



THE ACTION OF SEROTONIN ON BLOOD VESSELS

by

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Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge and belief contains no material previously published by another person, except where due reference is made in the text.

Part of the work has been published in the British Journal of Pharmacology and Chemotherapy 1966 vol.28, No. 3, and the Supplement III to Circulation Research, Vols XX and XXI, December 1967.

Some of this work has also been communicated to the 39th Meeting of the Australian and New Zealand Association for the Advancement of Science.

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Finally I wish to thank Professor R.F. Whelan in whose department this work was carried out.

Abbreviations

A	Adrenaline
Coc.	Cocaine
Conc.	Concentration
Expt. No.	Experiment Number
Ext.	External
5HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptamine
5HTP	5-hydroxytryptophan
Inj.	Injection
Int.	Internal
LSD	Lysergic acid diethylamide
MAO.	Monoamine oxidase
Max.	Maximum
NA	Noradrenaline
Perf ⁿ	Perfusion
S.F.	Sensitization Factor
TEAC	Tetraethylammonium chloride

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PREFACE

When perfused with Krebs bicarbonate solution the central artery of the rabbit ear is highly sensitive to catecholamines and many other vasoactive substances. The sensitivity to nerve stimulation and vasoconstrictor substances such as noradrenaline is increased by addition of serotonin (5HT) to the perfusion fluid (de la Lande and Rand 1965). The same phenomenon had already been demonstrated by several groups of workers in the whole ear when perfused in vitro (Ginzel and Kottegoda 1953, Savini 1956).

Since serotonin occurs naturally in, for example, the blood platelets of the rabbit, (see review by Erspamer 1961), the question may be asked whether the sensitizing action of serotonin is of physiological importance.

The experimental work in this thesis has been concerned with determining some of the conditions under which serotonin induced sensitization of vasoconstriction occurs in vivo and in vitro.

CHAPTER IINTERACTION OF SEROTONIN AND VASOACTIVE SUBSTANCES IN VIVO

A large number of pharmacological effects of serotonin on different species have been reported in the literature (these have been reviewed by Erspamer 1961). In this introductory survey emphasis is placed on results obtained on the dog, as this is the animal on which most experiments have been performed. However attention is drawn to published observation on the rabbit and other species where these are relevant.

Page (1952) demonstrated a species difference in the systemic blood pressure response to an intravenous injection of serotonin. In rabbits the response was purely depressor, whereas in the cat, dog and human a more complicated triphasic response was observed. An initial transient fall in pressure was followed by a rise and then prolonged depression of small magnitude (Page 1952, Reid 1952). The effects of serotonin on the blood pressure of the rat depended on a number of conditions. Small doses of serotonin or high blood pressure favoured the depressor effect, while large doses or low blood pressure led to a hyp^{er}tensive effect. (Outschoorn and Jacob 1960). Conclusions about the nature of the response in the cat, dog, human and rat were difficult to reach because in addition to a marked intraspecific difference in the action of serotonin on the blood pressure, a variation had also been noted in the response of individual

animals when serotonin was administered on different occasions. This differed from the response of other vasoactive substances such as noradrenaline or acetylcholine, which cause a predictable rise or fall in blood pressure. The response to intravenous injection of serotonin thus probably depended on more complex interactions with other factors in the circulation. Some of these are cited below:-

- I. the level of sympathetic nerve activity
2. release, diffusion, or action of noradrenaline at receptor
3. the blood pressure at the time of serotonin injection.

The first question posed by the variable blood pressure response to serotonin was whether or not the response was due to the level of the blood pressure at the time of administration of serotonin.

Page and McCubbin (1953) approached this problem by inducing an experimental hypertension in a number of dogs. They denervated the carotid sinus, and the blood pressure of dogs treated in this way, while usually above normal, was found to fluctuate both below and above this level. The response to intravenous injection of serotonin in all of these dogs in contrast to normal dogs was invariably depressor whereas the response to noradrenaline and other vasoconstrictors remained pressor. Response to serotonin however became pressor again if preceded by administration of tetraethylammonium chloride (TEAC), a ganglion blocking agent. The effect of section of the nerves to the carotid sinus is to inhibit the action of barorecep-

tors on the systemic circulation, thus leading to a rise in the level of activity in the sympathetic nervous system and a consequent increase in the degree of neurogenic vasoconstriction. Therefore Page and McCubbin postulated that the action of serotonin depended on the level of activity in the sympathetic nervous system. When this was high, as in the carotid denervated dog, serotonin had a depressor action. However when the level of activity was decreased by ganglion blockade with TEAC the action was again pressor as in the normal dog.

The reversal of the serotonin response could also be demonstrated after the level of activity of sympathetic nerves had been lowered by either cord section or intracisternal injection of procaine. The reversal could not be demonstrated after the blood pressure had been lowered by infusion of sodium nitroprusside, which acted solely on the peripheral vessels and left the level of activity in the sympathetic nerves raised above normal.

The normal depressor action of serotonin following carotid sinus denervation was not due to inhibition of the release or diffusion of noradrenaline across the synapse, nor to inhibition or antagonism of the action of noradrenaline on the smooth muscle of the blood vessels. When hypertension was elicited by infusion of noradrenaline, serotonin not only failed to decrease this hypertension but in fact enhanced it. Whether increase in the response to a hypertensive infusion of noradrenaline when serotonin was infused

simultaneously was due to addition of the effects of two vasoconstrictors, or to potentiation of the effects of noradrenaline was not distinguished.

Page and McCubbin (1953) were able to separate the depressor action of serotonin from that of TEAC. They perfused the leg of a hypertensive dog via its femoral artery with blood from a donor dog. The only connection between the perfused leg and body of the recipient was nervous tissue. TEAC injected into the recipient systemic circulation caused a fall in systemic pressure. A similar fall occurred in the isolated leg indicating that TEAC acted by way of nervous tissue. However when serotonin was injected into the recipient circulation, although the systemic blood pressure fell, the perfusion pressure in the leg remained unchanged. Thus the action of serotonin in lowering the blood pressure of the dog with the carotid nerves severed was unlikely to be mediated through ganglion blockade in the same way as that due to TEAC.

The possibility of a cholinergic component in the depressor response to serotonin in the dog was rendered unlikely by an experiment in which it was shown that atropine antagonized the depressor response to methacholine, an acetylcholine derivative, but did not antagonize the depressor response of these dogs to intravenous injection of serotonin.

In summary, the depressor response to serotonin did not appear to be due to interference with sympathetic transmission,

nor was there any cholinergic component in the response. It was not mediated via the central nervous system and appeared to be confined to the peripheral vessels.

The reversal of the pressor response to serotonin by decrease in sympathetic outflow was also demonstrated in three other species. In cats the normal depressor response following the pressor component of the serotonin response was abolished and reversed to a pressor one by prior administration of hexamethonium, another ganglion blocking agent. Serotonin injected intravenously into rabbits evoked a purely depressor response. There may have been a cholinergic component in these responses as they were reduced by atropine or by bilateral vagotomy. The responses to serotonin were reversed from depressor to pressor by prior administration of TEAC and hexamethonium (Page 1952) indicating that the responses to serotonin may have been similar to that recorded in the dog.

Trajkov, Glavas, Stojanova and Nikodijevic (1965) demonstrated reversal of the depressor component of the response of the rat blood pressure to serotonin by prior administration of either guanethidine or bretylium which cause in effect a 'chemical sympathectomy'. However dibenzyline which antagonizes the constrictor action of noradrenaline and adrenaline also inhibited the depressor or biphasic response of the rat blood pressure to serotonin before guanethidine or bretylium and the purely pressor response found after guanethidine or bretylium. Bretylium and guanethidine did not in-

hibit the known dilator effect of isoprenaline in the rat, and it was assumed that serotonin did not act on the same receptors as isoprenaline. These authors suggested that in the rat serotonin released small amounts of catecholamines and adrenaline, which is dilator in small doses in the rat, caused a dilatation of blood vessels and consequent fall in blood pressure. When the blood vessels were sensitized by guanethidine or bretylium, the depressor response to adrenaline was reversed to pressor, a large dose of adrenaline causing a pressor response.

The dilator response to serotonin in isolated tissues

The dilator response to infused serotonin during periods of increased vasoconstrictor activity in the sympathetic nerves was also demonstrated in several isolated tissues of the dog. McCubbin Kaneko and Page (1962) perfused the isolated kidney, a portion of mesentery and the partially isolated hind-limb of the dog. Increase in sympathetic activity was elicited by stimulation of the sympathetic nerves supplying these tissues. In all three preparations response to infusion of serotonin was constrictor before and either wholly dilator or containing a much larger dilator component during stimulation of the sympathetic nerves. McCubbin et al (1962) investigated the interaction of adrenaline, noradrenaline and tyramine in the hind-limb of the dog. Serotonin did not reverse the pressor response to either noradrenaline or tyramine, which acts by release of noradrenaline (Burn and Rand 1958). However the pressor response to adrenaline was partially inhibited. Ahlquist (1948) had already classified the actions of noradrenaline and adrenaline on vascular smooth muscle receptors. Noradrenaline acted almost entirely on constrictor or α receptors whereas adrenaline acted on both α and dilator or β receptors. From these interactions McCubbin et al (1962) suggested that during stimulation of vascular smooth muscle either by sympathetic

nerve activity or adrenaline action the β receptors were uncovered or in some other way became susceptible to the sensitizing action of serotonin.

Further evidence to support this suggestion was the antagonism by dichloroisoproterenol of the depressor response to both isoproterenol which acts at β receptors, and the depressor response due to serotonin. McCubbin et al do not appear to have investigated the interaction of isoproterenol and serotonin. If serotonin does sensitize β receptors to sympathetic nerve stimulation and adrenaline then it can be expected to sensitize β receptors to isoproterenol. This would provide convincing evidence in support of their theory that serotonin does not act directly on α receptors in the hind-limb of the dog to inhibit the pressure rise caused by adrenaline and sympathetic nerve stimulation, but acts indirectly by sensitizing the β receptors and lowering blood pressure by vasodilatation.

A different model for the action of serotonin in pressure regulations in the dog was proposed by Haddy, Gordon and Emanuel (1959). These authors measured pressure changes in different sized vessels in the dog fore-limb, i.e. the brachial artery and smaller arteries and veins. Noradrenaline, when administered alone, constricted both large and small arteries. On the other hand serotonin, which also constricted large arteries, dilated the smaller vessels. Serotonin administered during noradrenaline infusion

never-the-less caused a net increase in perfusion pressure.

Haddy et al (1959) suggested that the effects of noradrenaline and serotonin on large vessels were additive, while in small vessels they were antagonistic.

