lasted >5 minutes. After baclofen, the response to CCK was potentiated in one of 2 studies which responded with excitation. In the fibres which responded with inhibition to CCK under control conditions, baclofen caused the period of complete inhibition in 1 of 2 fibres to be shortened. The GABAb receptor agonist did not affect CCK responses in the other 2 fibres.

The responses shown in Figure 5-10 were obtained from the same efferent fibre as the one shown in Figure 5-4. CCK, under control conditions, evoked an increase in efferent discharge which lasted for <2minutes. After baclofen was administered, the excitatory response to CCK was of a greater magnitude and lasted for a longer period. The second fibre that responded with excitation to CCK under control conditions was not affected by systemic baclofen (not illustrated).

An example of the effect of GABAb receptor ligands on the inhibitory response to CCK is shown in Figure 5-11. The responses were obtained from the same fibre as the

one depicted in Figure 5-5. Under control conditions, CCK completely inhibited efferent discharge for approximately 7 minutes before activity slowly returned. Baclofen (7μmol/kg iv) did not reduce the depth of inhibition. However, the duration of complete inhibition was shortened. Subsequent CGP35348 administration did not reverse this shortened duration of complete inhibition. In the second fibre that responded with inhibition to CCK under control conditions, neither baclofen nor CGP35348 altered the duration of complete inhibition. In this study, the response to CCK was also maintained after subsequent bilateral vagal section.

In studies where the GABAb receptor ligands were administered both centrally and systemically, close intraarterial injection of CCK led to excitation of discharge in 1 fibre and complete inhibition of discharge in 4 other fibres under control conditions. In the fibre that was excited by CCK, neither GABAb receptor ligand, administered via either route, affected the efferent response to the octapeptide. Of the fibres that responded with inhibition to CCK under control conditions, central baclofen led to an reduction of the efferent response to CCK in all 4 fibres (Table 5-2). Baclofen attenuated the responses in 2 ways. In two fibres (Study 2 &3), the depth of inhibition was decreased, ie the inhibition of discharge seen was not complete after baclofen was administered centrally. In the other two fibres (Study 1 & 5), CCK still evoked a complete inhibition after central baclofen. However, the duration of complete inhibition in both efferent fibres in response to CCK was shortened.

The responses to CCK in the four fibres which responded with inhibition under control condirions were tested after central CGP35348 administration in 3 fibres. The depth of inhibition of one fibre's response to CCK had been attenuated by prior central baclofen administration (Study 3). In this study, central CGP35348 reversed the effects of baclofen such that a response similar to that obtained under control conditions was observed. In the other two studies (Study 1 & 5), central CGP35348 administration had no effect on the efferent response to CCK, ie the period of complete inhibition was unchanged by central GABAb receptor blockade.

The effects of systemic and central administration of baclofen and CGP35348 on the efferent responses to CCK were not consistent between the various fibres. The responses of individual fibres are described in the Appendix attached to the end of this chapter. There was, however, an effect which was seen consistently. CNQX (75-155nmol/kg icv) was administered in 3 studies where CCK evoked inhibitory responses under control conditions. In one study (Study 1), CNQX was administered after central and systemic baclofen and central CGP35348 administration. In 2 other studies (Study 2 & 5), CNQX was administered after central and systemic administration of both baclofen and CGP35348. In one of the 2 studies, CP99994 (12μmol/kg iv) was administered additionally after the GABAb receptor ligands. Prior treatment with GABAb receptor ligands only served to alter the duration of complete inhibition in response to CCK in 2/3 fibres while central baclofen attenuated the depth of response in the other fibre. Central CNQX, on the other hand, attenuated the depth of inhibition evoked by CCK in all 3 fibres tested.

4.6 Vagal efferent responses to bradykinin

Vagal efferent responses to bradykinin (BK, 18nmol) were obtained in 10 fibres (Tables 5-1 and 5-2). Under control conditions, 7/10 responses were inhibitory and the other 3/10 were excitatory. The responses were rapidly evoked, reaching a peak within the first 5 seconds, and lasted for <1minute (example on Figure 5-10). In 5 of these studies, baclofen was administered systemically as the only treatment. In the other 5 studies, treatments were administered both systemically and centrally.

In studies where baclofen was only administered systemically, the response of 1 fibre to BK was modulated such that the complete inhibitory response obtained under control conditions was abolished after baclofen (7μmol/kg iv). The responses of the other 4/5 fibres were unaffected by baclofen, even when administered at a dose of 14μmol/kg in 2 studies.

In studies where the GABAb receptor ligands were administered both systemically and centrally, neither baclofen nor CGP35348 given via either route modulated the efferent responses of 4/5 fibres tested. CNQX was administered as the last treatment in 2 of these 4 studies (Study 1 & 2). In both studies, central administration of CNQX led either to a partial or complete abolition in the response to BK. In the other 1 of 5 fibres (Study 5), BK completely inhibited efferent discharge under control conditions (see illustration on Figure 5-12). The direction of response was reversed to excitation with central baclofen, reversed a second time to complete inhibition with subsequent systemic baclofen. Central administration of CGP35348 then shortened the period of complete inhibition. The fourth treatment, systemic CGP35348, reversed the direction of response to one of excitation again. This profile of response to BK remained unchanged for the rest of the study, and was tested after treatment with each of the following drugs: CP99994 (12μmol/kg iv), CNQX (155nmol/kg icv), pirenzepine (5μmol/kg iv) and hCGRP8-37 (3.2nmol/kg icv).

4.7 Vagal efferent responses to capsaicin

Capsaicin (Cap, 65nmol) was administered close intraarterially in 9 studies (Tables 5-1 and 5-2), eliciting inhibitory responses in 6/9 fibres and excitatory responses in 3/9 fibres under control conditions. Like the efferent responses to bradykinin, the efferent responses to Cap were rapidly evoked and lasted for <1minute (example on Figure 5-10). In 5 of these studies, baclofen was administered systemically as the first and only treatment. In the other 5 studies, drugs were administered both systemically and centrally.

Of the four fibres which were tested only with systemic baclofen, the response to capsaicin of only one fibre was abolished by the GABA$_b$ receptor agonist. In this study, baclofen also abolished the response to bradykinin. The responses of the other 3/4 fibres to Cap were unaffected by baclofen.

In studies where the GABAb receptor ligands were administered both systemically and centrally, neither baclofen nor CGP35348 given via either route modulated the efferent responses of 4/5 fibres tested. In the only study (Study 5) where the bradykinin response was affected by GABAb receptor ligands, the response to capsaicin was also affected (see illustration on Figure 5-12). In this study, under control conditions, BK evoked a complete inhibition of discharge. The direction of response was reversed to one of excitation with central baclofen, reversed a second time to complete inhibition with subsequent systemic baclofen. Central CGP35348, the third treatment, then reversed the direction of response yet another time to one of excitation. This profile of response to Cap remained unchanged for the rest of the study, and was tested after treatment with each of the following drugs: systemic CGP35348 (100μmol/kg iv), CP99994 (12μmol/kg iv), CNQX (155nmol/kg icv), pirenzepine (5μmol/kg iv) and hCGRP8-37 (3.2nmol/kg icv).

5 DISCUSSION

In the previous chapter, GABAb receptors were found to be functionally expressed at the peripheral terminals of a proportion of corpus mechanoreceptors and not on mechanoreceptors from the oesophagus or antrum. In this chapter, the influences of GABAb receptors located elsewhere along the vagal reflex pathway were investigated. Results from this chapter suggest that 1) gastric mechanoreceptors may have presynaptic GABAb heteroreceptors located on their central terminals whereas those from CCK-sensitive afferents do not, 2) both populations of neurones release glutamate which then acts via non-NMDA receptors present on GABAergic interneurones, 3) the interneurones possess presynaptic GABAb autoreceptors and release GABA to act via GABAa receptors, 4) the strength of the heteroreceptors on gastric distension-sensitive neurones may be stronger than that of autoreceptors present on the interneurones, and 5) the central terminals of non-vagal colonic mechanoreceptors may possess presynaptic GABAb autoreceptors whereas those colonic mechanoreceptors following the vagal pathway may

have central presynaptic GABAb heteroreceptors. Lastly, as with data obtained in Chapter 3, there is heterogeneity in receptor expression within vagal efferent fibres.

5.1 GABAb receptor modulation of gastric mechanoreceptor inputs

It is assumed that the number of synapses involved in the vagal reflex pathway involving inputs from gastric mechanoreceptors is minimal as evidence for a monosynaptic vago-vagal reflex arc has been identified histologically[113, 243] and electrophysiologically[40, 44]. On the other hand, if the efferent response to a particular stimulus is inhibitory, it probably involves a GABAergic interneurone which activates GABAa receptors on vagal efferents in order to reverse the excitatory afferent response into an inhibitory efferent response. One can assume this because GABA is the only transmitter in the DVC which is capable of evoking rapid inhibition and because GABAa receptors have been identified within the DMVN[62].

Because the stomach was divided in only 3 of 13 vagal efferent studies and because the influences of central GABAb receptors were not investigated in isolation with the administration of baclofen centrally, it was not possible to ascertain whether central GABAb receptors influence the synaptic pathways of corpus tension receptors onto vagal efferent neurones differently to those of antral tension receptors. However, none of the efferent responses to antral distension were modulated by systemic baclofen whereas 2 of 3 efferent responses to corpus distension were markedly reduced, suggesting that the pathway involved in transmitting information from corpus mechanoreceptors onto vagal central neurones do vary from that arising from antral mechanoreceptors. The minor, if any, role of GABAb receptors in mediating inputs from antral mechanoreceptors is further verified by data from our afferent studies (see Chapter 4) in which baclofen did not modify the antral mechanoreceptor response to antral distension but had affected a proportion of corpus mechanoreceptor responses to corpus distension. Data obtained in this chapter also provides further confirmation that the effect of baclofen on blood pressure and gastric compliance is independent on its effect on vagal efferent and afferent activation.

There may be a tonic inhibitory influence of endogenous GABA. In Study 1, the excitatory response evoked by gastric distension under control conditions was abolished by central baclofen administration. However, subsequent central CGP35348 led to a response which was greater in intensity than the one obtained under control conditions. CGP35348 appeared to block the effects of the GABAb receptor agonist as well as any endogenous GABA which may have been tonically released. It also provides confirmation that the dose of CGP35348 was sufficient to block the GABAb receptors being studied.

It should also be noted that in no study were efferent responses to all stimuli tested affected by a particular drug treatment. This means that different neurotransmitters and/or neuromodulators are involved in modulating inputs from different populations of afferent fibres. It also reinforces one of the conclusions drawn from Chapters 2 & 3, ie that peripheral stimuli given activate different populations of afferents.

5.1.1 Heterogeneity of receptor expression

Consistent with the results of Chapters 3 & 4, evidence for the existence of a heterogenous population of fibres is also seen here, only here it is an heterogenous expression of receptors. When systemic baclofen was given without prior central administration, the responses to either whole gastric or corpus distension were greatly attenuated in 77% of studies (10/13 studies). However, baclofen was relatively ineffective in modulating efferent responses to gastric distension, even when administered at trice the standard dose, ie at of 21μmol/kg compared to 7μmol/kg. The ineffectiveness of baclofen in modulating efferent responses to gastric distension was independent of its effects on other parameters such as arterial blood pressure and intraluminal pressure.

More confirmation for the heterogeneity that exists within the vagal reflex pathway is found in the studies in which both GABAb receptor ligands were administered centrally and systemically. In 3 studies, a central dose of 3nmol/kg was sufficient to reduce the vagal efferent response to gastric distension. However, in the 2 other studies, central

baclofen administration at 6nmol/kg did not modulate efferent responses to gastric distension. The results suggest that neither central nor peripheral presynaptic GABAb receptors were involved in influencing inputs from gastric mechanoreceptors onto these particular vagal central neurones.

My data shows that in studies where baclofen was able to modulate afferent inputs from gastric mechanoreceptors, low doses of baclofen was sufficient. However, in other studies, increasing the dose of baclofen did not lead to a modulation of response. Together with my afferent data in Chapter 4 where only a proportion of corpus mechanoreceptors were affected by baclofen, this is consistent with the premise that GABAb receptors are present only on a proportion of vagal fibres arising from the stomach.

5.1.2 The relative importance of central GABAb receptors

Data from efferent studies showed that 77% (10 out of 13 fibres) of vagal efferent responses to gastric distension was markedly reduced systemic baclofen. This is a substantially higher than the 38% (3 of out 8 fibres) of gastric tension receptors which had reduced sensitivity to distension due to baclofen. The discrepancy between results from the afferent and efferent studies is due to either the efficacy of the drugs (unlikely from pharmacology) or the minor role of any GABAb receptors present on the peripheral terminals of the tension receptors compared with those which are positioned presynaptically at the central terminals of vagal gastric mechanoreceptors.

The relative importance of central GABAb receptors was confirmed by several findings: 1) when the efferent response to gastric distension was attenuated by central baclofen administration and subsequently reversed by central CGP35348, systemic administration of the same drugs did not affect the gastric distension response, 2) when the efferent response to gastric distension was not affected by central baclofen or CGP35348, neither systemic baclofen nor CGP35348 affected the response and 3) baclofen, administered systemically at a dose of 7μmol/kg, reduced the efferent responses

to gastric distension in 7/8 fibres tested whereas 14μmol/kg of sytemic baclofen was able to reduce the responses of only 3/8 gastric mechanoreceptors to distension (see Chapter 4).

Therefore, central GABAb receptors may modulate only a proportion of gastric mechanoreceptive inputs onto vagal central neurones. Where this modulation occurs, the relative contribution of peripheral GABAb receptors is minor. In those vagal efferent fibres with gastric mechanoreceptor inputs that are not influenced by central GABAb receptors, peripheral GABAb receptors also do not appear to play a role in the reflex pathway. This may be due to the large number of vagal afferent projections onto efferent neurones so that although some of the inputs may be attenuated peripherally, there remains to be an overwhelming influence of central GABAb receptors.

5.1.3 Interaction between central GABAb and non-NMDA receptors

The role of central presynaptic GABAb receptors in modulating inputs from gastric mechanoreceptors onto vagal central neurones was discussed in the section above. Also of interest was the neurotransmitter compound(s) whose release was inhibited by these central presynaptic GABAb receptors. As central non-NMDA receptor blockade attenuated a large proportion of efferent responses to gastric distension (Chapter 3), the involvement of non-NMDA receptors was investigated further in this chapter.