Although McCubbin et al (1962) found that noradrenaline constricted smaller vessels in their experiments with the perfused hind-limb of the dog they were unable to demonstrate a marked effect of noradrenaline on large arteries. Serotonin appeared to have no effect on the noradrenaline response in either large or small vessels in their preparation. It is difficult to form a unifying hypothesis for the two sets of observations. McCubbin et al (1962) simply commented that the difference must lie in the vasculature of the two different limbs of the dog. The differences observed lay in the response of the larger blood vessels. Haddy et al (1959) pointed out that the percentage of terminal flow in the brachial artery was greater than that carried by the femoral, and considered that this might effect their results.

The conclusions to be drawn from these studies is that interaction between serotonin and noradrenergic systems in the dog may involve both addition and inhibition of their effects. There is some evidence that serotonin may sensitize β receptors to the action of adrenaline.

CHAPTER 2SENSITIZATION BY SEROTONIN OF VASCULAR SMOOTH MUSCLE IN ISOLATED RABBIT ARTERIES AND ISOLATED AND PERFUSED RABBIT EARS AND ARTERIES

There do not appear to be any descriptions of interaction between serotonin and catecholamines in isolated arteries prepared as strips or rings. However examples of papers dealing with related topics are given below. Khairallah, Page and Turker (1966) reported potentiation by metanephrine of the response to serotonin, angiotensin and vasopressin in rabbit aortic strips and to serotonin only, in cat carotid strips which develop tachyphylaxis to angiotensin. Phentolamine, an α blocking agent, inhibited this potentiation and as responses of strips which had been depleted of noradrenaline by reserpine were not potentiated until the noradrenaline had been replaced, these authors suggested that the potentiation was due to release of noradrenaline by metanephrine and subsequent addition of the effects of the added and released vasoconstrictor.

Vacek (1962) compared the sensitivity of aortic strips obtained from normal rabbits to serotonin, histamine, and adrenaline with the sensitivity of strips obtained from rabbits made hypertensive by six weeks administration of sodium or potassium

chloride. The sensitivity to serotonin of strips from hypertensive animals was less than that of strips from normal animals whereas the sensitivity to noradrenaline and histamine was greater in the hypertensive strips.

Jelliffe (1962) demonstrated antagonism of the constrictor action of serotonin on isolated rabbit aortic chains by acetylcholine.

Isolated arterial strips and rings have also been used in a number of studies on arterial sensitivity to serotonin and other vasoactive substances and also on the action of partial agonists and antagonists of serotonin and tryptamine.

An example of the former use of these preparations is the study by Bohr, Goulet and Taquini (1961) who compared the sensitivity to serotonin and other vasoconstrictors, such as adrenaline, of strips prepared from the aortas and various resistance vessels of the dog and rabbit. Examples of the latter use of these preparations are two papers dealing with the use of sheep carotid rings to estimate the potencies of serotonin activity and anti-serotonin activity of a number of amino-indoles, (Woolley and Shaw 1953) and to measure antagonism of serotonin by yohimbine and some ergot alkaloids (Shaw and Woolley 1953).

Several workers have described sensitization of the isolated and perfused rabbit ear vessels to noradrenaline by serotonin. Ginzel and Kottegoda (1953) used the perfused rabbit ear prepar-

ation of Gaddum and Kwiatowski (1938). The ear was not isolated from the head but was perfused with undiluted rabbit blood or Locke solution via a cannula in the carotid artery at constant pressure and room temperature. They observed that the constrictor responses to both adrenaline and noradrenaline were potentiated by prior injection of either serotonin or tryptamine. Later Savini (1956) demonstrated that serotonin sensitized the vessels of the isolated rabbit ear to adrenaline. This preparation was also perfused at constant pressure and room temperature (the Bissenski (1889) method quoted by Rischbieter (1913)). The sensitization occurred in the absence of constriction as the catecholamines were injected when the rate of perfusion had returned to its resting value after a decrease caused by an injection of serotonin or tryptamine. Lysergic acid diethylamide (LSD) another indole derivative also sensitized the ear vessels to catecholamines (Gaddum and Hameed 1954, Savini 1956).

de la Lande and Rand (1965) demonstrated the same phenomenon in the isolated perfused segment of the central artery of the rabbit ear. They showed that serotonin enhanced the effects of sympathetic nerve stimulation as well as noradrenaline. Since dilator or β -receptors have not been demonstrated in the ear, the above findings indicate that serotonin sensitizes constrictor receptors in the ear vessels rather than antagonizing dilator or β -receptors.

The work in this thesis is an attempt to define the interaction of noradrenaline and serotonin in the rabbit ear artery. The following aspects were studied:

- 1) whether sensitization by serotonin was specific for catecholamines
- 2) whether the phenomenon was related to the constrictor activity of serotonin
- 3) whether sympathetic nerves or monoamine oxidase were involved
- 4) and whether the receptors which mediate the actions of serotonin in the artery are pharmacologically similar to those in other types of smooth muscle.
- 5) Experiments were also carried out on the anaesthetized rabbit to ascertain whether a similar type of interaction occurs in vivo

CHAPTER 3METHODSType of rabbit

Semi-lop eared rabbits were used in all experiments. They were not graded for size or separated for sex. The ears were approximately 15 - 20 cm long.

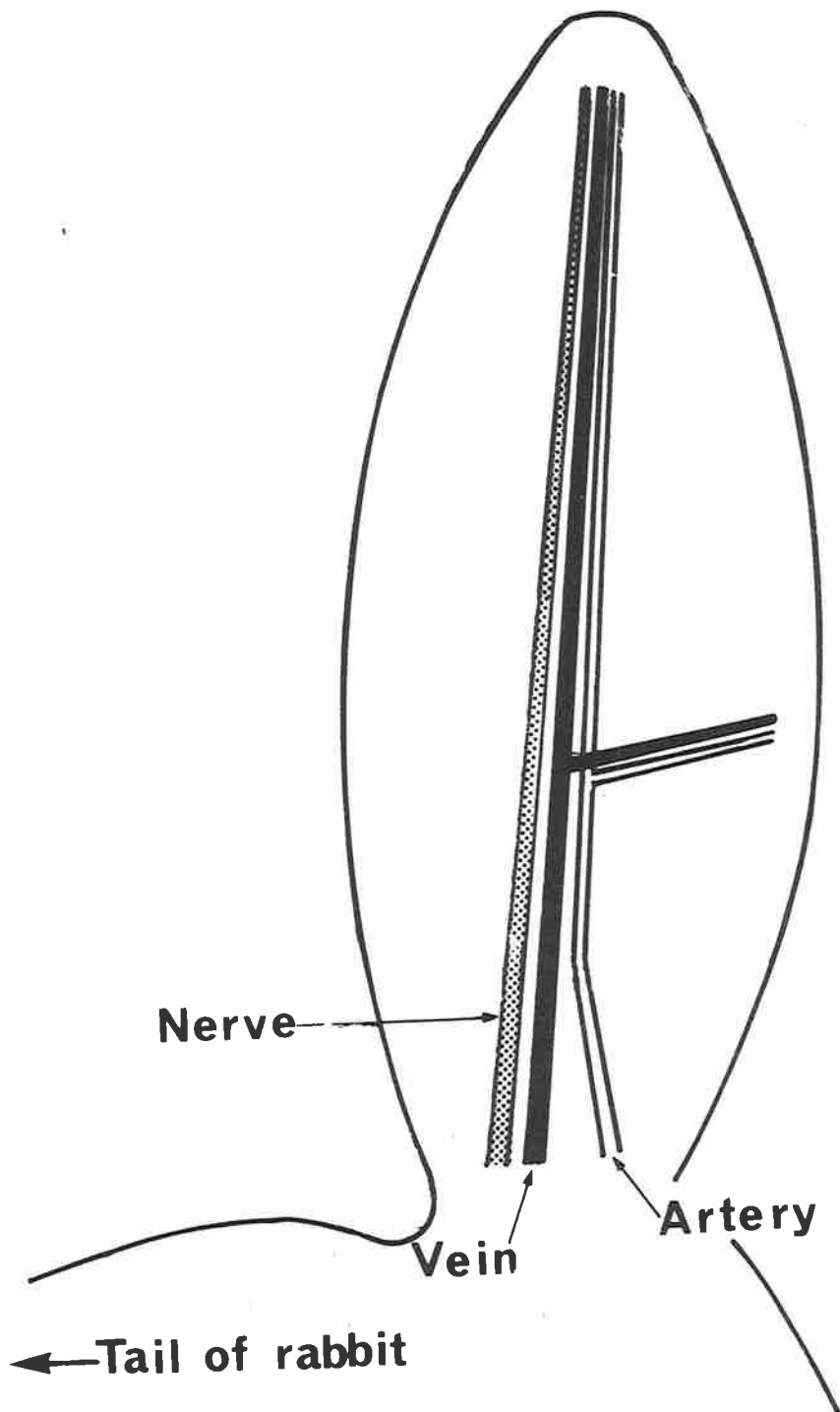
Anaesthetic

The rabbits were anaesthetized with ethyl carbamate (Urethane). Between 7 and 15 ml of a 25% solution was injected intraperitoneally. When surgical anaesthesia was attained a dose of heparin of 1000 units was injected into a marginal ear vein.

Dissection

The upper skin of the ear was cut to expose the artery and vein, and the artery was cleared of connective tissue and vein, 2-3 cm from the point of insertion in the muscle at the proximal part of the ear to the first major lateral branch of the ear vessels. Dissection of the artery is illustrated in Fig. I. Segments taken from this region do not possess lateral branches of macroscopic dimensions. Bleeding occurred in some dissections but care was taken to restrict this to a minimum in case the artery was exposed to serotonin released from ruptured platelets.

Fig. I. A diagrammatic representation of the vasculature of the rabbit ear.



Perfusion system

The apparatus used for perfusing isolated arteries is illustrated in Fig. 2.

The cannulated artery was connected to the perfusion tubing in an organ bath of 10-20 ml capacity. This was constructed with an outer jacket through which water at 37° was pumped by a circulating Braun pump, from a large heated reservoir. The artery was perfused at 37° with Krebs' bicarbonate solution (for composition see appendix 5) which was gassed with 95% oxygen and 5% carbon dioxide (Carbogen). The perfusion fluid was delivered at constant rate by means of a roller pump from a flask warmed in the large reservoir. Tubing between the pump and the artery was surrounded by a jacket through which water was passed at 37°. Constriction was recorded as a rise in perfusion pressure by means of a mercury manometer or a Statham Pressure Transducer. Perfusion was maintained at rates of 6-8 ml per minute. Perfusion pressures were of the order of 10-20 mm Hg when flow was permitted without the artery in the perfusion system.

The arteries were routinely perfused for at least one hour before drug dosage was commenced.

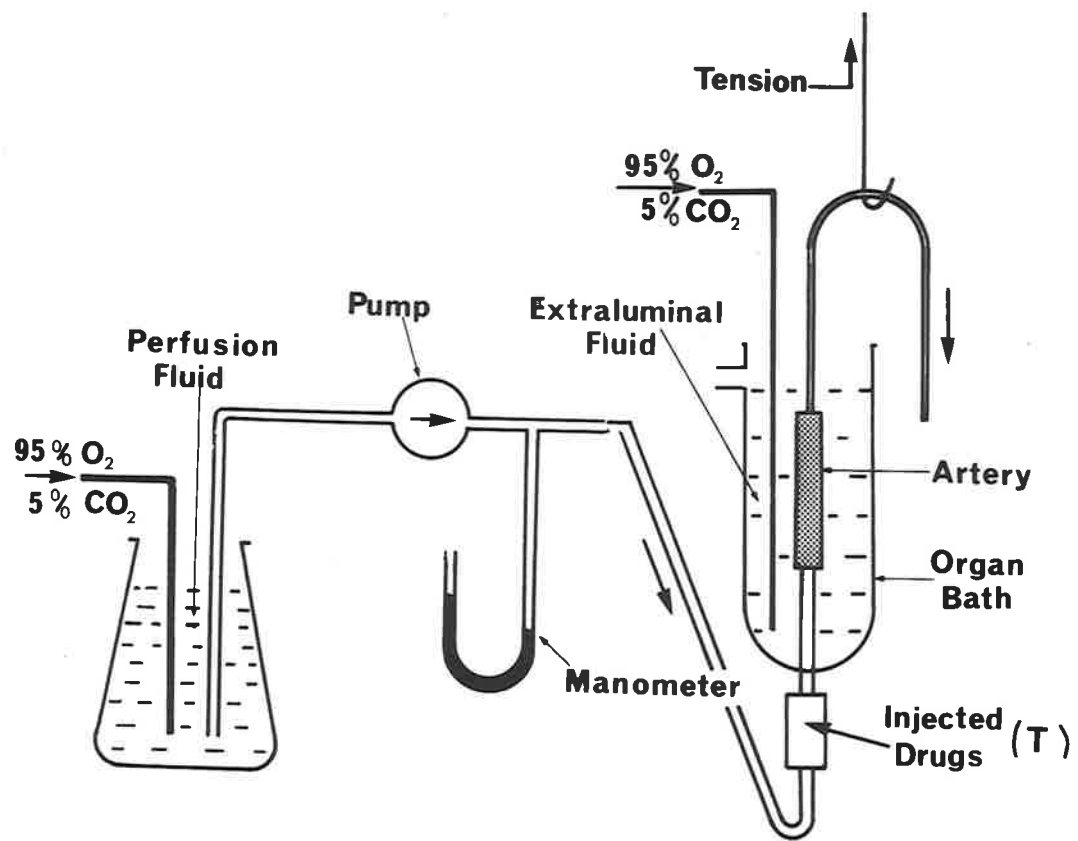
Cannulation

The arteries were cannulated with Sterivac Polythene tubing. Two methods of cannulation were used, and will be re-

Fig. 2. The apparatus used for perfusing isolated arteries.

T. Rubber injection tubing

The artery is double cannulated; the top cannula is bent to facilitate drainage.



ferred to respectively as single (de la Lande and Rand 1965) and double cannulation. Fig. 2 shows a double cannulated artery.

For single cannulation the proximal end of the artery was cannulated with tubing of 1.5 mm bore which was drawn out at the insert end to about 1 mm diameter. When in position in the organ bath Krebs' bicarbonate solution was pumped through the cannula and artery and drugs injected into this solution acted first on the intraluminal surface of the artery before escaping into the Krebs bicarbonate in the organ bath. This diluted drug then bathed the adventitia of the artery before being washed out by fresh perfusion fluid entering the organ bath via the lumen of the artery.

In double cannulation the distal end of the artery was also cannulated and thus it was possible to distinguish between the actions of drugs on the intraluminal surfaces and extraluminal surfaces of the artery. Drugs were either injected into the lumen of the artery or added to the Krebs bicarbonate solution in the organ bath. The distal end of the artery was cannulated with tubing of 0.5 mm bore which was drawn out at the insert end to about 0.5 mm diameter. This cannula was bent in a U shape to facilitate drainage over the edge of the organ bath.

Drug administration

Drugs were administered in three ways.

1) Intraluminal injection:- drug was added by injection into the perfusion stream through the rubber tubing (marked T in Fig. 2).

2) Intraluminal perfusion:- the drug was added to the perfusion reservoir and perfused through the artery.

3) Extraluminal perfusion:- drugs were added to the fluid in the organ bath.

Preparation of chronically denervated arteries

Rabbits were operated on at least one week before the arteries were to be removed for perfusion. They were prepared by an intraperitoneal injection of atropine and then anaesthetized with ether and local lignocaine in the neck. The neck was opened and the superior cervical ganglion of one side was located. This was removed together with a length of the pre- and post-ganglionic nerve. Penicillin powder was dusted into the incision and the neck was sewn up and the animal left to recover. The central arteries were removed from both control and sympathectomised ears and set up for perfusion by the method described on page 15. Both arteries were stimulated at 50 volts frequency 10 per second, duration 10-20 seconds by the method described by de la Lande and Rand (1965). When the artery from the non operated side was stimulated it constricted but if complete removal of the superior cervical ganglion had been achieved the artery from this ear did not respond to stimulation even when the voltage was increased to 150 volts.

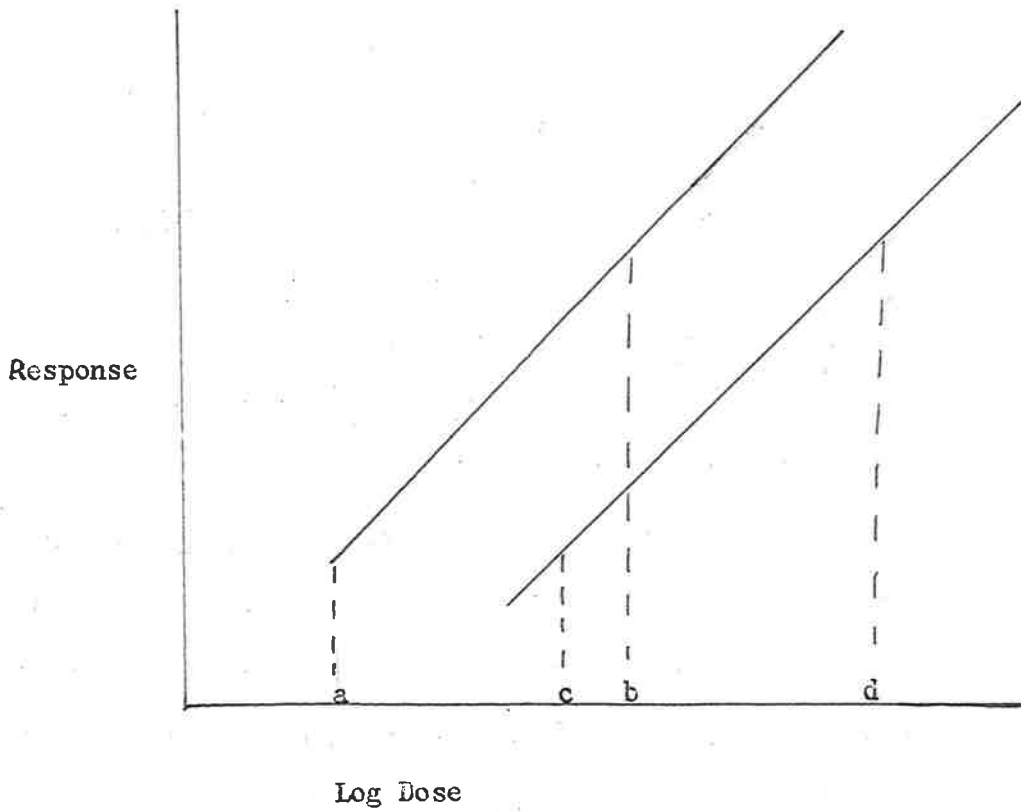
Administration of reserpine to rabbits to deplete the noradrenaline stores

Reserpine (see review by Shore 1962) was administered

the previous day to rabbits from which arteries were to be removed for perfusion by the method described on page 15. They were injected intraperitoneally with reserpine (Serpasil) at a dose of 2.5 mg/Kg.

Measurement of sensitivity

The interaction between serotonin and noradrenaline was quantitated by comparing the dose response curves to a vasoconstrictor drug (injected or perfused intraluminally, or perfused extraluminally) in the presence and absence of serotonin. Curves were obtained from a minimum of two responses each to the drug at two or more dose levels, the doses being restricted to those causing increase in perfusion pressure of between 10 and 150 mm mercury. Arithmetic means of the responses were used to plot dose response curves, since there was normally little variation in response to a particular dose of vasoconstrictor. Sensitization by serotonin was estimated by the shift of the vasoconstrictor dose response curve to the left. The ratio of the dose producing the same response in the absence and in the presence of serotonin is expressed as the sensitivity factor. The calculation is illustrated in Fig 3.



Sensitization factor (S.F.) = Mean of $\frac{b}{a}$ and $\frac{b}{c}$

Fig. 3. Calculation of the sensitization factor

CHAPTER 4PERFUSION OF THE ISOLATED CENTRAL ARTERY OF THE RABBIT EARResultsSection II) Response to noradrenaline

The responses of the artery to noradrenaline applied intraluminally or extraluminally were similar and are shown in Fig. 4. During the period of contact of drug and artery there was a rapid rise in perfusion pressure indicating a constrictor action of noradrenaline on the artery. In the majority of arteries the response was sustained at or near its maximum level. In others the response 'faded' rapidly. Each type of response was reproducible and concentration dependant. The characteristic response to intraluminal injection of noradrenaline was a transient rise in perfusion pressure which was also reproducible and dose dependant. (Fig. 4).