In studies where central non-NMDA receptor antagonism led to an attenuation of the efferent response to gastric distension, prior central baclofen and CGP35348 had reduced and reinstated the efferent response to gastric distension respectively. The influences of peripheral GABAb receptors in these responses were minimal as neither systemic baclofen nor CGP35348 affected the gastric distension responses. However, in the study where central non-NMDA receptor blockade did not affect the efferent response to gastric distension, prior GABAb receptor activation and blockade also did not modulate the efferent response.

In the cat, examples of adjacent glutamate and GABA immunoreactive terminals synapsing onto the same dendritic profile were quite frequent, and may provide an anatomical basis for the GABAergic mediated inhibition of glutamatergic excitatory inputs to the NTS[228]. Vagal afferent terminals which were immunoreactive for glutamate were also seen to form monosynaptic connections with dendrites or soma of neurones of the DMVN[229]. The interactions between excitatory and inhibitory amino acids, namely glutamate and GABA, have been studied previously by Bonanno et al[54] where presynaptic GABAb receptors are involved in modulating release of glutamate. The work was performed in rat cortical synaptosomes in which baclofen reduced potassium ion evoked release of glutamate. The effect in the synaptosomes was reversible with GABAb receptor antagonism. Furthermore, a study by Christenson and Grillner[70] in the isolated spinal cord of lampreys have shown that the primary afferents from skin mechanoreceptors used excitatory amino acids. These effects are mediated via the non-NMDA receptors and can be modulated by presynaptic GABAb receptors. Thus the evidence for non-NMDA receptors to interact closely with GABAb receptors is abundant in the literature.

My results seem to suggest the following: firstly, presynaptic GABAb receptors located on the central terminals of vagal gastric tension receptors are heteroreceptors, present on neurones which release glutamate to act via non-NMDA receptors. Secondly, the non-NMDA receptors are most probably present on GABAergic interneurones, which release GABA to act via GABAa receptors. Activation of the GABAa receptors will then reverse the excitation of discharge seen in the gastric mechanoreceptor response to gastric distension to the inhibition of discharge seen in some vagal efferent fibres in response to the same stimulus as IPSCs have been shown to be blocked by GABAa receptor antagonism[246]. Thirdly, where central GABAb receptor influences are not evident, neither peripheral GABAb nor central non-NMDA receptors are involved in modulating inputs from gastric mechanoreceptors.

5.2 GABAb receptor modulation of colonic mechanoreceptor inputs

The pathway taken by colonic mechanoreceptors may differ from that of gastric mechanoreceptors, the former involving mainly spino-bulbo afferent projections which may have distinct pharmacological features from vagal afferent pathways. CGP35348 reversed the effect of baclofen on all gastric distension responses whereas it was unable to reverse the effect of baclofen on the response to colonic distension in 1 of 2 studies. The differential effects of the CGP35348 on reversing the baclofen induced reduction of efferent responses may be due to the different GABAb receptor subtypes which have been identified[53, 163, 204]. A review by Bonanno[53] suggests that there are at least 4 GABAb receptor subtypes which are classified according to their sensitivity to baclofen, phaclofen\ which is another GABAb receptor antagonist, and CGP35348. These presynaptic GABAb receptors can either be autoreceptors, ie present on neurones which release GABA, or heteroreceptors, ie present on neurones which release either glutamate or somatostatin. Basically, autoreceptors are relatively insensitive to CGP35348, the antagonist used in my efferent studies, and may explain the results obtained here. Therefore, the presynaptic GABAb receptors present on pathways with inputs from colonic mechanoreceptors may be autoreceptors. However, I can only speculate on this as I have only investigated the effect of systemically administered GABAb receptor ligands on the inputs from colonic mechanoreceptors in 2 studies.

My data on GABAb receptor influences on colonic mechanoreceptor inputs is further complicated by the results obtained in 1 study where the response to colonic distension was abolished by bilateral vagotomy, performed as the last treatment. The loss of the colonic distension response with vagotomy implies that the afferent pathway taken by colonic mechanoreceptors is vagal, a finding that contradicts previous work[126, 129] using the same experimental model. In these studies by Grundy et al, the majority of vagal efferent responses to colonic distension were unaffected by bilateral vagotomy whereas only a few fibres showed a reduced response. It was concluded that the main source of afferent input from colonic mechanoreceptors was non-vagal. It should be noted that in my study where the response to colonic distension was abolished by vagotomy, systemic

CGP35348 was able to reverse the reduction in the colonic distension response caused by baclofen. Thus, it may be possible that presynaptic GABAb receptors present on the central terminals vagal gastric and colonic mechanoreceptors are heteroreceptors whereas those involving non-vagal colonic mechanoreceptors are autoreceptors. However, more work using different GABAb receptor ligands will need to be performed in order to further elucidate the neurotransmitters/neuromodulators involved in these pathways.

5.3 GABAb receptor modulation of oesophageal mechanoreceptor inputs

My data show that GABAb receptors are involved in modulating responses from oesophageal mechanoreceptors as 50% of vagal efferent responses (3/6 fibres tested) to oesophageal balloon distension were reduced by systemic baclofen. In one of these studies, prior central baclofen administration did not affect the efferent response to distension of the distal oesophagus, suggesting that the GABAb receptors involved were either located in the peripheral or located within the CNS outside the DVC, which is the presumed target of centrally administered drugs. If GABAb receptors are present centrally along the pathway from oesophageal mechanoreceptors, they are unlikely to be outside the DVC as I have shown that most, if not all, oesophageal mechanoreceptors which have inputs onto vagal central neurones are vagal (see Chapter 2). The other possibility, ie that GABAb receptors are located peripherally on oesophageal mechanoreceptors, may contradict the results obtained in the previous chapter (Chapter 4) where systemic baclofen did not affect the sensitivity of oesophageal mechanoreceptors to the same stimulus. However, it should be borne in mind that the number of afferent recordings is relatively small (n=2) while the sample size of the vagal efferent responses was larger (n=6). Also, recently obtained work from our laboratory with our *in vitro* electrophysiology preparation has shown vagal oesophageal mechanoreceptors are affected in their sensitivity to tension by baclofen. Obviously more work is needed to determine whether these GABAb receptors are mainly centrally or peripherally located. As the effects of CGP35348 on reversing the reduction caused by baclofen were not observed, I am unable to comment on whether these GABAb receptors are auto- or hetero-receptors.

5.4 GABAb receptor modulation of cholecystokinin-sensitive inputs

In Chapter 4, baclofen was suggested to modulate mucosal afferent responses to CCK indirectly through modulation of peripheral somatostatin levels. This is likely to apply similarly to excitatory efferent responses to CCK where baclofen potentiated the excitatory responses to CCK in 1/3 fibres.

Baclofen also modulated the inhibitory responses to CCK in 83% of fibres (5/6 fibres tested). This modulation was manifested as either a reduction in the depth of inhibition or a shortening in the period of complete inhibition. Subsequent CGP35348 administration reinstated the CCK response in only 1 of 6 fibres tested. Where GABAb receptor ligands were effective, the effect was seen with either route of administration. It should also be noted that central blockade of the non-NMDA receptors, regardless of the effects of prior administration of baclofen and CGP35348, was able to reduce the efferent response to CCK in all fibres tested.

The reduction in the duration of complete inhibition was only observed with the modulation of the CCK response by baclofen and contrasts with the effect of baclofen on the gastric distension response. Gastric distension generally evoked an efferent response which was rapidly evoked and maintained throughout the distension period, with efferent activity returning to predistension levels shortly after deflation. On the other hand, the CCK response is more slowly evoked and declined gradually over a period of several minutes. The gradual decline of the CCK response is easier to see with excitatory responses. Obviously, the difference in response profile may mean that drugs could affect the responses to the 2 stimuli differently. So whereas responses to gastric distension were obviously either reduced or unaffected by baclofen, the criteria used for the efficacy of the same drug may differ when considering slowly adapting responses.

There were several observations made from this portion of the study. Firstly, the effects of baclofen on CCK-sensitive inputs to vagal central neurones is likely to be central. This was inferred as baclofen modulated inhibitory CCK responses regardless of

the administration route. Secondly, these central GABAb receptors are likely to be autoreceptors as CGP35348, administered via either route, was largely ineffective in reversing the influence of baclofen[53]. Thirdly, the non-NMDA receptor may be involved in mediating at least a proportion of inputs from CCK-sensitive afferents. This is in agreement with the data described in Chapter 3 where, in experiments in which no GABAb receptor ligands were administered, the responses to CCK were modulated by CNQX in 3/5 fibres tested.

In an earlier section, I have shown that presynaptic GABAb heteroreceptors are located on neurones arising from gastric mechanoreceptors. These neurones probably release glutamate which act via non-NMDA receptors present on interneurones. In this current section, inputs from CCK-sensitive afferents, which are thought to be mainly vagal mucosal afferents, are also affected by central non-NMDA receptor antagonism. However, the presynaptic GABAb receptors present on the CCK-sensitive pathway appear to be autoreceptors, present on neurones which release GABA.

The discrepancy in the results above can be explained if we consider that the auto- and hetero-receptors are present on two synapses which are in series. The neurones arising from gastric mechanoreceptors may have presynaptic GABAb receptors located on their central terminals whereas those from CCK-sensitive afferents do not. Both populations of neurones release glutamate which then acts via non-NMDA receptors present on GABAergic interneurones. The interneurones also possess presynaptic GABAb receptors and release GABA to act via GABAa receptors. The strength of the heteroreceptors on neurones which receive gastric distension-sensitive inputs may be stronger than that of autoreceptors present on the interneuones. Therefore, CGP35348, which can block heteroreceptors present on glutamate releasing neurones, was able to reinstate the gastric distension response in all fibres tested. It may also explain why CGP35348 was able to only partially restore the inhibitory efferent responses to gastric distension in some fibres. As the autoreceptors present on the interneurones are CGP35348-insensitive, they would still be activated by baclofen and inhibit the release of GABA. On the other hand, the only

presynaptic GABAb receptors present on the CCK-sensitive pathway are on the interneurones which release GABA. Baclofen, administered either systemically or centrally, would block these autoreceptors. CGP35348 would then be unable to exert any discernible effects at the dose administered. It would also explain why CCK responses which were reduced by baclofen were also attenuated by central CNQX administration.

There was one other interesting observation about the CCK responses. In the one fibre where the efferent response to colonic distension was abolished by bilateral vagotomy (see section 5.2), the response to CCK was unchanged. This implies that the afferent arm of the pathway was non-vagal. I said previously that the data on this fibre's response to colonic distension contradicts previous findings. The data on the CCK response of the same fibre similarly contradicts findings by myself and others. In Chapter 1 and in studies by Blackshaw[38], close intraarterial CCK powerfully activates vagal mucosal afferents. However, this present study shows that non-vagal afferents can also be activated by cholecystokinin.

5.5 GABAb receptor modulation of bradykinin- and capsaicin-sensitive inputs

GABAb receptors appear to have a minor influence in the pathways receiving inputs from bradykinin- and capsaicin-sensitive afferents as only 2/10 efferent responses to bradykinin and capsaicin were modified by GABAb receptor activation. In both studies, efferent responses to both chemicals were affected, implying that bradykinin- and capsaicin-sensitive afferents may follow similar pathways.

In one study (Study 5), baclofen and CGP35348, rather than attenuating the response to these 2 chemicals, actually reversed the direction of response to both stimuli. The GABAb receptor ligands seemed to have opposing effects on the efferent responses to bradykinin and capsaicin depending on the administration route. The only other study where a reversal in the direction of response occurred with central blockade of both CGRP and non-NMDA receptors. This led to a reversal in the direction of the efferent response

to bradykinin and cholecystokinin and a marked reduction in the response to gastric and oesophageal distension (see Chapter 3). One piece of information inferred from that study was that up to 3 neurotransmitters may be involved in influencing afferent inputs.

The results from Study 5 may be explained when considering that inputs from bradykinin- and capsaicin sensitive afferents are most likely to follow a non-vagal, or spinal, pathway. When we consider the simplest scenario in which there are 2 GABAergic synapses involving the GABAb receptor along this pathway, one occuring within the spinal cord and another occuring within the DVC, the differential effects of central and systemically administered baclofen and CGP35348 may be explained. The first treatment was the administration of baclofen into the fourth ventricle. This would activate GABAb receptors located within the DVC, including those located presynaptically, and may lead to an inhibition in the release of particular neurotransmitter(s), allowing the effects of neurotransmitters released within other synapses which do not utilise GABAb receptors within the DVC to have a more predominant effect on vagal efferent neurones. These synaptic events would be reflected by the change of direction of the response. The second treatment, systemic administration of baclofen, may activate GABAb receptors located within the spinal cord and produce the same effect, ie inhibiting the release of one or more neurotransmitters and allowing the effects of others to have a larger involvement in signal transmission. Again, this may explain the second change in the direction of response. The third treatment, central CGP35348 administration, led to a shortening of the duration of complete inhibition evoked by bradykinin. This central GABAb receptor antagonism may lead to a restoration of the synaptic pathway within the DVC which has been previously disrupted by central baclofen. However, it may be that the influence of GABAb receptors in the spinal cord is greater than that within the DVC when considering inputs from bradykinin-sensitive afferents. This may also explain why systemic CGP35348, the fourth treatment, was able to reverse the direction of the BK response for the third time. However, the change in direction in the capsaicin response caused by the third treatment may reflect a difference in the balance of influence of vagal and non-vagal GABAergic pathway. Whereas the GABAb receptors located within the DVC may play a small role

overall with inputs from bradykinin-sensitive afferents, it may be possible that these same receptors may have a more dominant role with inputs from capsaicin-sensitivie afferents.

The question arises as to which neurotransmitters are involved in mediating inputs from bradykinin- and capsaicin-sensitive afferents. In study 5, antagonism of the NK-1, muscarinic M1, non-NMDA and CGRP receptors did not affect responses to either chemical. This means that in this particular study, none of the receptors were involved in mediating inputs from bradykinin- and capsaicin-sensitive afferents. Results from Chapter 3, however, indicate that CGRP and non-NMDA receptors are involved in mediating a large proportion of bradykinin- and capsaicin-sensitive inputs onto vagal central neurones.

5.6 CONCLUSIONS

It is obvious from the results of this and previous chapters that there is a highly complex organisation of neuronal networks present in the brainstem. Specifically from this chapter, it is proposed that presynaptic GABAb receptors are present on central terminations of primary afferent neurones as well as on interneurones. These play a major role in modulating inputs from gastric and colonic mechanoreceptors as well as from CCK-sensitive afferents which are likely to be mucosal fibres. The receptors appear to be both auto- and hetero-receptors, releasing GABA to act via the GABAa receptor and glutamate to act via the non-NMDA receptor respectively.