Comparison of the concentration curves of the drug applied intraluminally and extraluminally indicated that noradrenaline was more active by the intraluminal route. The ratio of activity of extraluminal to intraluminal noradrenaline was 0.13 ± 0.03 (SE).
in sixteen arteries

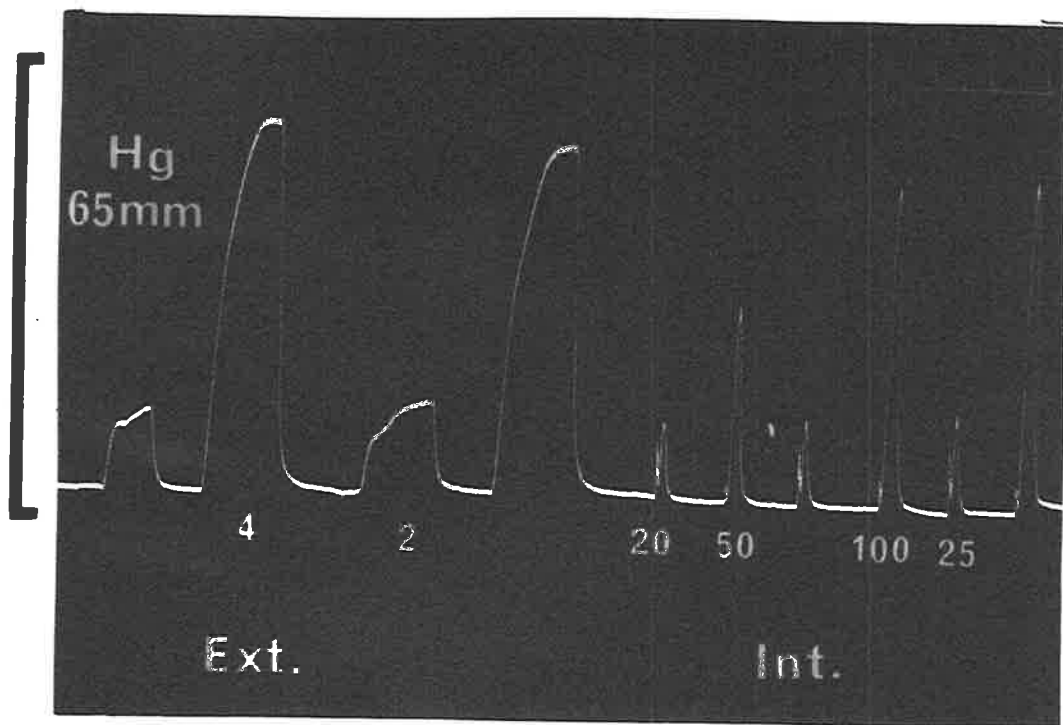
Fig. 4. A portion of a kymograph recording of the responses of a double cannulated artery to extraluminal perfusions and intraluminal injections of noradrenaline.

Ext. perfⁿ. Extraluminal perfusion 2 ug, 4ug.

Int. inj. Intraluminal injection 20 ng, 25 ng,
50 ng, 100 ng.

Time scale 2 min.

Pressure scale 150 mm Hg.



Ext. 2 , 4 μ g

Int. 20 , 25 , 50 , 100 ng

Time 2mins.

2) Response to serotonin

The action of serotonin resembled that of noradrenaline in that an intraluminal injection or extraluminal perfusion caused a transient rise in perfusion pressure and intraluminal or extraluminal perfusion caused a sustained rise.

Dose response curves to serotonin and noradrenaline are shown in Fig. 5. It will be noted that ~~the dose response curve to serotonin is much flatter than that to noradrenaline, and that over~~ all dose ranges its constrictor potency is less than that to noradrenaline.

3) Interaction of serotonin and noradrenaline

As well as constricting the artery, serotonin also enhanced the sensitivity of the artery to noradrenaline. The constrictor responses to intraluminal injections and extraluminal perfusions of noradrenaline were increased regardless of whether serotonin was added to the extraluminal or intraluminal perfusion media and regardless of whether serotonin itself caused constriction.

Fig. 6 shows the increase in response to intraluminal injections of noradrenaline during perfusion of serotonin at 20 ng/ml.

The sensitizing action of serotonin is apparent within seconds of contact with the artery and disappears within two minutes of its washout. The onset and offsets of action were characterised

Fig. 5. Log dose response curves for intraluminal injections of noradrenaline and serotonin. At all dose levels the constrictor potency of serotonin is less than that to noradrenaline.

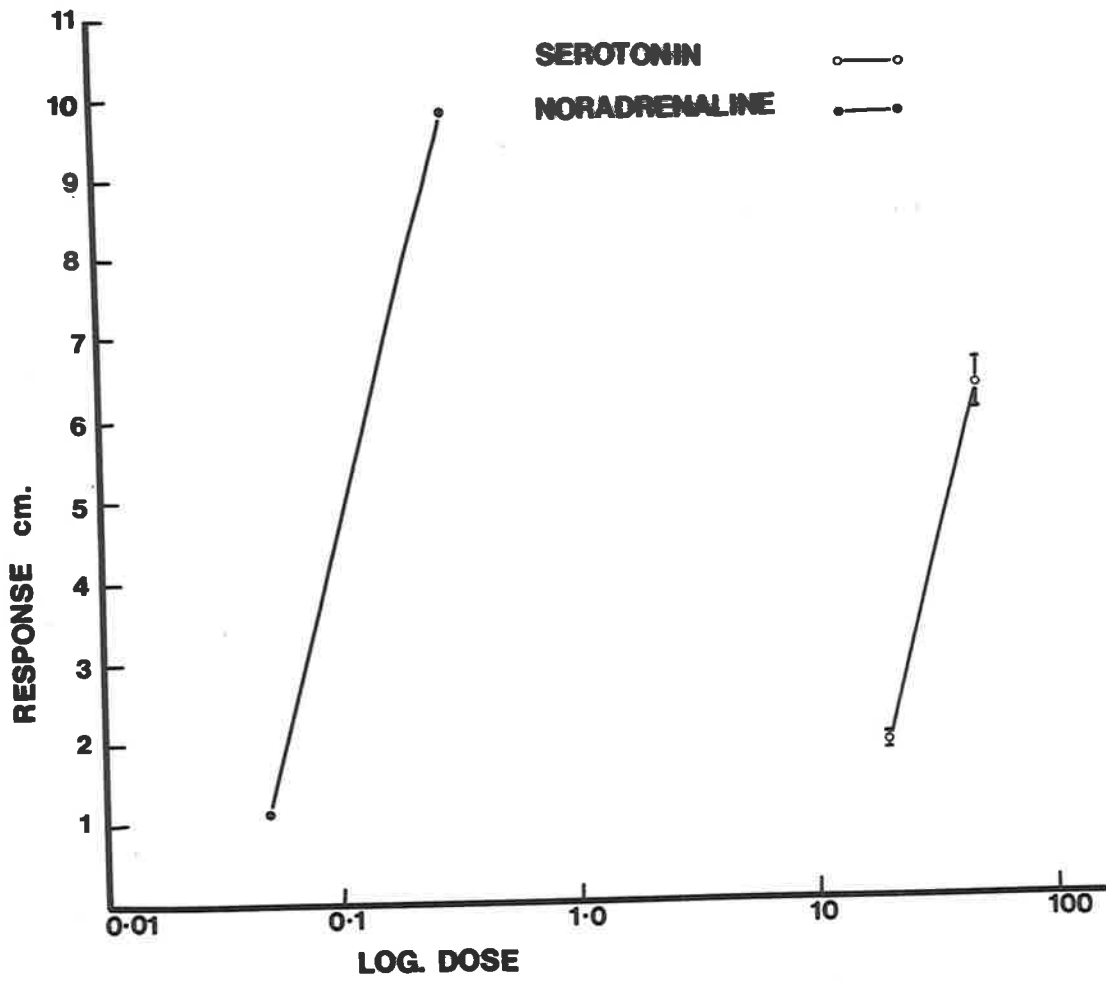


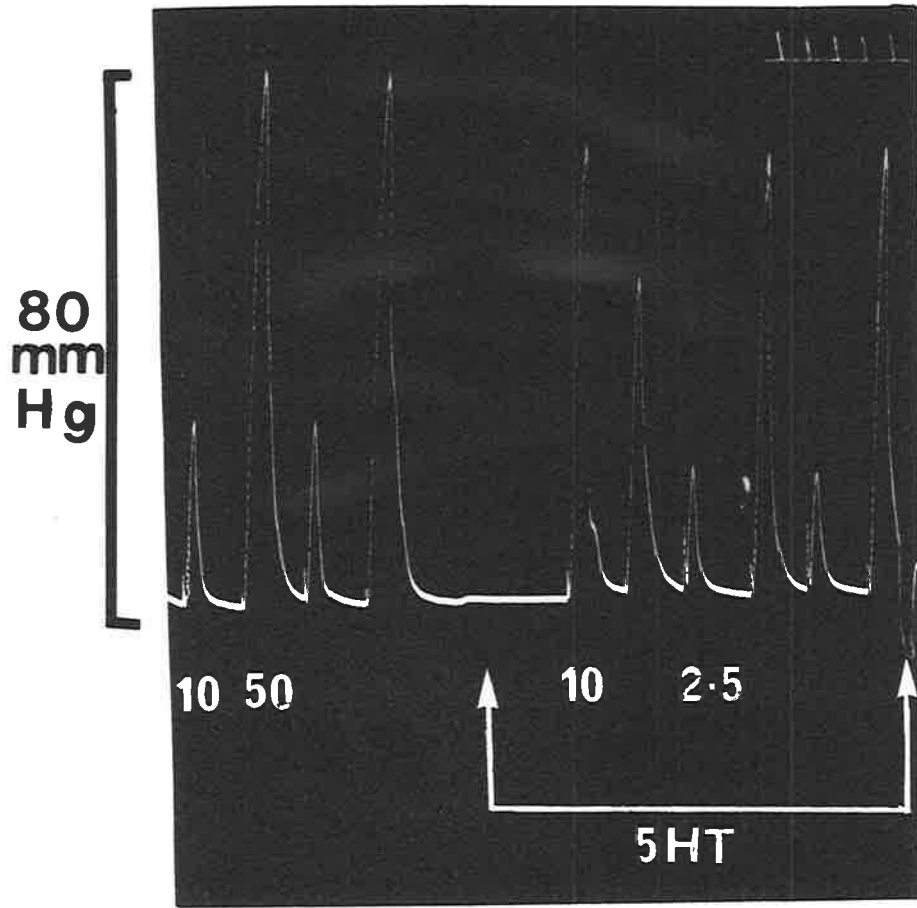
Fig. 6. A portion of a kymograph recording of the responses of a single cannulated artery to intraluminal injections of noradrenaline, in the absence and in the presence of serotonin 20 ng/ml in the Krebs bicarbonate perfusate.

Noradrenaline in the absence of serotonin

10 ng and 50 ng

Noradrenaline in the presence of serotonin 20 ng/ml

2.5 ng, 5 ng and 10 ng.



Int.	n g.
5HT	20ng/ml.
Time	2 mins.

by the increased sensitivity to injection of noradrenaline following perfusion of serotonin and by the speed with which sensitivity to injected noradrenaline changed following the addition and removal of serotonin from the extraluminal or intraluminal perfusing fluid.

The responses to noradrenaline during perfusion of serotonin were more prolonged, with slower onset and offset.