APPENDIX: Modulation of efferent fibre responses to peripheral stimuli

Due to the profound effects of systemically administered GABAb ligands on both afferent and efferent responses to gastric distension, the main objective of these studies was to elucidate the role of central GABAb receptors in the vagal efferent responses to gastric distension (GD). Thus, a different protocol was followed in each study. Therefore, the 5 studies will be described individually. In 4/5 studies, additional drugs were also given after the effects of central and systemic baclofen and CGP35348 on vagal efferent responses were ascertained. The effects of the various drugs on the efferent responses to each stimuli tested in the 5 studies are represented in Table 5-2.

STUDY 1

In this study, baclofen (3nmol/kg icv), CGP35348 (100nmol/kg icv), baclofen (14μmol/kg iv), hCGRP8-37 (3.2nmol/kg icv), and CNQX (75nmol/kg icv) were administered consecutively and the efferent responses to GD, BK, CCK and Cap were determined under control conditions and after each drug treatment.

Under control conditions, gastric distension (GD, 50ml saline) yielded a small, but consistent, increase in efferent discharge. Capsaicin (Cap, 65nmol close ia), bradykinin (BK, 18nmol close ia) and cholecystokinin (CCK, 100pmol close ia) led to complete inhibition of discharge. The period of complete inhibition due to Cap lasted for <1minute, to BK for 1.5 minutes, and to CCK for 7 minutes.

The excitatory response to GD obtained under control conditions was abolished with central baclofen. This was then reinstated with subsequent central CGP35348 administration. The response to GD after central GABAb receptor activation and blockade was more powerful than the control response. No changes in the efferent response to GD were evoked by subsequent systemic administration of baclofen and then CGP35348 even at twice the standard dose (14μmol/kg iv) of baclofen. Subsequent central hCGRP8-37

adminstration did not alter the response to GD. However, the final treatment with CNQX led to an abolition in the response to GD.

The period of complete inhibition of efferent discharge due to CCK was altered with the addition of various drugs. Under control conditions, CCK led to a period of complete inhibition that lasted for 7 minutes. Central baclofen administration reduced this period to 5 minutes. This was again reduced slightly after subsequent central administration of CGP35348 to 4 minutes. Systemic baclofen administered as the third treatment lengthened this period of complete inhibition to 7 minutes again.

Responses to Cap were maintained at each stage of the experiment, although it was not tested after systemic administration of baclofen or after central CNQX administration. The efferent response to BK was modulated only after central CNQX administration where the inhibition was significantly attenuated.

STUDY 2

In this study, baclofen (3nmol/kg icv), CGP35348 (100nmol/kg icv), baclofen (7μmol/kg iv), CGP35348 (100μmol/kg iv), and CNQX (75nmol/kg icv) were administered consecutively. Efferent responses to GD, BK, CCK and Cap were determined after each drug treatment. The effects of central GABAb receptor ligands and of central CNQX on the efferent responses to GD are illustrated on Figure 5-6.

Under control conditions, gastric distension (GD, 50ml saline), capsaicin (Cap, 65nmol close ia), bradykinin (BK, 18nmol close ia) and cholecystokinin (CCK, 100pmol close ia) led to an inhibition of discharge. The period of complete inhibition due to Cap and BK lasted for <15seconds, and to CCK for 2minutes.

The inhibition evoked by GD was complete and was revealed to consist of 2 components with central baclofen administration: an initial high threshold component,

which was not affected, and a secondary low threshold phase, which was abolished by central baclofen. This secondary component was reinstated with central of CGP35348 and persisted following peripheral administration of baclofen and CGP35348. Although peripheral administration of baclofen and CGP35348 did not further modulate the efferent response to GD, subsequent central CNQX again abolished the response to GD. This antagonism of the non-NMDA receptor abolished both phases of the inhibitory response to GD.

Responses to Cap and BK were maintained with central and systemic administration of baclofen and CGP35348. The response to BK was abolished after subsequent treatment with CNQX. The response to Cap was not tested after CNQX.

The response to CCK was attenuated by central baclofen and not tested after central CGP35348. Subsequent treament with systemic baclofen evoked a response that was similar to that obtained under control conditions, but with a slightly longer period of complete inhibition. Systemic CGP35348 administration did not affect the response profile although the period of complete inhibition was again lengthened. The last treatment with CNQX attenuated the response to CCK again, in a similar manner to that obtained after central baclofen administration.

STUDY 3

In this study, baclofen (3nmol/kg icv), CGP35348 (100nmol/kg icv), CGP35348 (100μmol/kg iv), and hCGRP8-37 (6.4nmol/kg icv) were administered consecutively. Bilateral vagotomy was performed as the last treatment in this study. Efferent responses to GD, BK, CCK and Cap were determined under control conditions and after each drug treatment.

Under control conditions, gastric distension (GD, 50ml saline), capsaicin (Cap, 65nmol close ia), bradykinin (BK, 18nmol close ia) and cholecystokinin (CCK, 100pmol

close ia) all evoked an inhibition of discharge. The period of complete inhibition due to Cap lasted for <15seconds, to BK for 2minutes and to CCK for 3minutes.

Like the efferent response to GD in Study 2, the inhibition of discharge in response to GD in this study was revealed by central baclofen to consist of 2 phases: an initial phase which was unchanged throughout the study, and a secondary response which was abolished by the central baclofen. This secondary phase returned with subsequent central application of CGP35348.

Responses to Cap and BK were unchanged with central and systemic administration of baclofen and CGP35348. After treatment with hCGRP8-37, the period of complete inhibition of discharge evoked by BK was shortened to <30 seconds. The response to Cap was unchanged after hCGRP8-37. Subsequent bilateral vagotomy did not alter responses to Cap or BK.

The effect of central baclofen and CGP35348 on the responses to CCK were similar to that on responses to GD. Central baclofen attenuated the CCK response which was then reinstated with subsequent adminstration of the antagonist centrally. The administration of systemic CGP3548 land central hCGRP8-37 did not alter the profile of the CCK response although the period of complete inhibition was altered by both treatments. Bilateral vagotomy, the last treatment in thid study, abolished the CCK response.

STUDY 4

In this study, baclofen (6nmol/kg icv), baclofen (14μmol/kg iv), CGP35348 (100nmol/kg icv) and CGP35348 (100μmol/kg iv) were administered consecutively. Efferent responses to GD, OBD, BK, CCK and Cap were determined under control conditions and after each drug treatment.

Under control conditions, gastric distension (GD, 50ml saline), oesophageal balloon distension (OBD, 2ml), capsaicin (Cap, 65nmol close ia), bradykinin (BK, 18nmol close ia) and cholecystokinin (CCK, 100pmol close ia) increased efferent discharge. The increases in efferent discharge due to Cap and BK were greater than that evoked by the 2 distension stimuli and CCK. These responses are illustrated in Figure 2-12.

The response to OBD was abolished with systemic administration of baclofen. None of the other responses were altered with any of the treatments, even with central baclofen administration at twice the standard dose.

STUDY 5

In this study, baclofen (6nmol/kg icv), baclofen (14μmol/kg iv), CGP35348 (100nmol/kg icv), CGP35348 (100μmol/kg iv), CP99994 (12μmol/kg iv), CNQX (155nmol/kg icv), pirenzepine (5μmol/kg iv) and hCGRP8-37 (3.2nmol/kg icv) were administered consecutively and the efferent responses to GD, OBD, BK, CCK and Cap were determined under control conditions and after each drug treatment. The effects of GABAb receptor ligands on the responses to BK and Cap are illustrated on Figure 5-12.

Under control conditions, gastric distension (GD, 50ml saline) and oesophageal balloon distension (OBD, 2ml air) yielded an increase in efferent discharge. Capsaicin (Cap, 65nmol close ia), bradykinin (BK, 18nmol close ia) and cholecystokinin (CCK, 100pmol close ia) led to a complete inhibition of discharge. The period of complete inhibition due to Cap and BK lasted for <15 seconds, and to CCK for 4.5minutes.

The responses to GD and OBD were unchanged by central and peripheral activation of GABAb receptors, even when baclofen was administered at twice the standard dose via both routes. GD was not influenced by treatment with any other drug. The response to OBD was attenuated by CNQX but not by any other treatment.

The responses to CCK were affected by both GABAb receptor ligands in that the duration of complete inhibition was altered by central and systemic administration of both baclofen and CGP35348. Central baclofen shortened the period of complete inhibition. Systemic baclofen, the second treatment, lengthened it to twice the duration of the response obtained under control conditions. The duration of complete inhibition was unchanged by central CGP35348 but shortened again by the systemic administration of the GABAb receptor antagonist. Subsequent systemic administration of CP99994 did not alter the response. Central CNQX, on the other hand, attenuated the depth of inhibition in response to CCK. Pirenzepine, did not affect the CCK response.

The responses to BK and Cap were greatly affected by central and peripperal administration of baclofen and CGP35348. Central baclofen reversed the direction of response to both chemicals from one of inhibition to one of excitation. Subsequent systemic baclofen again changed the direction of response to both BK and Cap from excitation to one of inhibition. When the third treatment, central CGP35348, was administered the duration of complete inhibition evoked by BK was shortened with this treatment. The direction of response to Cap was again reversed, this time from an inhibitory response to an excitatory one. With systemic CGP35348 administration, the direction of response to BK was reversed from one of inhibition to one of excitation. The Cap response was unaffected by systemic GABAb receptor antagonism. Subsequent cumulative administration of CP99994, CNQX, pirenzepine and hCGRP8-37 did not affect responses to either BK or Cap.

Table 5-1. Effect of the GABAb receptor ligands on vagal efferent fibres responses to peripheral stimuli. The direction and intensity of efferent response under control conditions is indicated by these symbols:
+ denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase: - denotes a partial inhibition; -- denotes a complete inhibition lasting <5minutes. Doses used are in μmol/kg iv. * indicates reinstatement of original response.

The majority of responses to oesophageal balloon distension (OBD, 1.5ml air), gastric distension (GD, 40-60ml saline), corpus distension (CorpD, 15-20ml saline), and antral distension (AD, 4ml saline) were excitatory while colonic distension (ColD, 3ml) elicited inhibitory responses. Cholecystokinin (CCK, 100pmol close ia), bradykinin (BK, 18nmol close ia) and capsaicin (65 close ia) evoked both excitatory and inhibitory responses.

Baclofen (7-21μmol/kg iv) was the first treatment administered in all studies. It had no effect on the majority of responses to OBD and AD. It reduced or abolished the response to GD in 8/10 fibres, to CorpD in 2/3 fibres, and to ColD in 2/2 fibres. With the efferent response to CCK, baclofen shortened the duration of complete inhibition in 1/2 fibres (indicated by §) which responded with inhibition of discharge and potentiated the response of 1/2 fibres which responded with excitation of discharge. It also abolished the response to BK and Cap in 1/5 fibres.

The GABAb receptor antagonist CGP35348 (100μmol/kg iv) was administered after baclofen in 2 studies. The efferent responses to gastric distension were reinstated by CGP35348 in 2/2 fibres (indicated by *). CGP35348 also reversed the effects of baclofen on the ColD response in 1/2 fibres. It did not affect any responses to CCK.

Bilateral vagotomy, was performed after baclofen and CGP35348 administration in 1 study (7/8/97). It abolished the responses to GD and ColD while leaving the response to CCK unaffected.

Unit ID	Dose	Subsequent Treatments	OBD		GD		CorpD		AD		ColD		CCK		BK		Cap	
				%Cont		%Cont		%Cont		%Cont		%Cont		%Cont		%Cont		%Cont
28/5/96	14				++	0												
24/6/96	7		++	100	++	0									--	0	-	0
12/7/96	7				--	0									--	100	--	100
19/7/96	14				++	100									-	100		
7/8/96	14		+	0	+	0												
13/8/96	21				++	100												
9/7/97	7				--	0												
16/7/97	7	CGP35348, BiVX			--	0*					--	0*	--	80§				
7/8/97	7	CGP35348			--	0*					--	0	--	100				
18/9/97	7		+	100	--	0												
7/11/96	7		+	50			+	0	+	100			+	130	+	100	+	100
8/11/96	14						++	30	++	100			++	100	+	100	+	100
25/11/96	7						+	100	+	100								
Total No. of Responses Studied			**4**		**10**		**3**		**3**		**2**		**4**		**5**		**4**	
Drug Effect		-None	2		2		1		3		0		2		4		3	
		-Reduced >50%Control	0		0		0		0		0		1§		0		0	
		-Reduced ≤50%Control	1		0		1		0		0		0		0		0	
		-Blocked	1		8		1		0		2		0		1		1	
		-Potentiated	0		0		0		0		0		1		0		0	

Table 5-2. Effects of GABAb ligands and other drugs on vagal efferent responses to peripheral mechanical, chemical, and pharmacological stimuli.
The direction and intensity of efferent response under control conditions is indicated by these symbols: + denotes 50-100% increase from basal discharge rates: ++ denotes >100% increase; -- denotes a total inhibition: (x) denotes the period of total inhibition in minutes.

The effect of each drug treatment on the efferent response to each peripheral stimulus is indicated by the symbols: ↔ no change in efferent response, ↓ attenuation in efferent response, ↓↓ total abolition of the efferent response, ∅ return of efferent response to original response, ℜ reversal of the direction of the efferent response, n/t not tested.

The responses of 5 vagal efferent fibres to oesophageal balloon distension (OBD 1.5-2ml air), gastric distension (GD, 40-60ml saline), cholecystokinin (CCK, 100pmol close ia), bradykinin (BK, 18nmol close ia) and capsaicin (Cap, 65 nmol close ia) were tested under control conditions and after each drug treatment.

The treatments administered were baclofen (GABAb receptor agonist), CGP35348 (GABAb receptor antagonist) hCGRP8-37 (CGRP receptor antagonist), pirenzepine (M1 receptor antagonist), CNQX (non-NMDA receptor antagonist), CP99994 (NK-1 receptor antagonist), and BiVX (bilateral vagotomy).

The efferent responses to GD of the studies on the left column were reduced or abolished by central baclofen and reversed by central CGP35348. These were also unaffected by subsequent systemic baclofen or CGP35348 but were later abolished again by central CNQX administration, which was the last treatment in the study. The efferent responses to GD of the studies on the right column remained unchanged by either GABAb receptor ligand administered via both routes.

STUDY 1

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	0	+	--(7.0)	--	--
Baclofen icv (3nmol/kg icv)		↓↓	↔(5.0)	↔	↔
CGP35348 icv (100nmol/kg icv)		∅	↔(4.0)	↔	↔
Baclofen iv (14µmol/kg iv)		↔	↔(7.0)	↔	↔
hCGRP8-37 icv (3.2nmol/kg icv)		↔	↔(5.0)	↔	↔
CNQX icv (75nmol/kg icv)		↓↓	↓	↓	n/t

STUDY 2

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	0	--	--(2.0)	--	--
Baclofen icv (3nmol/kg icv)		↓↓	↓	↔	↔
CGP35348 icv (100nmol/kg icv)		∅	n/t	↔	↔
Baclofen iv (14µmol/kg iv)		↔	↔(1.0)	↔	↔
CGP35348 iv (100µmol/kg iv)		↔	↔(3.5)	↔	↔
CNQX icv (75nmol/kg icv)		↓↓	↓	↓	n/t

STUDY 3

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	0	--	--(3.0)	--	--
Baclofen icv (3nmol/kg icv)		↓↓	↓	↔	↔
CGP35348 icv (100nmol/kg icv)		∅	∅(3.0)	↔	↔
CGP35348 iv (100µmol/kg iv)			↔(7.0)	↔	↔
hCGRP8-37 icv (6.4nmol/kg icv)			↔(3.5)	↔	↔
BiVX			↓↓	↔	n/t

STUDY 4

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	+	+	+	+ +	+ +
Baclofen icv (6nmol/kg icv)	↔	↔	↔	↔	↔
Baclofen iv (14µmol/kg iv)	↓↓	↔	↔	↔	↔
CGP35348 icv (100nmol/kg icv)		↔	↔	↔	↔
CGP35348 iv (100µmol/kg iv)		↔	↔	↔	↔

STUDY 5

Treatment	Peripheral Stimuli				
	OBD	GD	CCK	BK	Cap
Direction of Control Response	+	+	--(4.5)	--(0.5)	--(0.5)
Baclofen icv (6nmol/kg icv)	↔	↔	↔(1.0)	ℜ	ℜ
Baclofen iv (7µmol/kg iv)	↔	↔	↔(9.5)	ℜ(3.0)	ℜ(0.5)
CGP35348 icv (100nmol/kg icv)	↔	↔	↔(8.5)	↔(0.5)	ℜ
CGP35348 iv (100µmol/kg iv)	↔	↔	↔(1.5)	ℜ	↔
CP99994 iv (12µmol/kg iv)	↔	↔	↔(1.5)	↔	↔
CNQX icv (155nmol/kg icv)	↓	↔	↓	↔	↔
Pirenzepine iv (5µmol/kg iv)	↔	↔	↔	↔	↔
hCGRP8-37 icv (3.2nmol/kg icv)	↔	↔	n/t	↔	↔

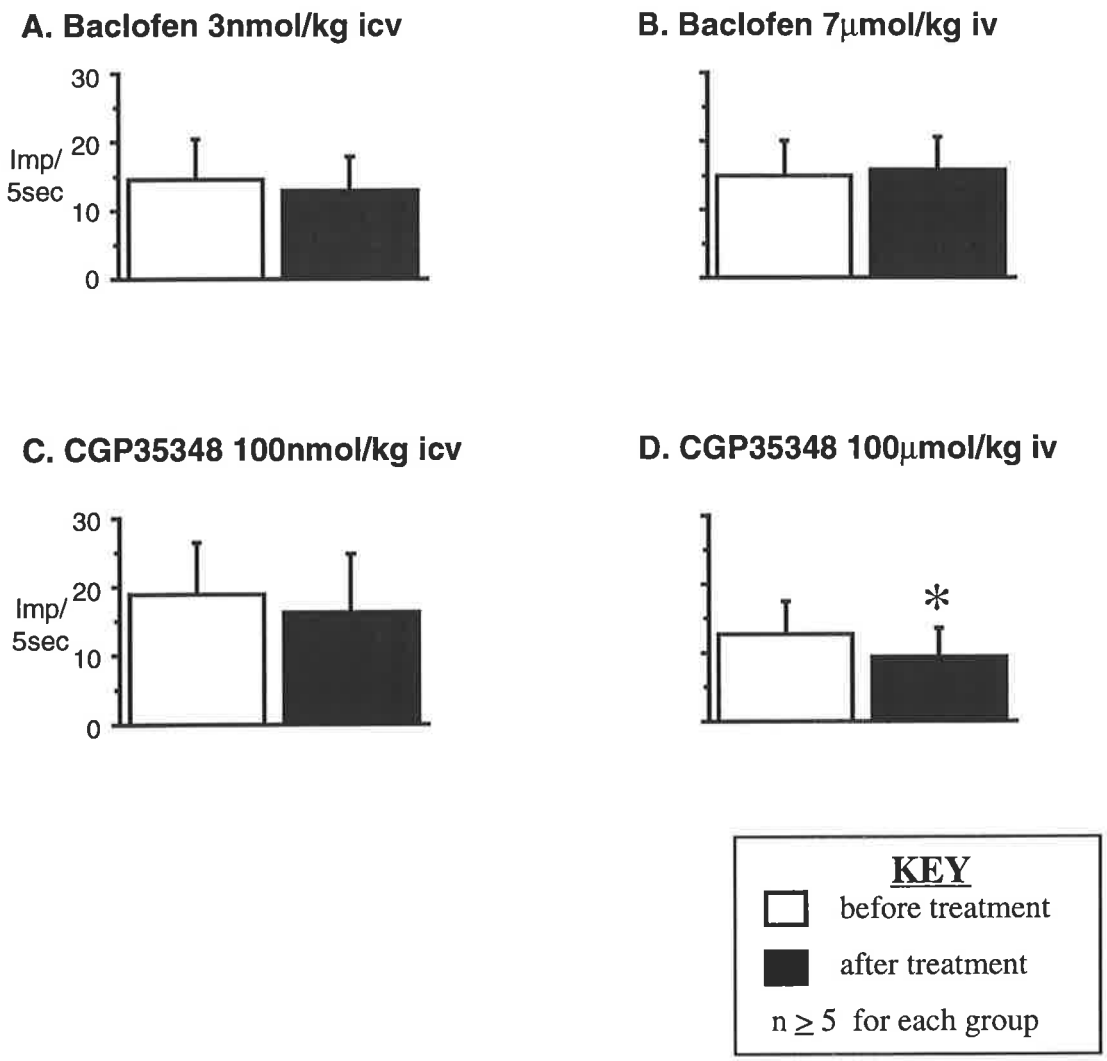


Figure 5-1. Group data showing GABAb receptor influences on basal vagal efferent discharge.

A-B. Basal vagal efferent discharge remained unchanged with both central and systemic administration of baclofen.

C-D. Basal vagal efferent discharge remained unchanged with central administration of CGP35348, but was significantly reduced after systemic CGP35348.

* $p \leq 0.05$ using paired t-test

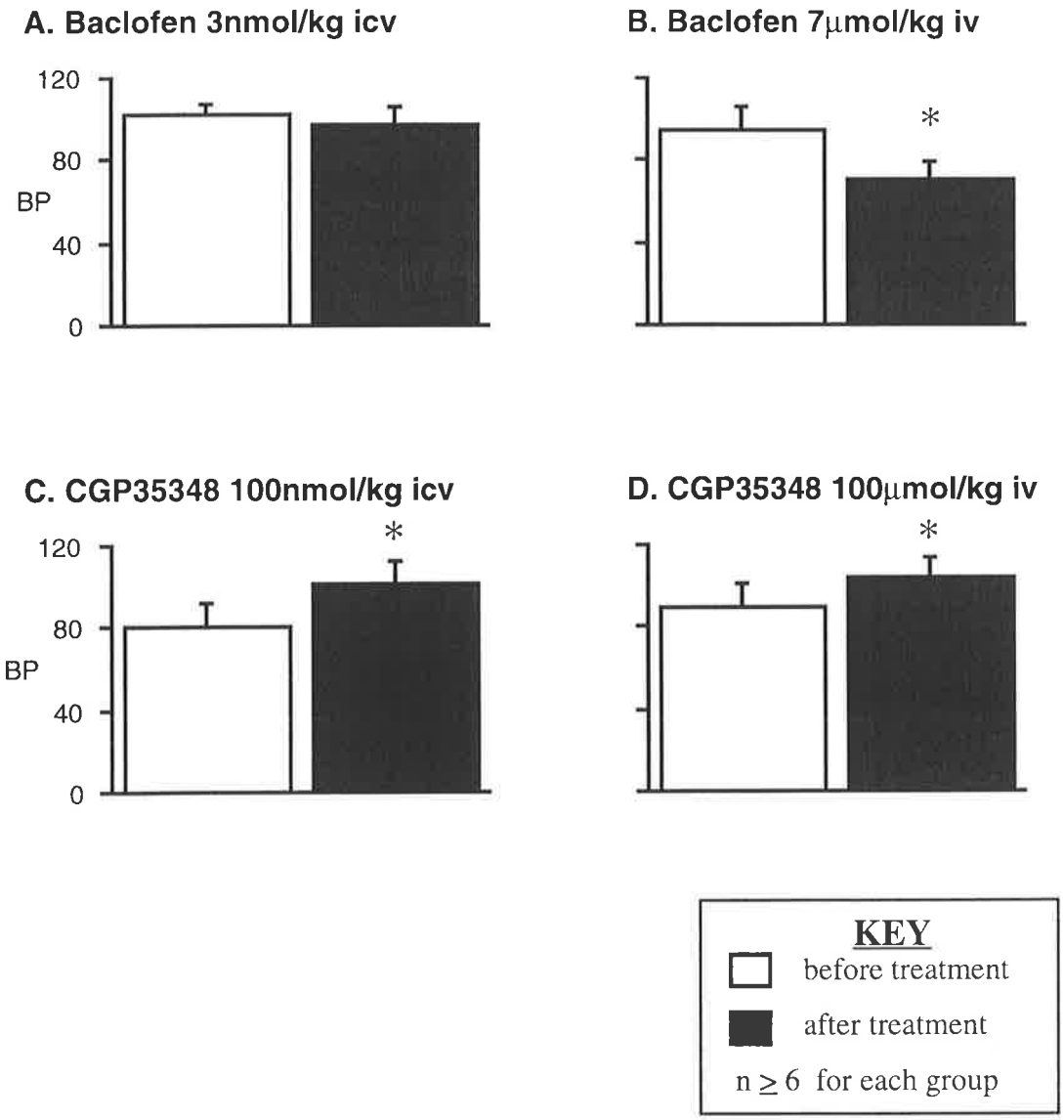


Figure 5-2 Group data showing GABAb receptor influences on mean arterial pressure.

A-B. Blood pressure was reduced following both central and systemic administration of baclofen, although this reduction was not significant with central GABAb receptor agonism.

C-D. The mean arterial pressure was significantly potentiated with both central and systemic administration of CGP35348.

* $p \leq 0.05$ using paired t-test

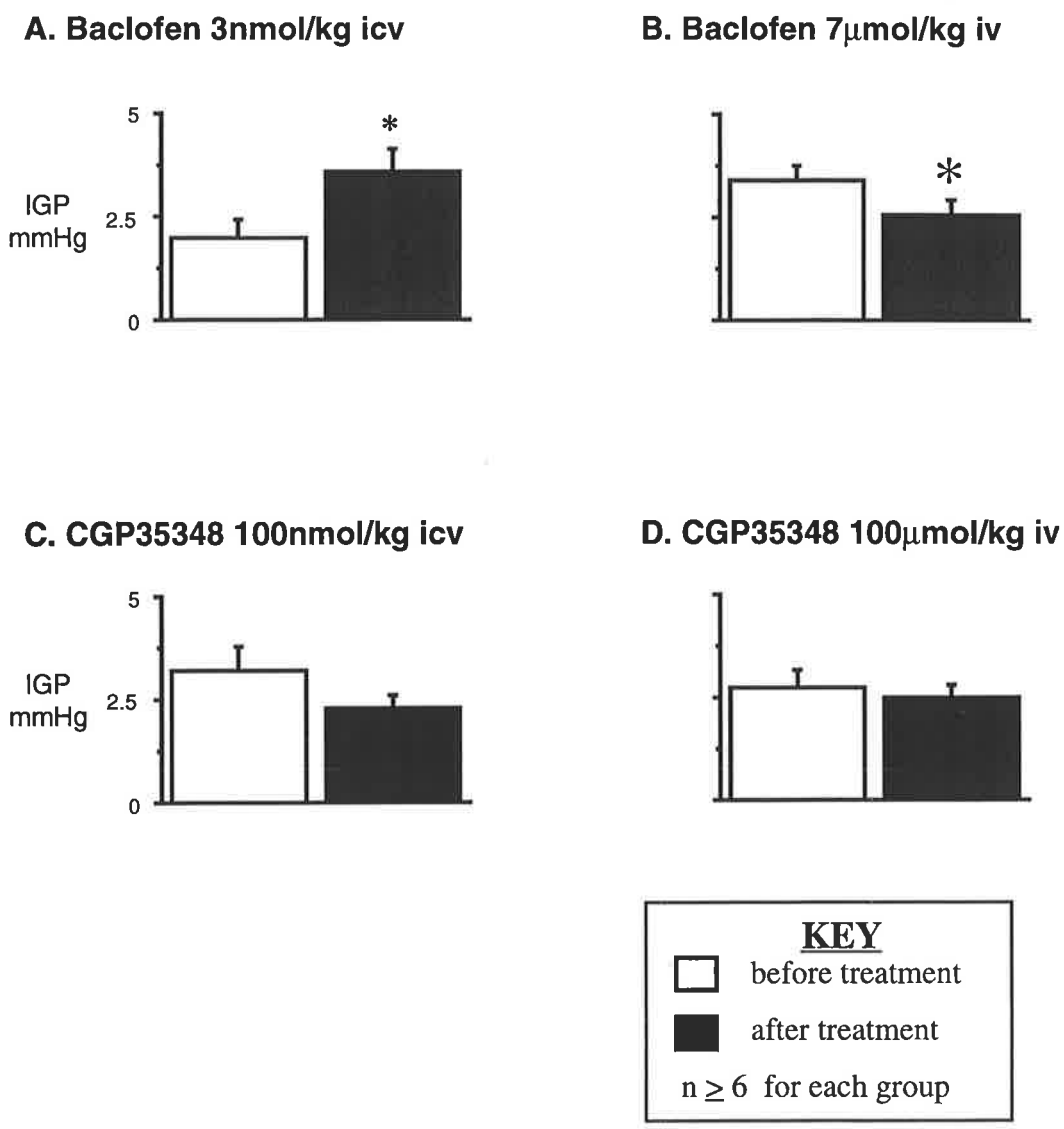


Figure 5-3. Group data showing GABAb receptor influences on basal gastric pressure.
Basal gastric pressure was measured immediately before and after each treatment.