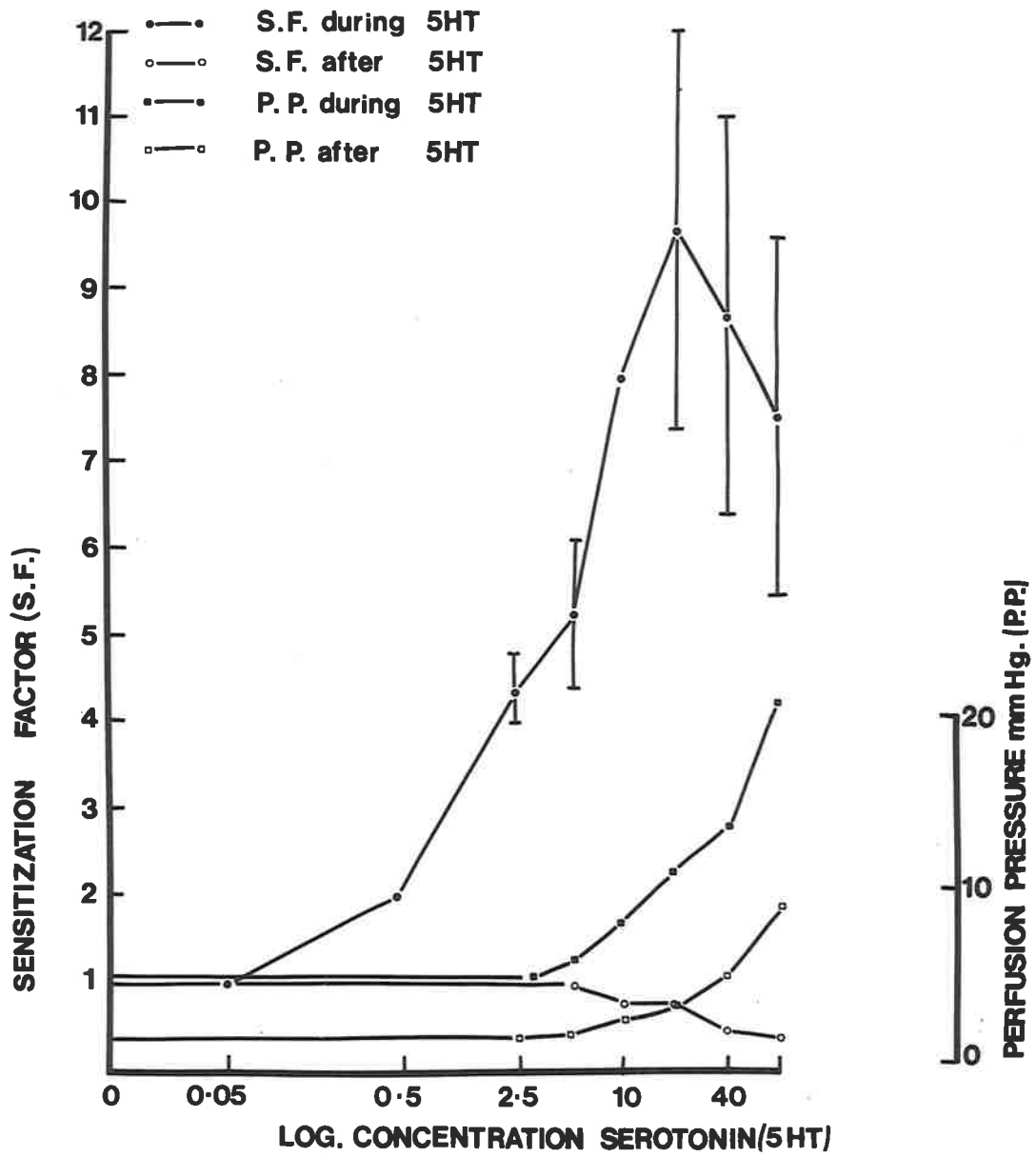
The relation between the constrictor and the sensitizing action of serotonin

As serotonin both constricted the artery and sensitized it to the action of noradrenaline it was necessary to determine whether these two actions were related.

In three experiments the concentration of serotonin was varied and the effect on the sensitizing and constricting actions were recorded.

Two procedures were used. In two experiments a dose response curve was obtained for intraluminal injections of noradrenaline from 0.01 ng/ml to 40 ng/ml inclusive in one experiment and 5 ng/ml to 80 ng/ml inclusive in the second. Dose response curves were obtained at each concentration for intraluminal ^{administration} injections of ^{serotonin} noradrenaline. In the third experiment, doses of serotonin were not cumulative; instead serotonin was washed out after each dose. The concentration was varied between 0.05 ng/ml to 80 ng/ml inclusive. The relation between the concentration of serotonin, the degree of constriction estimated in terms of the perfusion pressure and the degree of sensitization estimated from curves in the presence of serotonin and those obtained on each washout were calculated for the non-cumulative method of serotonin addition. The relation is shown in Fig. 7. Values for concentration, constriction, and the degree of sensitiz-

Fig. 7. Graph showing the relation between concentration of serotonin between 0 - 80 ng/ml, the sensitization factor for intraluminal injections of noradrenaline and the pressure rise due to serotonin constriction of the artery.



ation were similar in all experiments, however in one experiment the maximum sensitization was obtained at concentrations above 80 ng/ml serotonin.

Serotonin sensitized the perfused central artery of the rabbit ear to the constrictor action of noradrenaline at concentrations below those necessary to cause constriction. Sensitization was first observed at a concentration of 0.5⁻ ng/ml, at which concentration the sensitization factor was 2. The threshold for sensitization was between 0.05 ng/ml and 0.5 ng/ml of serotonin. The threshold for constriction was about 5 ng/ml.

Discussion

Sensitization of the artery to the constrictor action of noradrenaline took place at concentrations of serotonin approximately one hundred times less than those needed to cause constriction. The two actions of serotonin on the artery were apparently separate in these experiments as the constriction became greater after the maximum sensitization had been reached. It should be pointed out however that although constriction of the artery only became apparent at 5 ng/ml of serotonin, it may be argued that a small degree of constriction might be present at lower concentrations of serotonin which would not have been measured by the mercury manometer. This point is answered in part in appendix I of this thesis dealing with the spiral strip preparation of the artery.

The sensitization of the artery to the constrictor action of noradrenaline by serotonin occurs with very little change in the slope of the dose response curve. On the other hand when dose response curves are plotted for serotonin alone and for serotonin in the presence of noradrenaline the latter slope was greatly increased as well as being shifted to the left along the axis. The increase in the slope of the serotonin dose response curve in the presence of noradrenaline suggested that the artery was sensitized to the constrictor action of noradrenaline by serotonin. The other possibility

that noradrenaline sensitized the artery to the constrictor action of serotonin is a less likely explanation in view of the very small increase in slope of the dose response curve for noradrenaline compared to the shift to the left along the axis (de la Lande et al 1966).

From these experiments it was concluded that the increase in sensitivity of the artery to noradrenaline in the presence of serotonin is probably a true sensitization of the artery to vasoconstriction by serotonin and this sensitization may be separated from the constrictor action of serotonin by lowering the concentration of serotonin perfused through the artery to a sub threshold concentration for serotonin constriction.

Interaction between serotonin and vasoconstrictors other than noradrenaline

Introduction

To find out whether the sensitizing action of serotonin was specific for noradrenaline, the interaction between serotonin and other vasoconstrictors was investigated.

Methods

The methods used in this section were the same as those used for the interaction of serotonin and noradrenaline.

RESULTS

Response to Histamine

The normal response to intraluminal injections of histamine was similar to that to noradrenaline. A rapid rise in pressure was followed by a rapid fall when histamine was removed. The responses were dose dependant. The potency of histamine appeared to be of the order of one twentieth that of noradrenaline, but a precise comparison was not made.

The effect of serotonin on these responses was examined by a similar procedure to that used for noradrenaline (page 23).

The sensitizing action of serotonin on the central artery of the rabbit ear was not specific as it sensitized the artery to histamine in three experiments. In one serotonin was added at a concentration of 20 ng/ml and the sensitization factor was 10.7. In the second experiment serotonin was added at a concentration of 50 ng/ml and the sensitization factor was 3.5 immediately after serotonin addition but rose to 24.5 nearly two hours after addition. In the third artery serotonin was added cumulatively in concentrations from 1ng/ml to 8 ng/ml. In this artery sensitization was greatest at 4 ng/ml, the sensitization factor was 15.5 whereas at a concentration of 8 ng/ml the sensitization factor was 13.5 ng/ml. The reason for the difference in the maximum sensitizing concentration in the two methods of serotonin addition was not investigated. These results confirmed those reported by de la Lande et al (1966).

Response to Potassium chloride

Potassium chloride caused a transient vasoconstriction when injected in amounts of 3.12 μg to 6.24 μg . Dose response curves were obtained for the constriction due to intraluminal injections of potassium chloride both before and during simultaneous perfusion of serotonin at 20 ng/ml. Sensitization factors were calculated. In two experiments responses were also obtained on the same artery to noradrenaline in one before and in one after those to potassium chloride. Sensitization factors for noradrenaline were calculated in two experiments (by the method on page 19).

The serotonin sensitization factor for potassium chloride ranged between 1.3 and 2.5 in four arteries. In two of these arteries the values for noradrenaline were 2.4 and 3.6. The results are shown in Table I.

The possibility was investigated that part of the constrictor action of potassium chloride might have been indirect and caused by release of noradrenaline. To test this possibility, two arteries were treated with an antagonist of the constrictor action of noradrenaline. Dose response curves were obtained for potassium chloride and noradrenaline. 5 μg of phenoxybenzamine hydrochloride (dibenyline) was injected intraluminally after which the pump was turned off and the artery allowed to remain in contact with phenoxybenzamine for five minutes. The drug was then washed out and

further dose response curves were obtained for potassium chloride while the dose of noradrenaline was systematically increased (to 1 mg) to provide a measure of the antagonism. Table I lists the results.

TABLE I

Expt No.	5HT S.F. for KCL	Phenoxybenzamine hydrochloride present	5HT S.F. for NA
I	I.4	+	
2	I.3	+	
3	I.9	-	3.4
4	I.6	-	3.0

NA noradrenaline
S.F. sensitization factor

Responses to potassium chloride were unaffected by phenoxybenzamine hydrochloride but there was no response to 1 mg nor-

adrenaline. This shows that noradrenaline was antagonized by a factor of more than 10^5 , and that the constrictor action of potassium chloride was direct, that is it was not mediated by release of noradrenaline.

Discussion

Serotonin sensitized the perfused central artery of the rabbit ear to both histamine and potassium chloride. In a parallel study it was shown that the artery was sensitized by serotonin to the effects of stimulation of the sympathetic nerve supply (de la Lande and Rand 1965). In another parallel study de la Lande et al (1966) demonstrated that serotonin sensitized the perfused artery to histamine in the presence of dibenylamine and angiotensin, and the perfused human digital artery to noradrenaline. Sensitization of the rabbit ear perfused at constant pressure had previously been shown by Ginzel and Kottegoda (1953) and Savini (1956).

Sensitization of the artery to a number of different vasoconstrictors which are believed to act at different sites suggests that serotonin acts on a basic mechanism in the contractile sequence and not at any one specific site of drug action. Injections of potassium chloride constricted the artery by direct depolarization of the muscle membrane and, as serotonin sensitized the artery to potassium chloride, it can be assumed that its site of action is at the membrane. However although serotonin sensitized the artery to the transient constrictor action of injected potassium chloride, provision of a high potassium level in the fluid surrounding the artery, completely inhibited the sensitization of

the artery to this constrictor action, even though it had little effect on the sensitivity of the artery to catecholamines (de la Lande et al 1966). Assuming that the high potassium level caused a prolonged depolarization it seems possible that serotonin acts on the ionic fluxes that lead up to depolarization rather than on any later phase in the action potential; the receptors that mediate the sensitizing action of serotonin must be linked with the membrane potential.