A-B. Basal gastric pressure was significantly increased following central administration of baclofen, and reduced significantly with systemic baclofen.

C-D. Basal gastric pressure was attenuated with both central and systemic administration of CGP35348.

* $p \leq 0.05$ using paired t-test

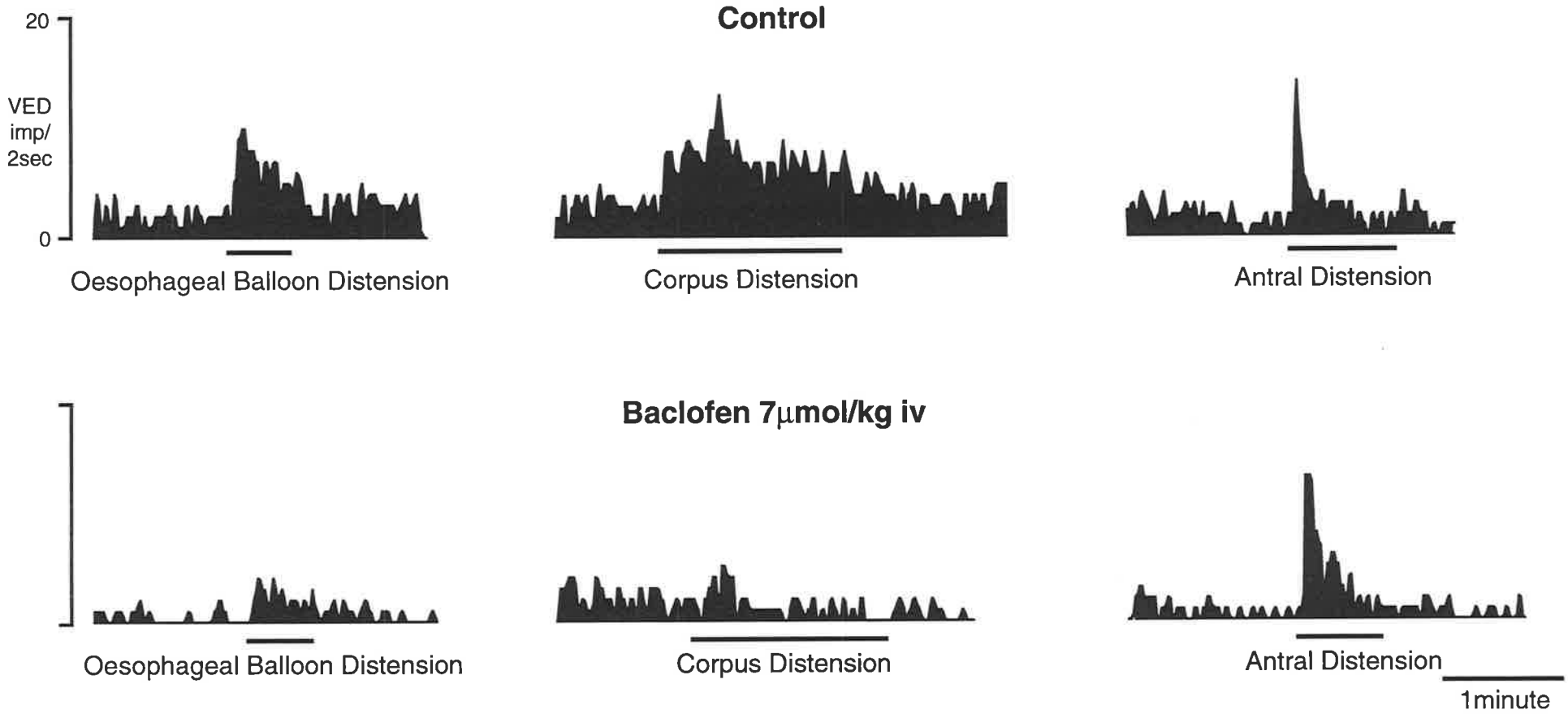


Figure 5-4. Effect of baclofen on the response of a vagal efferent fibre to distension.
Oesophageal balloon distension (OBD. 2ml air), corpus distension (CorpD, 20ml saline), and antral distension (AD, 20ml saline) evoked increases in vagal efferent discharge.

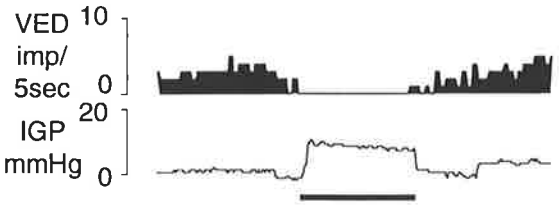
Baclofen (7μmol/kg iv) attenuated the response to OBD, abolished the response to CorpD, but did not affect the response to AD.

Figure 5-5 Effects of GABAb receptor agonist and antagonist on vagal efferent and intraluminal pressure responses to gastric and colonic distension.

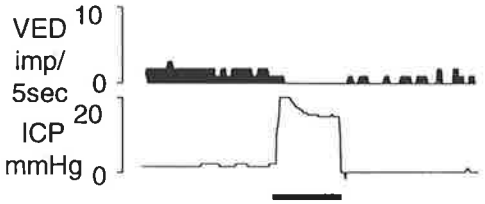
Top trace: Under control conditions, both gastric and colonic distension completely inhibited vagal efferent discharge. These responses were blocked by baclofen (7μmol/kg iv). Subsequent administration of CGP35348 (100μmol/kg iv) reversed the efferent response to gastric distension but not to colonic distension.

Bottom trace: Mean gastric pressure during gastric distension was increased after baclofen (7μmol/kg iv) and was unaffected by subsequent CGP35348 (100μmol/kg iv) administration. Neither baclofen nor CGP35348 affected the intraluminal pressure response to colonic distension. The pressure tracings were truncated at the start and end of the distension period in order to display the changes in intragastric pressure during distension more clearly. The increase at the start of distension was caused by the high positive pressure generated when fluid was infused via the pressure transducer into the stomach. The decrease at the end of the period was due to the negative pressure caused by the rapid removal of gastric fluid with a large syringe.

Control

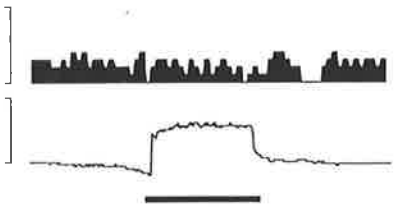


Gastric Distension
40 ml saline

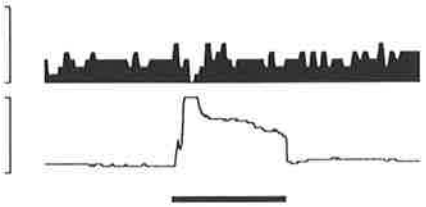


Colonic Distension
3ml saline

Baclofen 7μmol/kg iv

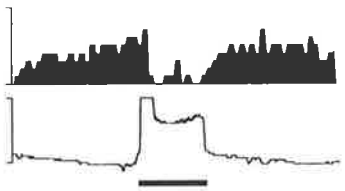


Gastric Distension
40 ml saline

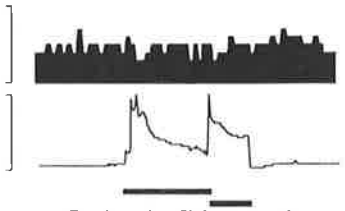


Colonic Distension
3ml saline

CGP35348 100μmol/kg iv



Gastric Distension
40 ml saline



Colonic Distension
3 + 1ml saline

30 sec

Figure 5-6 GABAb and non-NMDA receptor influences on vagal efferent and intraluminal responses to gastric distension. Data obtained from STUDY 2.

Gastric distension (50ml saline) inhibited the efferent discharge and caused an increase in intraluminal pressure under control conditions. Central baclofen (3nmol/kg icv) administration blocked the efferent response and decreased the pressure response to distension. This effect on efferent discharge was reversed with central administration of the antagonist CGP35348 (100nmol/kg icv). Both GABAb receptor ligands were then administered systemically (not illustrated here). Neither baclofen (14μmol/kg iv) nor CGP35348 (100μmol/kg iv) affected the reponses to gastric distension. Subsequent central blockade of the non-NMDA receptor with CNQX again led to the abolition of the efferent response.

The pressure tracings were truncated at the start and end of the distension period in order to display the changes in intragastric pressure during distension more clearly. The increase at the start of distension was caused by the high positive pressure generated when fluid was infused via the pressure transducer into the stomach. The decrease at the end of the period was due to the negative pressure caused by the rapid removal of gastric fluid with a large syringe.

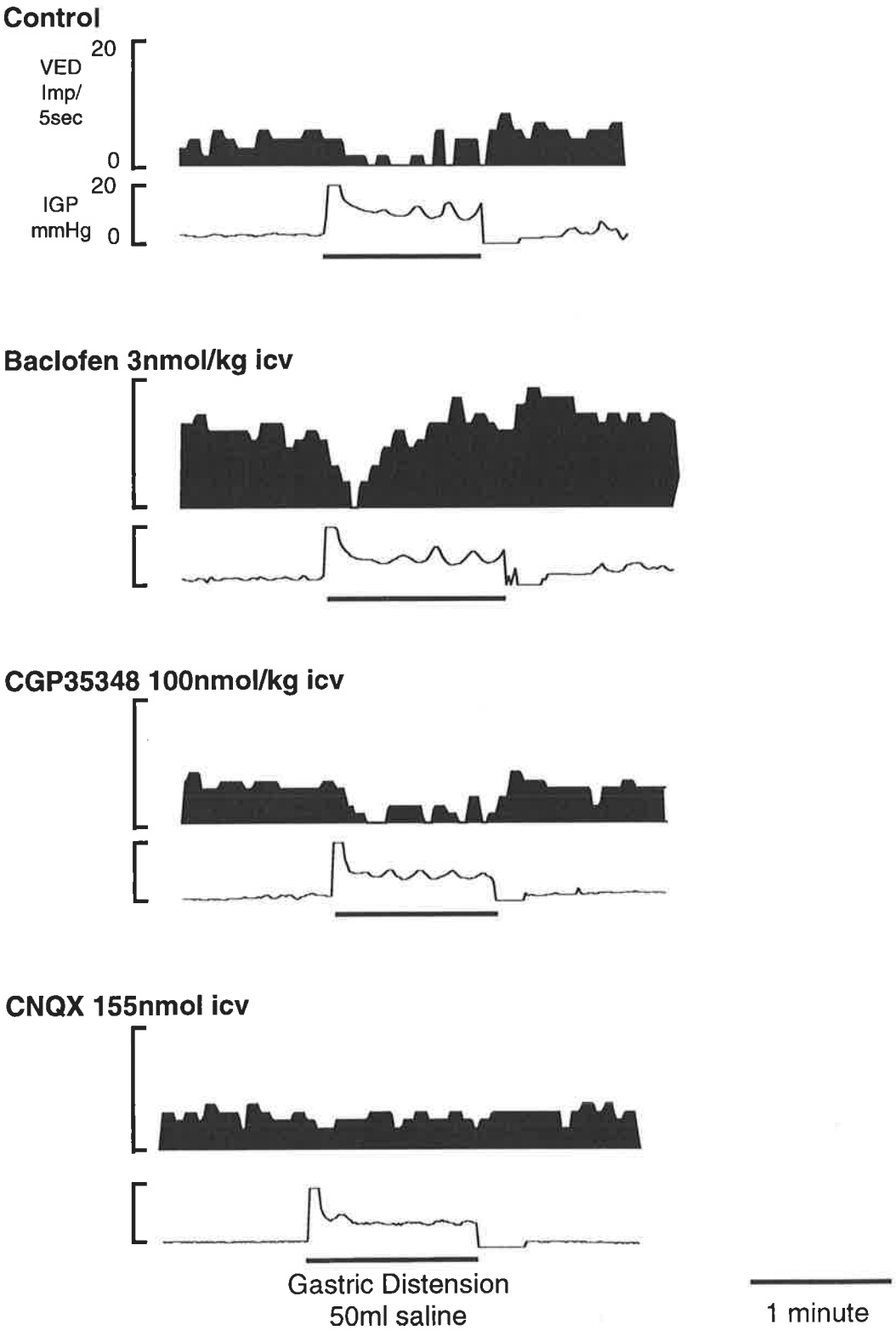

Control
20
VED
Imp/
5sec
0
20
IGP
mmHg
0
Baclofen 3nmol/kg icv
CGP35348 100nmol/kg icv
CNQX 155nmol icv
Gastric Distension
50ml saline
1 minute

Figure 5-7. Effects of baclofen on vagal efferent discharge, intraluminal pressure and blood pressure in response to gastric distension.

The effects of baclofen on vagal efferent discharge, intraluminal pressure, and blood pressure under resting conditions and during gastric distension (40ml saline) were observed in this experiment. Baclofen was adminstered intravenously in boli of 7μmol/kg iv.

Top trace: Vagal efferent responses to gastric distension were largely unaffected by baclofen administration, even up to a cumulative dose of 21μmol/kg iv. Note that the basal efferent discharge decreased consistently with increasing doses of the GABAb receptor agonist.

Middle trace: Intragastric pressure during distension increased with increasing doses of baclofen. The pressure tracings were truncated at the start and end of the distension period in order to display the changes in intragastric pressure during distension more clearly. The increase at the start of distension was caused by the high positive pressure generated when fluid was infused via the pressure transducer into the stomach. The decrease at the end of the period was due to the negative pressure caused by the rapid removal of gastric fluid with a large syringe.

Bottom trace: Blood pressure consistently decreased with increasing doses of baclofen.