Section 2

Interaction of indoles, other than serotonin, with noradrenaline

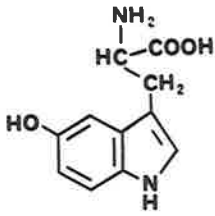
In the previous section it was demonstrated that the serotonin sensitization was not specific for noradrenaline or even for one class of vasoconstrictors. This section describes the interaction of indoles other than serotonin with noradrenaline. The study was undertaken in order to test whether other indoles besides serotonin caused sensitization, and to compare the pharmacological properties of the serotonin receptors mediating sensitization in the artery with those in other tissues.

The indole derivatives were as follows:-

- 1) A precursor and a metabolite of serotonin.
- 2) Indole derivatives which have been shown to be antagonists of serotonin on other tissues. (One non-indole derivative has been included).
- 3) Miscellaneous indoles.

The chemical structures of the compounds is shown in Fig. 8.

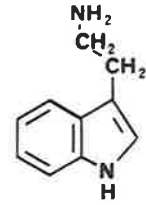
Fig. 8. Chemical structures of the indole derivatives perfused in place of serotonin or together with serotonin.



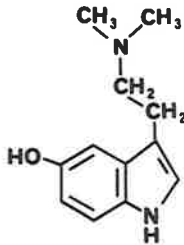
**5-HYDROXYTRYPTOPHAN
(5 HTP)**



**5-HYDROXYINDOLEACETIC ACID
(5HIAA)**



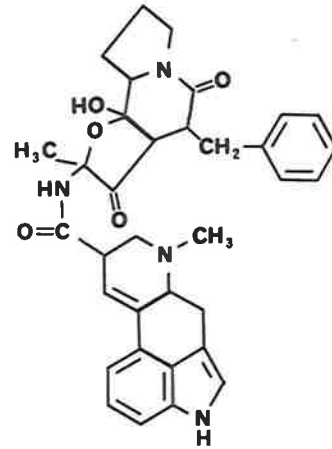
TRYPTAMINE



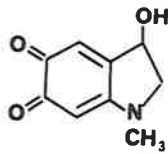
BUFOTENINE



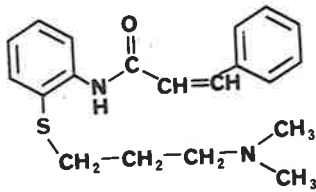
**5-HYDROXYTRYPTAMINE
OR SEROTONIN (5HT)**



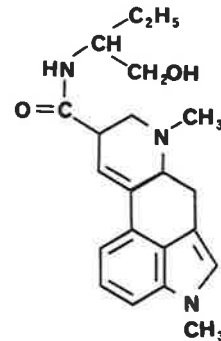
ERGOTAMINE



ADRENOCHROME



**2'-(3-DIMETHYLAMINOPROPYLTHIO)
CINNAMANILIDE (SQ 10,643)**



**1-METHYL-8-LYSERGIC ACID
BUTANOLAMIDE (METHYSERGIDE)**

Procedure

The methods of both cannulation and noradrenaline administration are the same as those described on pages I5 to I7 . .

Dose response curves were obtained for noradrenaline before, during and after perfusion of serotonin at 20 ng/ml. After serotonin had been washed out further dose response curves were obtained for noradrenaline before, during and after perfusion of the indole derivative at 20 ng/ml. In some arteries the indole derivative was constrictor at 20 ng/ml and in these experiments the concentration was decreased until a dose response curve could be obtained for noradrenaline. In experiments where the noradrenaline response was unaffected by perfusion of the indole derivative, the concentration of indole was progressively increased as indicated in the appropriate table. In some experiments where the indole derivative was inactive it was perfused together with serotonin in order to detect possible interaction between the two indoles.

RESULTS

When serotonin was added to the Krebs bicarbonate solution at a concentration of 20 ng/ml the sensitization factor was 6.39 ± 3.78 in 18 experiments.

I) A serotonin precursor and metabolite

5-hydroxytryptophan (5HTP) a precursor of serotonin and 5-hydroxyindoleacetic acid (5HIAA) were perfused through the artery.

The results of these experiments are shown in Tables 2 and 3.

In three experiments 5HTP perfused at concentrations between 20 ng/ml and 80 μ g/ml and serotonin in a concentration of 20 ng/ml did not constrict the arteries. The 5HTP sensitization factor for intraluminal noradrenaline was less than that of serotonin in all three experiments. In two arteries 5HTP perfused simultaneously with serotonin reduced the previously determined serotonin sensitization factor.

In concentrations between 20 ng/ml and 15 μ g/ml 5HIAA did not constrict four arteries, however serotonin at a concentration 20 ng/ml did constrict three of these arteries. The sensitization factor for 5HIAA and serotonin perfused simultaneously was less

than that due to serotonin perfused alone, but there was no obvious effect on the sensitivity to intraluminal injections of noradrenaline when 5HIAA was perfused alone.

TABLE 2

Expt No.	5HTP conc	5HT S.F. Int.Inj	5HT + 5HTP S.F. Int.Inj	5HTP S.F. Int.Inj
1	10 µg/ml	4.6	-	1.5
	80 µg/ml	-	3.2	1.3
2	10 µg/ml	8.8	6.0	1.2
3	10 µg/ml	5.1	-	1.8

S.F. sensitization factor

TABLE 3

Expt. No.	5HIAA conc.	5HT S.F. Int. Inj	5HT + 5HIAA S.F. Int. Inj	5HIAA S.F. Int. Inj
1	5 $\mu\text{g/ml}$	14.7	9.5	1.3
2	5 $\mu\text{g/ml}$	7.5	3.9	0
3	5 $\mu\text{g/ml}$	3.9	2.8	1.1
4	15 $\mu\text{g/ml}$	6.9	4.8	0

S.F. sensitization factor

2) Antagonists of serotonin

Methysergide and ergotamine are potent antagonists of serotonin on several isolated tissues. Methysergide inhibited oedema formation due to injection of serotonin into the rat paw (Doepfner and Cerletti 1958). Ergotamine antagonised the constrictor action of serotonin on the isolated rat uterus (Fingl and Gaddum 1953). These two substances were perfused through the artery in place of serotonin in this study to attempt to antagonise either the constrictor or the sensitizing action of serotonin and thus to separate these two actions.

RESULTS

The results of the experiments perfusing ergotamine and methysergide are shown in Tables 4 and 5.

Ergotamine was slightly constrictor in three of four arteries at a concentration of 0.5 ng/ml but in one the constriction was not sustained. A comparison was not made between serotonin and ergotamine but ergotamine increased the sensitivity of the artery to noradrenaline administered by all routes, and a comparison of these values with values for serotonin in

other experiments suggests that ergotamine is more active in sensitizing the artery to noradrenaline at a concentration of 0.5 ng/ml than serotonin perfused at a concentration of 0.5 ng/ml.

Methysergide sensitized the artery to noradrenaline in three arteries at a concentration of 40 ng/ml, in one of these sensitization was greater at 160 ng/ml than that at 40 ng/ml. Methysergide constricted this artery at a concentration of 40 ng/ml but at a concentration of 160 ng/ml in this artery and 40 ng/ml in the other two arteries there was no constriction. Serotonin at a concentration of 40 ng/ml constricted the artery in two experiments.

TABLE 4

Expt No.	Ergotamine conc.	Ergotamine S.F.		
		Int. Inj.	Ext. Perf ⁿ .	Int. Perf ⁿ .
I	0.5 ng/ml		2.7	7.6
2	0.5 ng/ml	18.3	10.8	
3	0.5 ng/ml	4.8	3.8	
4	0.5 ng/ml	3.9	2.9	

TABLE 5

Expt No.	Methysergide conc	5HT conc	5HT S.F. Int. Inj.	Methysergide S.F.
I	40 ng/ml 160 ng/ml	20 ng/ml	7.5	3.2 4.1
2	40 ng/ml	40 ng/ml	6.7	6.4
3	40 ng/ml	40 ng/ml	30.5	7.9

S.F. sensitization factor

Fingl and Gaddum (1953) reported antagonism of the constrictor action of both serotonin and adrenaline in the perfused rabbit ear by ergotamine. de la Lande and Rand (1965) and de la Lande and Harvey (1965) both reported the sensitization of the perfused artery of the rabbit ear to adrenaline by serotonin. On this basis it might have been expected that ergotamine would have sensitized the perfused rabbit ear of Fingl and Gaddum. There is no satisfactory explanation for this difference in action of ergotamine in the two series of experiments but Fingl and Gaddum do not state the conditions under which the ear was perfused. In earlier experiments (Gaddum and Hameed 1954) rabbit ears were perfused at constant pressure and room temperature but as shown in appendix 2 these conditions do not prevent sensitization of the isolated and perfused artery of the rabbit ear.

A non-indole antagonist of serotonin

Only one non-indole antagonist of serotonin was investigated. 2'-(3-dimethylaminopropylthiocinnamanilide) hydrochloride (SQ 10,643) which is a potent antagonist of the constrictor action of serotonin on the isolated rat uterus and guinea-pig ileum (Report from the Pharmacology Section, The Squibb Institute for Medical Research).

Procedure

The artery was single cannulated and dose response curves were obtained for intraluminally injected noradrenaline under the following conditions:-

- 1) Before and during perfusion of serotonin at 20 ng/ml,
- 2) before and during perfusion of SQ IO,643 at concentrations varying between 20 ng/ml and 1.5 µg/ml in three separate experiments,
- 3) before and during perfusion of serotonin at 20 ng/ml together with SQ IO,643 at concentrations between 20 ng/ml and 10 µg/ml.

Sensitizing factors were calculated for serotonin, and SQ IO,643 at the concentrations shown in Table 6.

RESULTS

It can be seen from this table that SQ IO,643 was not a specific antagonist of serotonin in this system, as noradrenaline was also antagonized. No further experiments were performed. SQ IO,643 in one of the three experiments appears to have increased the sensitivity of the artery very slightly to intraluminally injected and extraluminally perfused noradrenaline in the presence of serotonin.

Table 6

Sensitization factors for Serotonin and SQ IO,643

Expt No.	5HT 20 ng/ml S.F.		SQ IO,643 S.F.			SQ IO,643 + 5HT S.F.		
	Noradrenaline Int.Inj	Ext.Perf ⁿ	SQ Conc	Noradrenaline Int.Inj	Ext.Perf ⁿ	SQ Conc	Noradrenaline Int.Inj.	Ext.Perf ⁿ
1	11.8	-	1ug/ml	0.4	-	1ug/ml	2.8	-
2	4.7	2.7	20 ug/ml	0	1.3	20 ug/ml	5.9	4.0
3	2.6	3.6	1.5 ug/ml	0.3	0.5	5ug/ml	0.3	0.5

S.F. Sensitization factor

3) Miscellaneous indoles

The results of perfusion of bufotenine, adrenochrome, and tryptamine are summarized in Tables 7, 8 and 9.