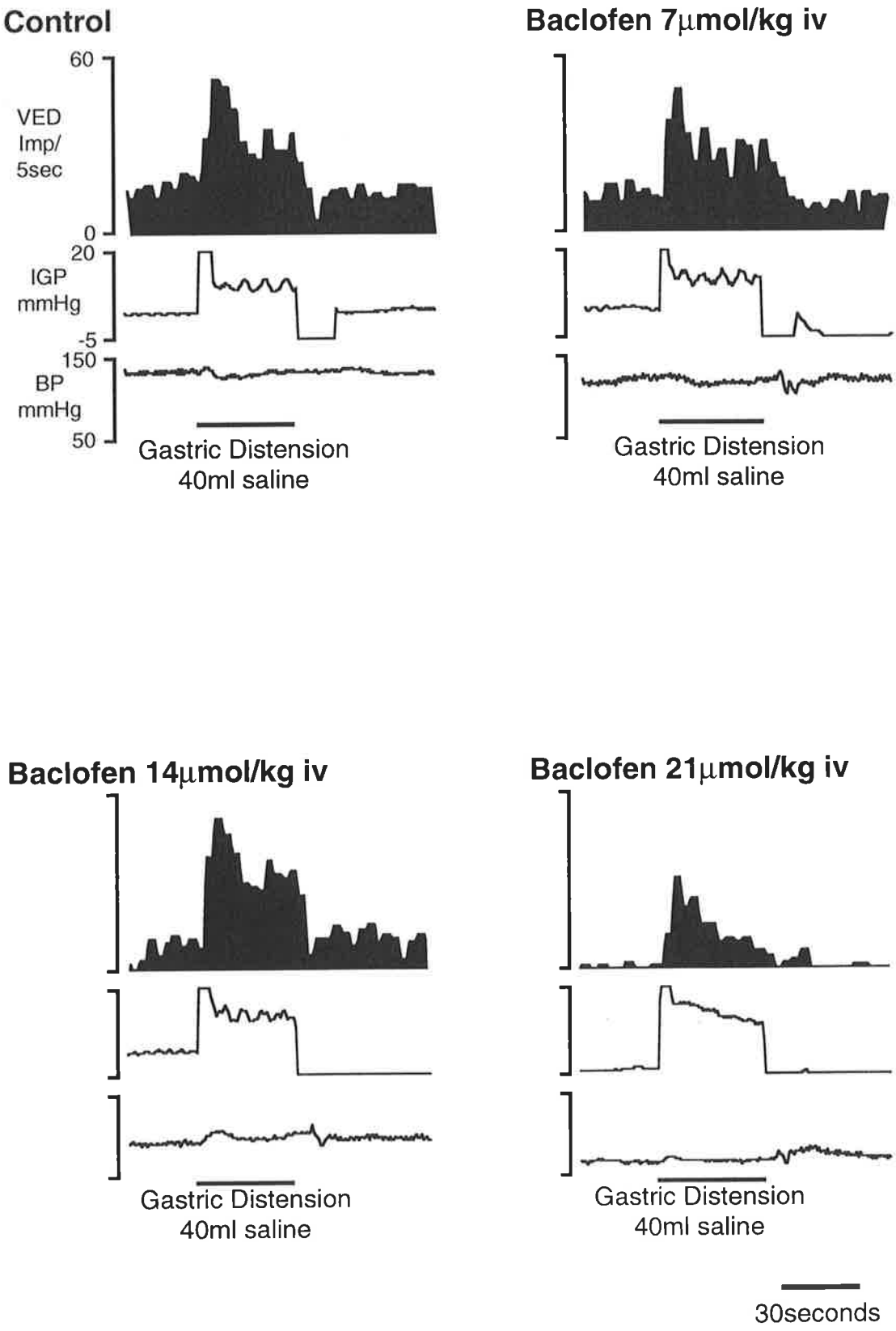

Control
60
VED
Imp/
5sec
0
20
IGP
mmHg
-5
150
BP
mmHg
50
Gastric Distension
40ml saline
Baclofen 7μmol/kg iv
Gastric Distension
40ml saline
Baclofen 14μmol/kg iv
Gastric Distension
40ml saline
Baclofen 21μmol/kg iv
Gastric Distension
40ml saline
30seconds

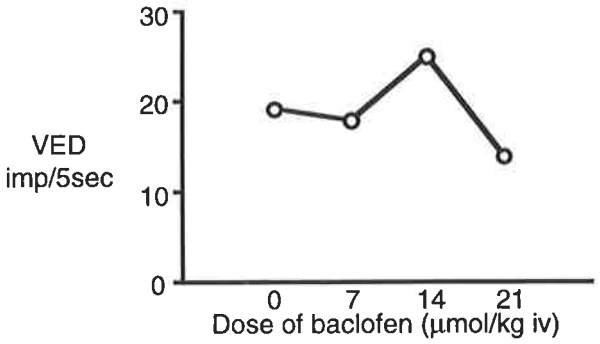
Figure 5-8. Effect of baclofen on vagal efferent discharge, intragastric pressure and mean arterial pressure in a single experiment.
These measurements were obtained from the study depicted in Figure 5-7.

A. Baclofen (7-21μmol/kg iv) slightly attenuated the vagal efferent response (VED) to gastric distension (60ml saline).

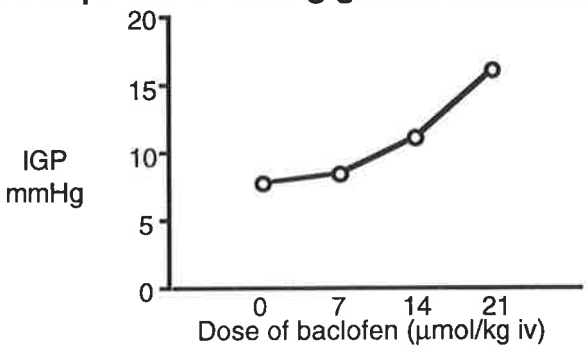
B. Intragastric pressure (IGP) during gastric distension increased with increasing doses of baclofen.

C. Mean arterial pressure (MAP) decreased dramatically with increasing doses of baclofen, indicating that the GABAb receptor agonist was effective in this experiment.

A. Vagal efferent discharge during gastric distension



B. Intragastric pressure during gastric distension



C. Basal blood pressure

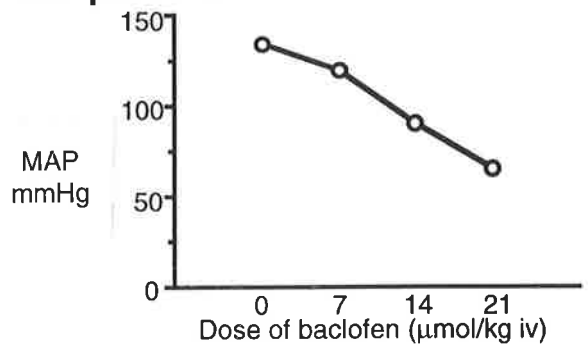
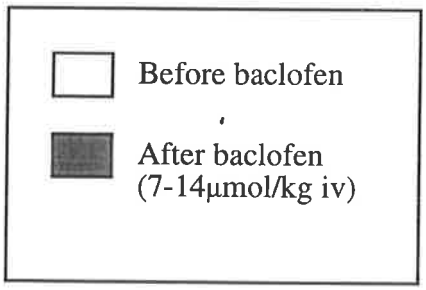
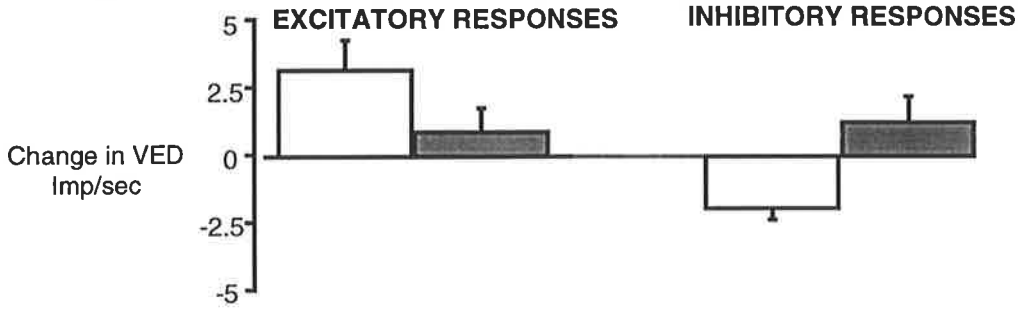


Figure 5-9. GABAb receptor influences on gastric mechanics and vagal efferent sensitivity during gastric distension(40-60ml saline).

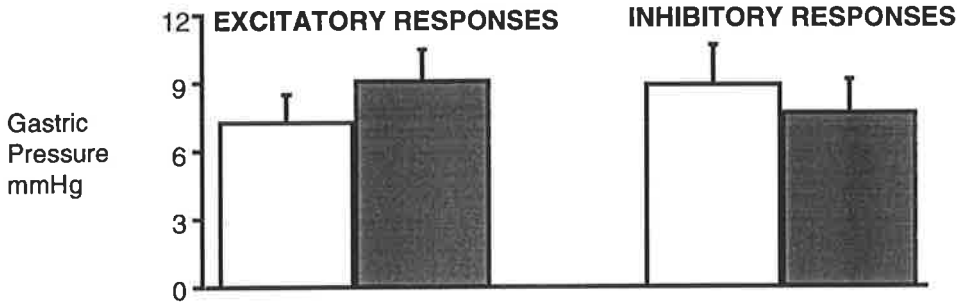
A. Baclofen (7-14μmol/kg iv) attenuated both excitatory (n=3) and inhibitory (n=4) vagal efferent responses to gastric distension.
B. Intraluminal pressure during gastric distension was relatively unchanged after baclofen administration.
C. The relationship between efferent discharge and intraluminal pressure was changed after baclofen administration, although this was not significant (using a combined Wilcoxon test).



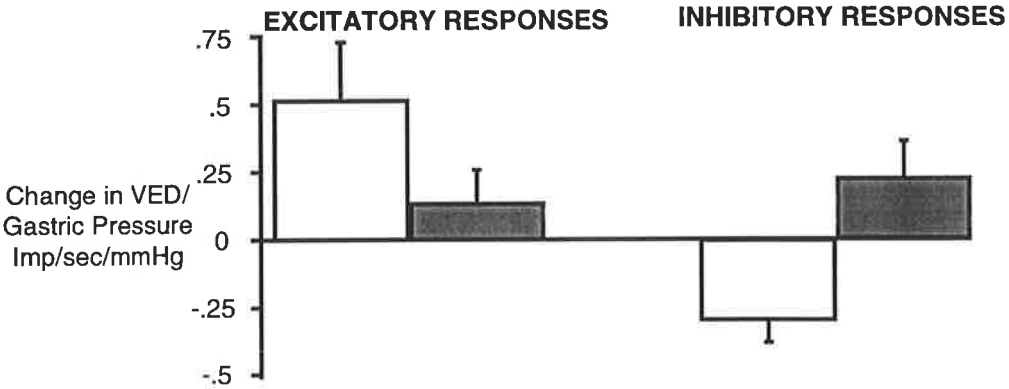
A. Vagal Efferent Responses



B. Intra-luminal Pressure Responses



C. Vagal Efferent Discharge-Intraluminal Pressure Relationship



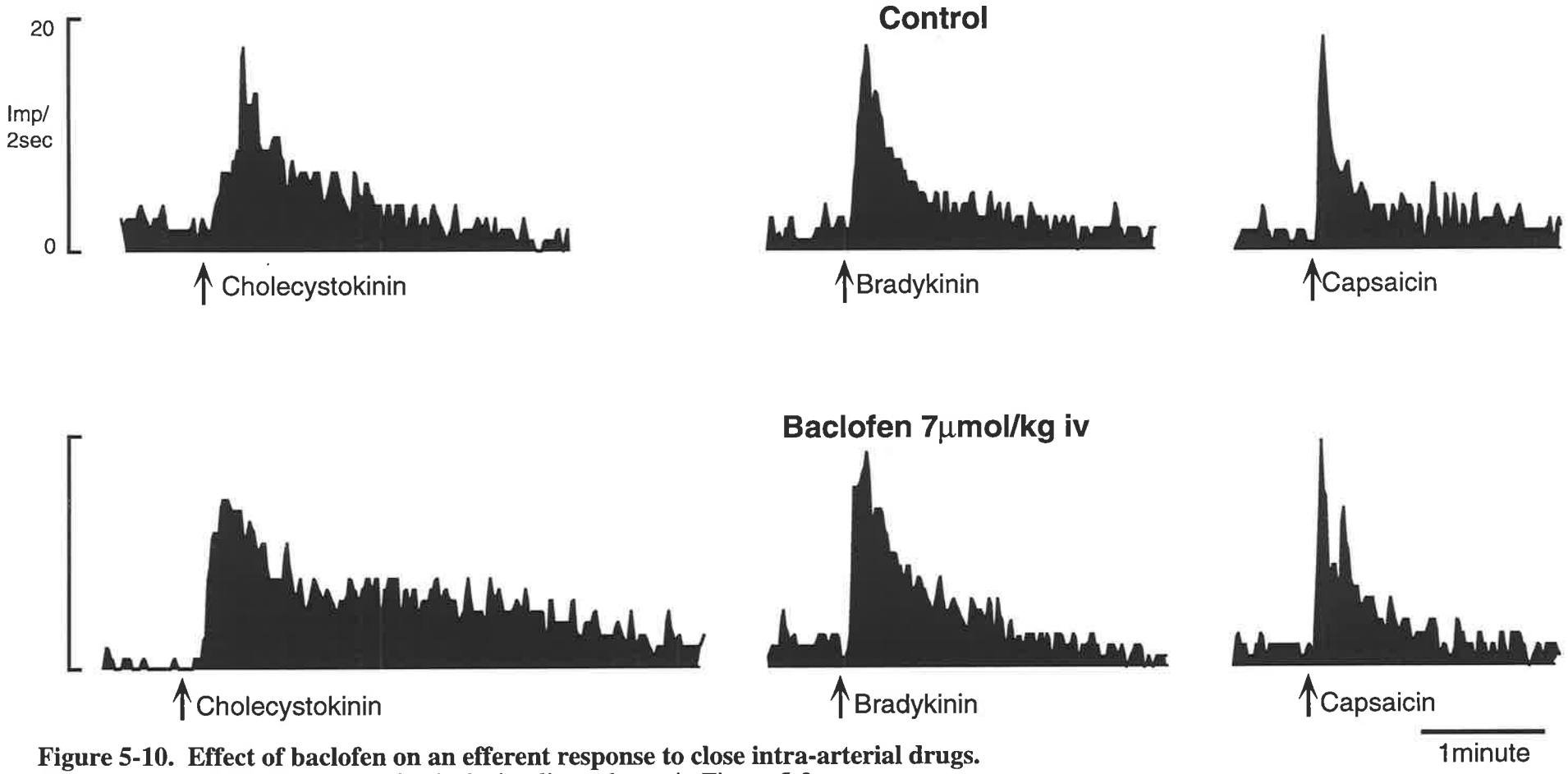


Figure 5-10. Effect of baclofen on an efferent response to close intra-arterial drugs.
The responses of this fibre to mechanical stimuli are shown in Figure 5-2.

Cholecystokin (CCK, 100pmol), bradykinin (BK, 18nmol) and capsaicin (Cap, 65nmol) evoked rapid increases in vagal efferent discharge.

Baclofen (7μmol/kg iv) increased the intensity and duration of the efferent response to CCK but did not affect the responses to BK or Cap.