The sensitization factors for bufotenine and serotonin were similar when noradrenaline was administered by all routes. In four arteries serotonin did not cause constriction, in one of these arteries bufotenine caused a transient very small constriction.

Adrenochrome had no measurable effect on the sensitivity to noradrenaline in three arteries, but reduced the sensitization factor for serotonin.

Serotonin constricted three arteries in a concentration of 20 ng/ml however in the same three arteries adrenochrome did not cause constriction at a concentration of 10 μ g/ml.

The sensitization factor for tryptamine perfused through the artery at a concentration of 20 ng/ml was lower than that for serotonin at 20 ng/ml in three arteries. At a concentration of 1 μ g/ml the sensitization factor for tryptamine was less than that for serotonin at a concentration of 20 ng/ml. Serotonin at this concentration constricted the artery in two of three experiments and tryptamine constricted the artery in two experiments at a concentration of 1 μ g/ml.

TABLE 7

Expt No.	Bufotenine Conc.	5HT S.F.			Bufotenine S.F.		
		Int. Inj	Ext Perf ⁿ	Int Perf ⁿ	Int Inj ¹	Ext Perf ⁿ	Int Perf ⁿ
I	20 ng/ml	11.9	4.4		9.1	6.9	
2	20 ng/ml	4.9	4.1		9.1	3.8	
3	20 ng/ml		4.0 approx	4.5		3.7	4.4
4	20 ng/ml		7.0	10.4		2.9	8.0 approx

TABLE 8

Expt No.	Adrenochrome conc	5HT S.F.	5HT+Adrenochrome S.F.	Adrenochrome S.F.
I	10 µg/ml	13.2	3.3	0.9
2	10 µg/ml	3.2	3.4	0
3	10 µg/ml	3.9	2.6	0.8

S.F. Sensitization factor

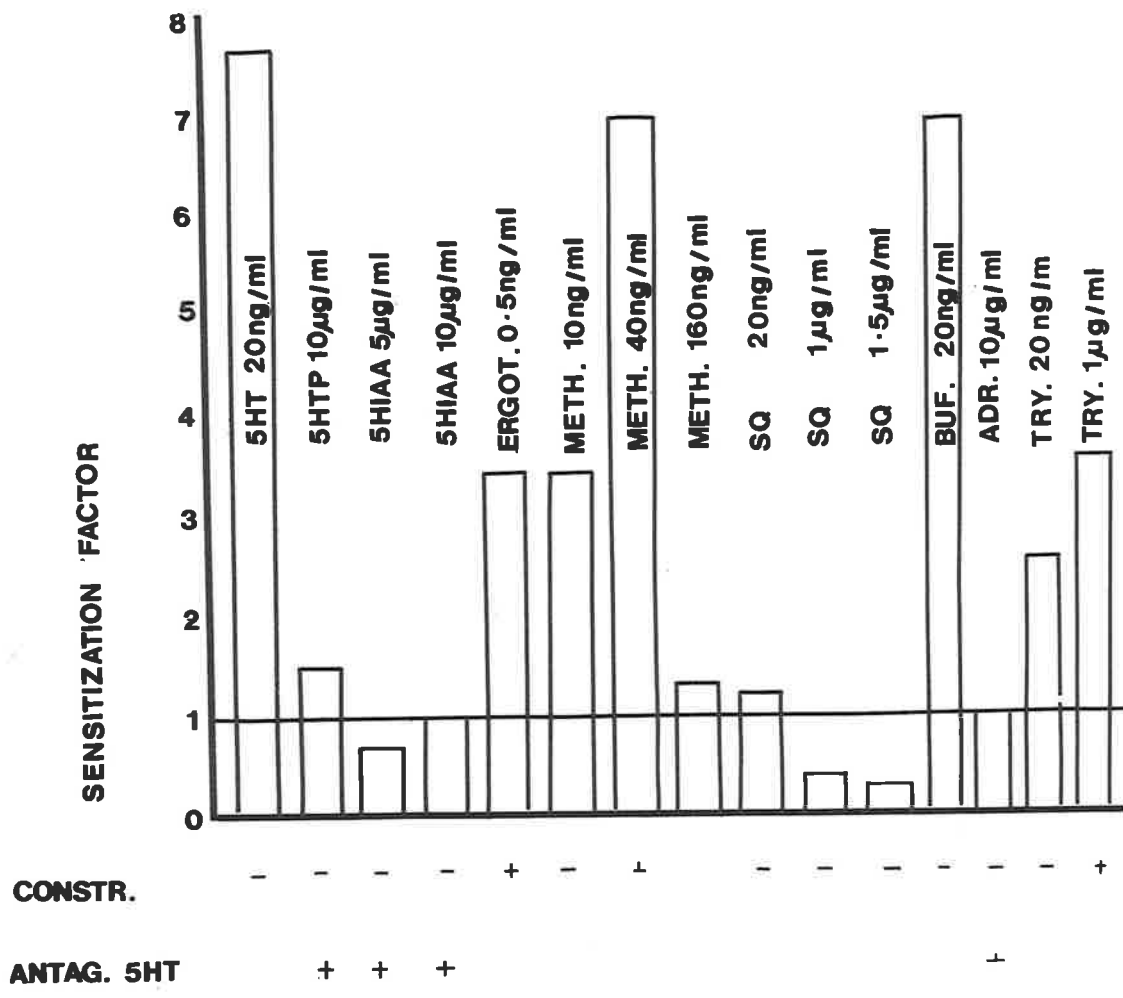
TABLE 9

Expt No.	Tryptamine conc	5HT S.F.	Tryptamine S.F.
I	20 ng/ml I µg/ml	4.1	1.9 4.0
2	20 ng/ml	3.5	1.5
3	20 ng/ml I µg/ml	5.0	1.9 3.0

S.F. sensitization factor.

Histogram I

The height of each column represents the sensitization factor. + denotes constriction or antagonism
I denotes possible constriction



Discussion (Histogram I is a summary of these results)

The results reported in this section show that sensitization of the artery to noradrenaline is not a property specific to serotonin. It is displayed by many indole derivatives of which serotonin at a concentration of 20 ng/ml is the most active. In terms of potency (i.e. concentration required to produce sensitization) ergotamine is highly active, even at concentrations as low as 0.5 ng/ml.

It is of interest that among the substances causing sensitization are ergotamine and methysergide which antagonize the action of serotonin on other tissues such as the rat uterus (Fingl and Gaddum 1953) and oedema formation in the rat paw (Doepfner and Cerletti 1958). It is possible that these substances are partial agonists, but the usual tests e.g. depression of serotonin's sensitizing action, were not applied owing to the sensitization caused by the compound in question. However the main feature is the failure to obtain an antagonist of the sensitizing action of serotonin amongst the compounds tested. This clearly shows that the receptors 'mediating sensitization' in the artery differ in their structural requirements from those causing constriction in other types of smooth muscle. The possibility that receptors responsible for con-

striction in the artery may be similar to the latter types of receptor was not examined in the present study.

Tryptamine and 5-hydroxytryptophan, a precursor of serotonin, in this series of experiments acted as partial agonists of serotonin in that they sensitized the artery to noradrenaline but to a lesser extent than serotonin. Tryptamine was less active than serotonin as a sensitizing agent in four out of five experiments although there was a definite degree of sensitization ranging from sensitivity factors of 1.9 to 4.0. The sensitization due to 5-hydroxytryptophan was small, the sensitivity factors ranging from 1.4 to 1.6. The one metabolite investigated, 5-hydroxyindoleacetic acid was totally inactive as a sensitizing agent, the sensitivity factors in four experiments ranging from 0 to 1.1. From the recorded data in these experiments it would seem that serotonin and not a breakdown product (5-hydroxyindoleacetic acid), or a precursor (5-hydroxytryptophan) is the active substance in this sensitization phenomenon.

An initial impression of the structure of the serotonin receptor can be gained by examining the structure of the compounds perfused through the artery in this section. The receptor requires an intact indole ring structure; adrenochrome, which lacks one of the two double bonds in the five-membered ring is totally inactive as a sensitizing agent.

All of the sensitizing agents investigated in this section had a substitution in the position 3 of the indole structure and a positive charge on the side-chain at position 2. For example serotonin itself or bufotenine (in which the amine has two methyl substituents) were both active sensitizing agents, whereas 5-hydroxyindoleacetic acid which is negatively charged was inactive as a sensitizing agent but was a weak inhibitor of the serotonin sensitization of the artery to noradrenaline. 5-hydroxytryptophan, a zwitterion, sensitized to a small degree, but it was also a weak inhibitor of serotonin sensitization. The positively charged side-arm at position 3 may either be necessary for binding of the molecule at the receptor or the indole may be bound at the receptor and the side-arm required to react with some other structure.

Ergotamine, which in other tissues is an antagonist of the constrictor action of serotonin (Fingl and Gaddum 1953) in this preparation was more active than serotonin in both constricting and sensitizing the artery. The ring structure of the molecule has a much more rigid structure than that of serotonin and if it acts at the same receptor as serotonin the rigidity of the molecule must hold the positive charge in a particular position, which is likely to be optimal for sensitization.

The hydroxyl group at position 5 is not essential for

sensitization as tryptamine shows some activity; but it does increase the activity, as both serotonin and bufotenine, which both possess this hydroxyl group are more active than tryptamine. Ergotamine appears to be a more active sensitizing agent than serotonin although it does not possess a hydroxyl group at position 5. This extra activity may be due to the rigidity of the molecule binding it more strongly to the receptor. Two other possibilities are that additional binding occurs either through the peptide side chain at position 6 or the two extra ring structures. The third possibility is a remote one as methysergide, a manufactured ergotamine derivative lacking the peptide side chain at position 6 but containing the two extra ring structures, is approximately eighty times less active as a sensitizing agent. Methysergide was also much less active as a constricting agent than ergotamine. This may be due to the substitution of a methyl group on the nitrogen at position I of methysergide or absence of a peptide side chain equivalent to the one on ergotamine.

A great deal more work is necessary before any definite conclusions are possible about the nature of the serotonin receptor but in summary these experiments suggested:-

- 1) an indole is necessary for sensitization
- 2) a hydroxyl group at position 3 or a rigid

molecule increases the activity.

3) substituents at position 3 which confer a positive charge are necessary for activity; negatively charged substances are weak inhibitors of the sensitizing action of serotonin.