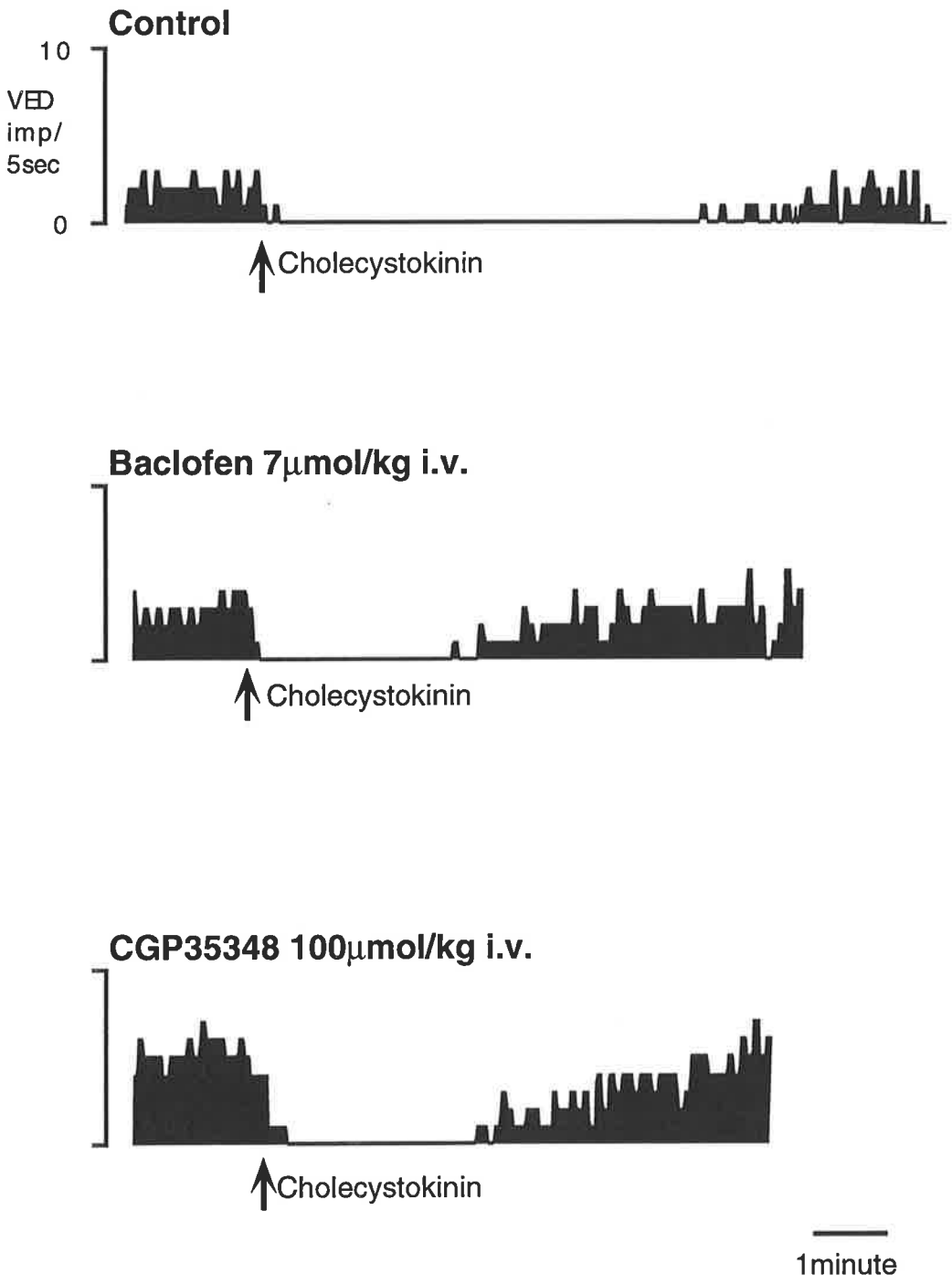


Figure 5-11. GABAb receptor influences on a vagal efferent fibre response to close intraarterial cholecystokinin.
The response of this fibre to mechanical distension is shown in Figure 5-3.

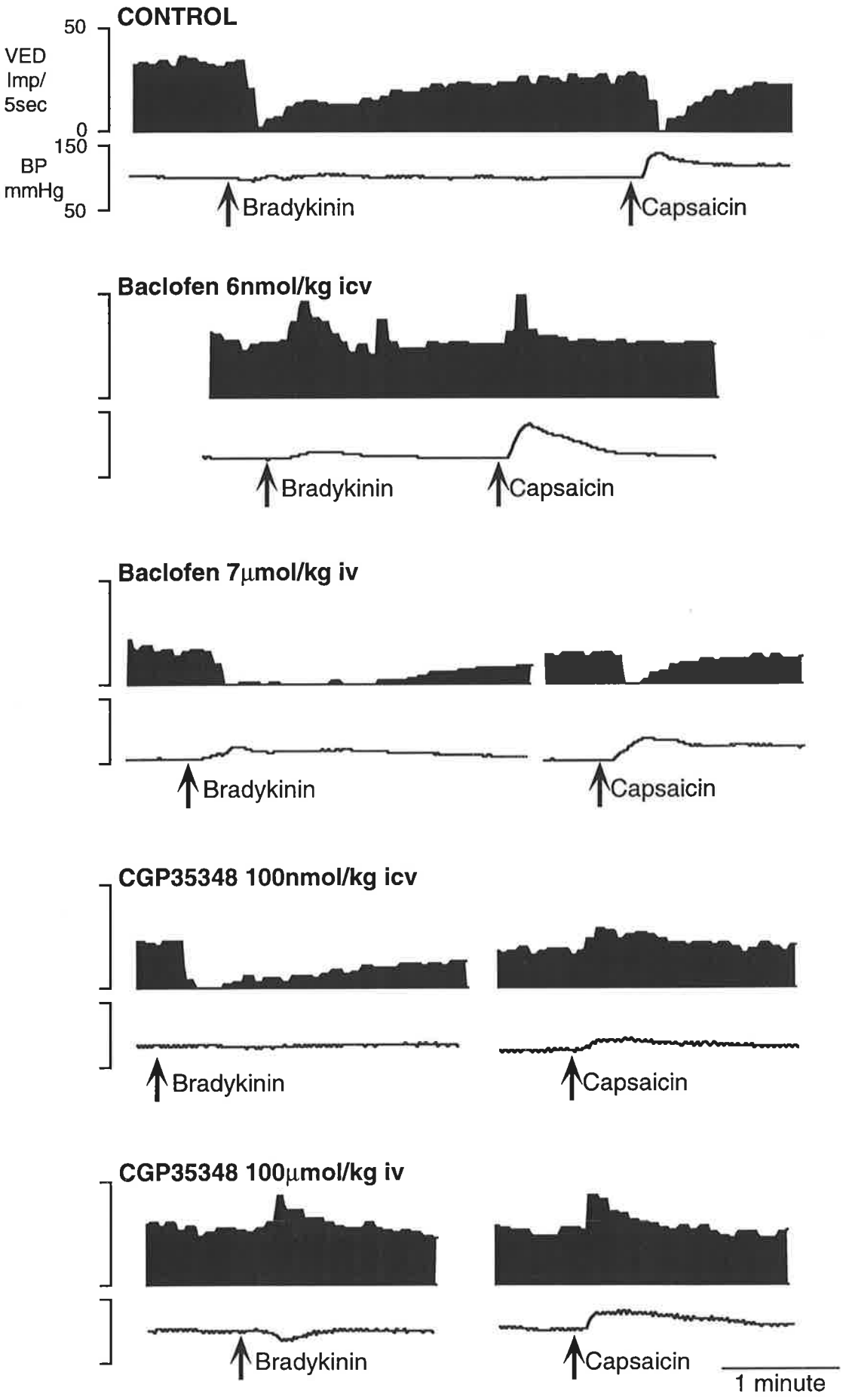
Cholecystokinin (100pmol close ia) induced a long lasting complete inhibition of fibre discharge. Baclofen administration (7μmol/kg iv) reduced the period of complete inhibition. This was not reversed by subsequent CGP35348 administration (100μmol/kg iv).

Figure 5-12 GABAb receptor influences on a vagal efferent fibre response to close intraarterial administration of bradykinin and capsaicin.

The responses of this fibre are documented in Table 5-2 as STUDY 5.

Under control conditions, bradykinin (18nmol, close ia) and capsaicin (65nmol, close ia) each induced a rapidly evoked, short lasting complete inhibition of vagal efferent discharge (VED). Central administration of baclofen (6nmol/kg icv) reversed the direction of efferent response to both stimuli to one of excitation. Subsequent systemic administration of the GABAb receptor agonist (7μmol/kg iv) again reversed the direction of response to both stimuli, yielding an inhibition of discharge. Central CGP35348 (100nmol/kg icv) shortened the duration of complete inhibition caused by bradykinin. The efferent response to capsaicin was again reversed, evoking a small excitatory response. The intravenous administration of CGP35348 (100μmol/kg iv) led to excitatory responses with both bradykinin and capsaicin.

Efferent responses to bradykinin and capsaicin were also obtained after cumulative adminstration of CP99994 (12μmol/kg iv), CNQX (155nmol/kg icv), pirenzepine (5.0μmol/kg iv), and hCGRP8-37 (3.2nmol/kg icv). The responses were unchanged after each of these antagonists.


CONTROL
50
VED
Imp/
5sec
0
150
BP
mmHg
50
Bradykinin
Capsaicin
Baclofen 6nmol/kg icv
Bradykinin
Capsaicin
Baclofen 7μmol/kg iv
Bradykinin
Capsaicin
CGP35348 100nmol/kg icv
Bradykinin
Capsaicin
CGP35348 100μmol/kg iv
Bradykinin
Capsaicin
1 minute

Concluding remarks

The vagus nerve is involved in the control of a wide variety of gastrointestinal functions, and plays a central integrative role between the gut and other systems such as the respiratory and cardiovascular systems. This coordination of normal autonomic events which occur below the threshold of consciousness is possible due to the extensive interconnections projecting to and from vagal central terminations.

The peripheral endings of vagal afferents have been well characterised using electrophysiological and histochemical techniques. It is known that these afferents have terminations within the mucosal, muscular and serosal layers of the gut wall and are present along the entire length of the gastrointestinal tract. The sensitivity of these afferents is characterised according to the location of their peripheral endings, that is 1) mucosal afferents show fine tactile discrimination and are directly sensitive to chemical stimuli applied either on the mucosal surface or intraarterially, 2) muscular afferents respond to changes in wall tension and muscle length, and are generally not directly activated by chemicals, and 3) serosal afferents tend to respond only to increases in intraluminal pressure and muscle length which may be above the normal physiological levels. As these different groups of afferents respond in distinct ways to a range of peripheral stimuli, it has been relatively easy to assign putative roles for them. For example, mucosal afferents in the duodenum are considered to be involved in regulating the rate of gastric emptying, muscular afferents are likely to be involved in monitoring tone and contractility and serosal afferents may be involved in mediating sensations such as bloating and emesis.

In the same way that characterisation of the peripheral endings of afferents have been performed, the location of the central terminations and interconnections of vagal neurones have also been identified. This is true of both vagal afferents and efferents which are known to have their main sites of termination within the NTS and AP and the DMVN and NA respectively. The central terminations are arranged in a rough viscerotopic organisation. For example, gastric afferents terminate mainly within the subnucleus gelatinosa of the NTS whereas those arising from the oesophagus have their endings in the subnucleus centralis.

Along with the viscerotopic organisation of the central connections of the vagus, the distribution of neurotransmitters, neuromodulators and their respective receptor subtypes within the brainstem has been ascertained with immunohistochemical and autoradiographic techniques. However, there is a paucity in the available literature as to whether there exists a type of chemical coding within the CNS, ie whether specific subpopulations of afferents release certain neurotransmitters along the reflex pathway onto central neurones when activated peripherally. It is in this area that I have chosen to concentrate my efforts.

In this work, I have used an established electrophysiological technique to record from single fibres to provide detailed information communicated by both the afferent and efferent limbs of the vagal reflex pathway. As the receptive fields of mucosal and muscular receptors are small (often $<10mm^2$), data obtained from vagal afferent studies together with simultaneous recordings of intraluminal pressure provides us with information about how changes occuring within the local environment of the peripheral endings affect the degree of excitability of the afferent. It is then possible to evaluate how changes in tone and motility affect the vagal afferent input to the CNS. Recording from single vagal efferent fibres, on the other hand, is similar to obtaining a representative sampling of afferent responses as these central neurones have been shown to receive convergent inputs from different populations of afferents. Although the exact destination of the efferent endings in the periphery was unknown as the vagal efferent fibres were truncated at the level of the recording site, the endings were surmised to be located within the upper GI tract as powerful responses were obtained by peripheral stimulation of that region.

Recording from both vagal afferents and vagal efferents means that the information which can be extracted from the data obtained is much more powerful than if either one of the methods were used in isolation. This is especially true as my experiments were performed in vivo using the same anaesthetic and general conditions. Also, the same stimuli were used to evoke changes in nerve activity. The only essential technical difference between afferent and efferent recordings was the direction that single nerve fibres were teased: for efferent studies, the proximal cut end of the vagal nerve strand was placed on the

recording electrode whereas for afferent studies, recordings were made from the distal portion of the vagal nerve strand. Apart from this fundamental difference, the set up was virtually identical for both types of recordings.

One of the primary aims of this thesis is the investigation of central processing of afferent inputs within the brainstem. Recordings of vagal gastric and oesophageal tension receptors and of vagal mucosal afferents in Chapters 1 & 4 show that the responses of these afferents to maintained distension and to chemical stimuli is very similar to the excitatory vagal efferent responses evoked by the same stimuli in Chapters 2, 3 & 5. The virtually identical responses generated by both arms of the reflex pathway indicates that there is a strong vago-vagal reflex and that the degree of central modulation which occurs in this pathway was minimal. This is in agreement with histological data showing that monosynaptic connections between vagal afferents and vagal efferents do exist[221, 243]. The presence of one or a few synapses occuring within the reflex pathway is also seen in one study[40] where the latencies of the reflex response of vagal efferents following electrical stimulation of the vagus were measured. Fibres which showed short latency responses were thought to be involved in reflex pathways via the brainstem which contained only one or a few synapses whereas the longer latency responses may be mediated though pathways which involve a larger number of synapses or a more complicated synaptic arrangement.

The advantages in recording from both afferents and efferents was most apparent when the chemosensitivity of oesophageal afferents was being investigated. Previous data on this is scarce, with the exception of two reports. Although I managed to record only from one oesophageal mucosal receptor due to the inherent technical limitations of the preparation, the responses of this vagal afferent to oesophageal acidification and capsaicin infusions were similar in latency, duration and intensity to the activation by the same stimuli of vagal central neurones. We can thus infer that the majority of inputs to vagal efferent neurones which were activated by oesophageal chemical infusion were mucosal receptors. Recording from both ends of the reflex pathway also means that additional information, such as the site of sensitisation seen in the response to acid, could be ascertained. In this

case, sensitisation was thought to occur peripherally as the oesophageal mucosal receptor and some efferent fibres did not respond to the first infusion of oesophageal acid but responded to subsequent infusions. In addition, efferent responses to other gastro-oesophageal stimuli remained unchanged.

Another example where data from afferent studies complemented those obtained from efferent studies occurred when the site of GABAb receptors along the vagal reflex pathway was being determined. From my afferent data, GABAb receptors were seen to be functionally present on the peripheral ends of a small group of vagal afferents. These afferents were all mechanoreceptors arising from the corpus. In contrast, my efferent data show that most inputs from gastric mechanoreceptors are modulated by GABAb receptor activation when the GABAb receptor ligands were administered systemically. This may seem to contradict the afferent data except that when only central GABAb receptors were activated, the same pattern of modulation was seen with gastric distension. My conclusion from the combined data is that while GABAb receptors exist on the peripheral terminations of gastric afferents, they play a minor role when compared to the influence of central presynaptic GABAb receptors. It is obvious that the power of interpretation would be significantly weakened if the effects of GABAb receptor modulation were only investigated on either vagal afferents or vagal efferents.

As stated previously, a major aim of this thesis is to analyse whether there is functional specificity inherent within the central distribution of receptor subtype when different populations of afferents are activated. The efferent studies performed in Chapter 3 and 5 demonstrate the complexity and the heterogeneity that exists within the CNS in the vagal reflex pathways studied. Some receptors, eg NK-1, NMDA and M2 muscarinic receptors, do not play a large role in reflex mechanisms arising from the proximal gut while others are involved in mediating inputs from a specific group of afferents. The specific attenuation of gastric mechanoreceptive inputs by M1 muscarinic receptor blockade is an example of the receptor subtype which seems to be selectively present on a subpopulation of

neurones. Still other receptor subtypes, eg non-NMDA, CGRP1 and presynaptic GABAb receptors, are involved in modulating inputs from more than one group of afferents.

This range of involvement of the various receptor subtypes is not surprising considering the large number of neurotransmitter and neuromodulatory compounds and their receptors present in every step of the vagal reflex pathway[166]. Due to the extensive network of fibres within and projecting from the DVC, it is likely that compounds play some role in the generation of functional specificity of the system. However, just as the functional differentiation of the system cannot be precisely and accurately determined from the spatial positioning of the neural connections, from my studies, it is unlikely that a single compound will be responsible for mediating a single function. There is probably a high degree of redundancy and overlap in the system.

So, what does this mean? The efferent studies performed in Chapter 2 clearly show that central vagal neurones receive convergent information from different types of afferents. This confirms findings made by previous electrophysiological studies[33, 40, 41, 126] as well as histological studies on the vagus nerve consisting of over 90% afferent fibres[18]. The efferents receive inputs from the most proximal portion of the gastrointestinal tract, ie the oesophagus, to the most distal, ie the colon. The afferent inputs may have their peripheral terminations within the mucosal, muscular, or serosal layers of the gut wall. These afferents may follow different pathways. For example, the majority of oesophageal mechanoreceptors and CCK-sensitive mucosal afferents which converge onto vagal central neurones are vagal in origin, whereas those arising from colonic mechanoreceptors or from bradykinin-sensitive afferents follow mainly a non-vagal route. However, individual efferents do not necessarily receive convergent information from the same combination of afferents.

Vagal preganglionic motor neurones form less than 10% of the total number of vagal fibres. The number of fibres is halved when considering only the abdominal branches of the vagus, ie those that are most likely to project to the gut. There is extensive convergence

of inputs not only from the gut, but also from cardiovascular and respiratory afferents within the brainstem as well as from higher brain centres. This ensures that integration of neuroendocrine and behavioural functions occurs in response to external environmental factors. This implies that this is an efficient method of effecting motor and secretory changes based on information received from different areas of the gut and from other systems. Perhaps the best example of the integrative role of the vagus is with the induction of the emetic response. Here, stimulation of vagal afferents peripherally leads to reflex changes which involves the cardiovascular, respiratory and gastrointestinal systems.

With this complex and intricate arrangement in mind, it is no wonder that there is a loose chemical specificity that exists within the ferret brainstem. Just as there is great diversity in the anatomical terminations and connections that exist within the brainstem, it is also unlikely that the neuronal circuits are stringently chemically coded. This situation is similar to the one found in dorsal root ganglion cells where although there is no simple correlation between the presence of specific peptides and the functional identity of these primary afferents neurones, there does seem to be preferential involvement of some neurotransmitters in certain modalities, eg substance P in nociception. In the same way, in this thesis, we can see both general and specific trends emerging which would hopefully contribute towards increasing our understanding of vagal innervation of the gastrointestinal tract.

Publications arising from this thesis

Full papers and review articles

Partosoedarso ER, Blackshaw LA. Involvement of central neurotransmitters in vagal reflex pathways activated by upper gastrointestinal stimuli. In progress.

Partosoedarso ER, Blackshaw LA. Central involvement of the $GABA_b$ receptor in modulating inputs from the upper gastrointestinal tract onto vagal efferent neurones. In progress.

Blackshaw LA, Partosoedarso ER. Peripheral involvement of the $GABA_b$ receptor in modulating inputs from vagal tension and mucosal receptors. In progress.

Blackshaw LA, Partosoedarso ER, Page, AJ. Acute capsaicin sensitivity of gastro-intestinal vagal afferents *in vitro* and *in vivo*. In progress.

Partosoedarso ER, Blackshaw LA, Dent J. Vagal efferent fibre responses to gastric and oesophageal mechanical and chemical stimuli in the ferret. *J. Auton. Nerv. Syst.* 1997, 66: 169-178

Abstracts

Partosoedarso ER, Blackshaw LA. Central neurotransmitter mechanisms in gastro-intestinal vagal reflexes. *Neurogastroenterol. Motil.* 1998; 10:90.

Partosoedarso ER, Page AJ, Blackshaw LA. Acute capsaicin sensitivity of gastrointestinal vagal afferents. *J. Auton. Nerv. Syst.* 1997; 65:156

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APPENDIX
Rationale for drugs and stimuli used in vagal efferent studies

A. Peripheral stimuli

Stimuli were administered to activate the peripheral endings of afferents. The distension stimuli activated tension receptors located only within the region or organ being distended. With the exception of acid, chemical stimuli were administered close intraarterially into the abdominal aorta at the coeliac axis to activate only the peripheral ends of gastroduodenal afferents. The latencies of response seen in some studies were very short (<1second) so responses could not have been due to a central action of the chemical being administered.

1) **Oesophageal balloon distension (OBD)** was performed by distending a silicone rubber balloon mounted on an orally inserted oesophageal assembly which was positioned within the distal oesophagus. Distension was performed by rapid injection of 1-2ml air for a period of 30seconds. This procedure has been shown to selectively activate vagal and non-vagal (spinal) oesophageal tension receptors[76, 107, 207, 210, 232, 239, 240].

2) **Oesophageal acidification (ACID)** was performed by injecting 2ml of 150mM HCl as a bolus via a sidehole positioned 1cm below the silicone rubber balloon. This was used in both vagal afferent and efferent preparations to activate oesophageal afferents. The concentration of acid used in these studies was able to evoke relaxation of the lower oeosphageal sphincter (LOS)[34].

3) Either **Gastric distension (GD),** or **corpus distension (CD) and antral distension (AD)** was performed to selectively activate tension receptors within the whole stomach (GD) or those located within the corpus (CD) and antrum (AD) respectively[12,36,38,45,91,183,236]. The distension volume used for GD was 40-60ml, for CD 20ml, and for AD 10ml based on levels used in previous studies in ferrets[12]. The distension period lasted for 60 seconds. CD and AD were performed in

some studies instead of GD as tension receptors within the corpus and antrum are known to respond differently when activated[12,36,38]. Thus, corpus and antral distension was performed in order to study the effects of the drugs listed below on the afferent inputs from the corpus and antrum respectively. These afferents can follow either a vagal or a non-vagal, probably spinal, pathway.

4) **Colonic distension (ColD)** was performed with 3ml isotonic saline infused into a segment of colon for 1 minute. This activated a population of vagal and non-vagal mechanoreceptors[126], and was done to investigate the effects of GABAb receptor ligands on afferent pathway from the distal portion of the gastrointestinal tract.

5) **Cholecystokinin (CCK)** was administered close ia at a dose of 100pmol to directly stimulate vagal mucosal receptors in the gastroduodenal region although it may indirectly excite gastroduodenal tension receptors thorugh changes in motility[38,39].

6) **Bradykinin (BK)** was administered close ia at a dose of 18nmol to activate vagal and splanchnic afferents in the upper GI tract[174,240].

7) **Capsaicin (Cap)** was administered close ia at a dose of 65nmol to activate all unmyelinated C-fibres afferents[141] within the gastroduodenal region. This was done in the original belief that all populations would be sensitive, although this generalisation was brought into question over the course of the experiments. The afferents activated by Cap was thus assumed to include a broader range than that stimulated by BK.

B. Surgical and pharmacological interventions

The dose of some drugs were titrated against its effect on the efferent response to at least one stimuli, eg the dose of baclofen used both centrally and systemically was titrated against its effect on the efferent response to gastric distension.

The types of afferent inputs investigated were 1) vagal and non-vagal oesophageal mechanoreceptors (via OBD), 2) vagal and non-vagal corpus and antral mechanoreceptors (via GD, CD, and AD), 3) vagal and non-vagal colonic mechanoreceptors (via ColD), 4) vagal gastroduodenal mucosal receptors (via CCK), 5) a broad population of vagal and splanchnic muscular and serosal afferents (via Cap and BK close ia).

1) **Bilateral vagotomy (BiVx)** – to selectively eliminate inputs from vagal afferents. This allowed subsequent observation of any inputs from afferents which followed a non-vagal pathway.

2) **Bilateral splanchnectomy** – to selectively eliminate inputs from afferents travelling via the greater splanchnic nerves. Performed in only one study, these nerves were severed at the crus of the diaphragm after the effects of bilateral vagotomy on efferent responses were investigated.

3) **NK-1 receptor antagonism** with CP99994 (12μmol/kg iv) and CP96345 (8μmol/kg iv) was performed as substance P-immunoreactivity has been identified in the gastrointestinal tract, the nodose ganglion and the dorsal medulla[58,248,250]. NK-1 receptors are the main subtype of neurokinin receptor sensitive to substance P. Substance P binding sites were found in the brainstem[271,182] and NK-1 receptors specifically in the AP and NTS[265]. Both antagonists could block induced emetic responses[95,265]. CP99994 also reduced the excitatory post-synaptic potentials evoked by vagal nerve stimulation and chemoreceptor input from left ventricular receptors to the NTS[211]. The doses used in these studies were similar to that used in reducing the inhibition of colonic motility induced by rectal distension in the rat[150] (5-10mg/kg or 10-

20μmol/kg ip) and in blocking the LOS responses to intraoesophageal capsaicin and acid after bilateral vagotomy[34] (1-5 mg/kg or 2-10μmol/kg iv).

4) **CGRP1 receptor antagonism** with hCGRP8-37 (3.2-6.4nmol/kg icv) was performed as CGRP is contained in afferents and CGRP receptors have been found in the dorsal horn of the spinal cord, DMVN, NTS and NA[145,225,226,231]. CGRP is released locally in response to gastric acid backdiffusion and mediates gastric hyperemia[142,173]. Central CGRP also inhibits stimulated gastric acid secretion and gastric emptying and contractility. In the spinal cord, the peptide is involved from pain transmission in the gut[177,215]. The same dose as I have used administered intrathecally (10μg or 3.2 nmol) was effective in reversing the sensitizing effects of acetic acid on colorectal distension-induced visceral pain in rats as measured by the number of abdominal contractions seen during the distension period[215].

5) **NMDA receptor antagonism** was performed with CGS19755 (13μmol/kg iv). Both glutamate and the NMDA receptor have been identified in the NTS, AP, and DMVN[62,79,97,229]. The NMDA receptor is implicated in the triggering of transient LOS relaxations[165] and in the generation of oeosphageal peristalsis[264]. The dose and route of administration used in my studies has been shown to reduce the number of transient LOS relaxations in the first 90 minute period after administration[165].

6) **Non-NMDA receptor antagonism** was performed with CNQX (75-155nmol/kg icv) as the non-NMDA receptor is functionally involved in the primary synaptic transmission within the rat brain stem[4] and also in the CNS control of respiration and cardiovascular responses[55,56,269,276]. The doses used in this study were effective in causing a fall in mean arterial pressure, hence indicating that a sufficient quantity of the drug had reached areas within the brainstem responsible for regulating cardiovascular reflexes. CNQX was administered centrally as the cost involved in administering this drug intravenously was prohibitive and the technique for central injections had been mastered by this stage.

7) **Muscarinic M1 and M2 receptor antagonism** were performed with pirenzepine (2.5-5.0μmol/kg iv) and methoctramine (7-14μmol/kg iv) respectively as muscarinic receptors and the synthesizing enzyme for acetylcholine exist in the NTS[245], DMVN[17], and NA[160], ie those areas of the brainstem that are involved with vagal reflexes. Both antagonists were assumed to cross the blood brain barrier. Initial studies confirmed that the doses used would cause a decrease in intraluminal pressure during gastric distension.

8) **GABAb receptor agonism and antagonism** were performed with baclofen (7-14μmol/kg iv or 3-6nmol/kg icv), and CGP35348 and CGP36742 (100μmol/kg iv or 100nmol/kg icv) respectively. GABA, the main central inhibitory neurotransmitter, and the GABAb receptor have been identified in the cell bodies and the central terminals of vagal afferent fibres[139,149,244,252]. In vagal afferent studies, baclofen, CGP35348 and CGP36742 were used to determine whether GABAb receptors were functionally present on the peripheral terminals of tension receptors located in the oesophagus, corpus and antrum and on mucosal receptors located in the gastroduodenal region. The initial dose of baclofen used (7μmol/kg iv) reduced the number of transient LOS relaxations seen in other studies in our laboratory while CGP35348 reversed the effects of baclofen. Baclofen and CGP35348 were also used in vagal efferent studies to determine 1) the presence of GABAb receptors functionally within the brainstem nuclei which receive inputs from GIT neurones and 2) the relative contribution of central and peripheral GABAb receptors in mediating inputs from each type of GIT afferents onto vagal efferent neurones.