Section 3

Comparison of the effects of serotonin, cocaine and reserpine on the rabbit ear artery

In the present study, further evidence that the action of serotonin does not involve an adrenergic mechanism was sought by measuring the effect of sympathetic denervation, reserpine and of cocaine on serotonin action. The first section showed that the action of serotonin was not specific for noradrenaline. Nevertheless, it seemed desirable to compare serotonin's sensitizing action with those of other types of procedure which are known to cause sensitization to noradrenaline, and to assess whether serotonin still acted on such sensitized arteries. Sympathetic denervation and cocaine were used since their ability to enhance the sensitivity to noradrenaline, and the mechanism involved, is well documented. Reserpine was included in this study since in addition to depleting noradrenaline from sympathetic nerves it is known to release serotonin from its binding sites in other tissues (platelets, brain) (see review by Shore 1962). Thus it was possible that the reserpine treated artery might respond to serotonin in a different fashion to the normal artery.

In addition these experiments provide a further test of the role of adrenergic mechanisms in the action of serotonin.

The interaction of serotonin and cocaine in the perfused artery

Procedure

After initial dose response curves for intraluminally injected and extraluminally perfused noradrenaline had been obtained two procedures were followed:-

1) a) serotonin was perfused intraluminally and extraluminally at a concentration of 20 ng/ml and dose response curves were obtained as above for noradrenaline in the presence of serotonin.

b) cocaine was then added to the perfusate at a concentration of 10 µg/ml both intraluminally and extraluminally and dose response curves were obtained for noradrenaline as above in the presence of serotonin and cocaine.

c) serotonin was washed out and dose response curves were obtained as above for noradrenaline in the presence of cocaine.

d) cocaine was washed out and dose response curves were obtained for noradrenaline during perfusion of Krebs bicarbonate.

2) The addition of serotonin and cocaine was reversed in the

second procedure.

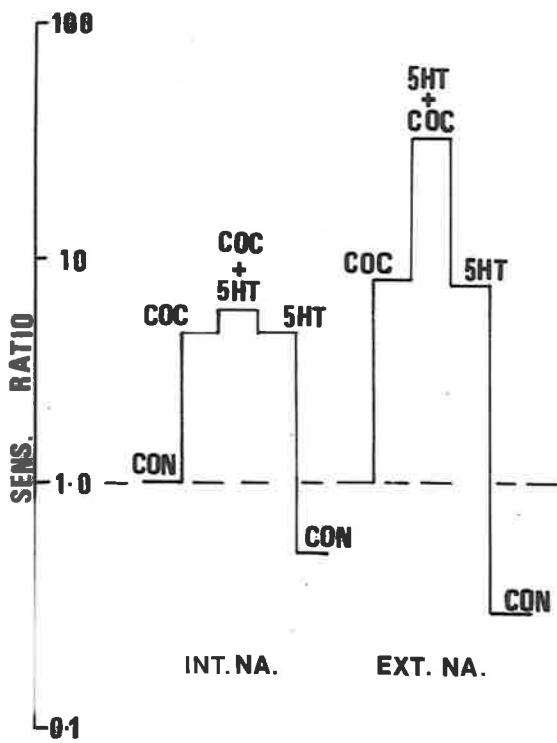
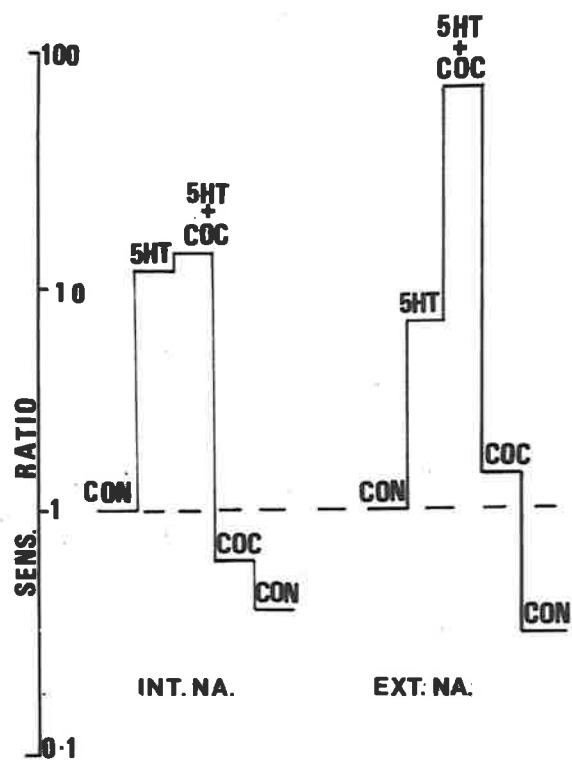
The sensitization factor for serotonin, serotonin in the presence of cocaine, cocaine and cocaine in the presence of serotonin, were calculated by the method described on page 19, for intraluminally injected and extraluminally perfused noradrenaline. The procedure is summarized in the diagram in Fig. 9.

RESULTS

Serotonin 20 ng/ml perfused intraluminally and extraluminally caused an increase in perfusion pressure on its own in one of seven arteries. In the same artery constriction to serotonin was increased in the presence of cocaine and the constriction was sustained. In one of seven arteries serotonin did not constrict the artery on its own but constriction occurred when cocaine was added to the perfusate although it was not sustained. In two arteries a non-sustained constriction occurred when serotonin was added to a cocaine perfused artery. In three arteries no constriction occurred in the presence of either of the sensitizing agents.

Irrespective of the effect of serotonin on the perfusion pressure the constrictor responses to noradrenaline were

Fig. 9 Diagrammatic representation of addition of serotonin and cocaine and calculation of the sensitization factors for cocaine and serotonin and combinations of the two drugs. The height of the columns denotes the sensitization factors.



increased in each artery. The magnitude of the increases in sensitivity to intraluminal and extraluminal noradrenaline were 10.19 ± 4.50 and 14.10 ± 14.70 respectively. Values for seven experiments are shown in Table IO. These estimates include those based on testing noradrenaline sensitivity prior to adding and in the presence of cocaine, and those based on the reduction in sensitivity following washout of serotonin.

In all experiments cocaine in the absence of serotonin increased the sensitivity to extraluminal noradrenaline much more than to intraluminal noradrenaline, the increase amounting to 9.47 ± 7.19 and 1.74 ± 1.02 respectively. The corresponding increases in the presence of serotonin were 8.7 ± 6.08 and 3.44 ± 5.41 respectively, indicating that serotonin had not significantly altered the characteristic selective action of cocaine on extraluminal noradrenaline sensitivity. The results of seven experiments are also shown in Table IO. Paired t-tests using geometric means were carried out on these results to assess whether sensitization factors for cocaine alone and serotonin alone differed significantly from those values for cocaine in the presence of serotonin and serotonin in the presence of cocaine. Table II lists the results. The t-tests were used to determine whether the combination of route of administration of noradrenaline (column I) and the presence of the sensitizing agent or agents (column Ia)

Expt. No.	Procedure	Sensitization Factors							
		Noradrenaline Int. Inj.		Noradrenaline Ext. Per.		Noradrenaline Int. Inj.		Noradrenaline Ext. Per.	
		5HT	5HT in Coc	5HT	5HT in Coc	Coc	Coc in 5HT	Coc	Coc in 5HT
1	0 — 5HT — 5HT + Coc — Coc — 0 —	20.8	9.2	12.0	13.6	-	1.8	-	12.2
2	0 — 5HT — 5HT + Coc — Coc — 0	7.0	9.0	4.0	8.5	1.3	1.6	4.2	21.1
3	0 — Coc — Coc + 5HT — 5HT — 0	7.4	2.1	26.3	4.4	2.5	1.2	6.3	4.7
4	0 — Coc — Coc + 5HT — 5HT — 0	10.3	8.4	64.7	25.7	1.5	2.3	26.0	2.5
5	0 — Coc — Coc + 5HT — 5HT — 0	7.7	11.5	9.4	4.9	0.9	2.1	13.0	10
6	0 — Coc — Coc + 5HT — 5HT — 0	8.9	6.6	6.6	4.1	1.5	1.2	4.8	2.8
7	0 — 5HT — 5HT + Coc — Coc — 0	7.3	12.4	-	6.1	1.1	1.4	5.0	8.7

Table IO

Table II

Comparison No.	NA route of ad. I	Ia	NA route of ad. 2	2a	Significance	Probability
1)	I	5HT	I	5HT in Coc	-	0.05
2)	E	5HT	E	5HT in Coc	-	0.05
3)	I	Coc	I	Coc in 5HT	-	0.05
4)	E	Coc	E	Coc in 5HT	-	0.05
5)	I	5HT	I	Coc	+	0.005
6)	E	5HT	E	Coc	-	0.05
7)	I	5HT	E	5HT	-	0.05
8)	E	5HT	I	Coc	-	0.05
9)	I	Coc	E	Coc	+	0.05
10)	I	5HT in Coc	E	5HT in Coc	-	0.05
11)	I	Coc in 5HT	E	Coc in 5HT	+	0.02
12)	I	5HT in Coc	I	Coc in 5HT	+	0.001
13)	E	5HT in Coc	E	Coc in 5HT	-	0.05

T tests were carried out for the values in columns I and Ia and 2 and 2a.

I Intraluminal

E Extraluminal

ad. Administration

was significantly different from the combination in columns 2 and 2a. From the table it can be seen that the only comparisons which were significantly different were 5,9,11,12.

These results confirm that cocaine was more active in sensitizing the artery to extraluminally applied noradrenaline than to intraluminally injected noradrenaline (de la Lande 1966) and showed that serotonin was equally active in sensitizing to noradrenaline applied by either route.

Effect of sympathetic denervation on serotonin action

In three experiments, the effect of serotonin on a chronically denervated artery was compared with that of a control (innervated artery) taken from the opposite ear of the same rabbit. The artery was denervated by removal of the homolateral superior cervical ganglion approximately seven days previously (see methods page 18), and double cannulated. Dose response curves were obtained for intraluminal injections and extraluminal perfusion of noradrenaline. Serotonin was perfused intraluminally and extraluminally and as it displayed greater constrictor activity in the denervated than in the control artery, the concentration employed was reduced to 5 ng/ml. Dose response curves were obtained for

for noradrenaline as above both during and after perfusion with serotonin.

RESULTS

Sensitization factors for serotonin in three sympathectomised and three control arteries are shown in Table I2.

TABLE I2

Expt No	Sympathectomised			Control		
	Int Inj	Int Perf ⁿ	Ext Perf ⁿ	Int Inj	Int Perf ⁿ	Ext Perf ⁿ
I	6.8	6.7	7.8	7.3	3.2	3.6
2	4.2	7.0	3.2	3.5	4.3	4.3
3	2.0	2.7	3.4	2.2	2.0	2.9

From these results, it was concluded that there was no obvious difference between the response of the control and the sympathectomised artery to serotonin.

The action of serotonin on reserpinized arteries

Procedure

The experiments were carried out on arteries from rabbits treated with reserpine. Preparation of the animals is described on page 18. Arteries were single cannulated. Dose response curves were obtained for intraluminal injections of noradrenaline before, during and after perfusion of serotonin at 20 ng/ml. Sensitization factors were calculated for serotonin.

RESULTS

Serotonin sensitized the artery to the constrictor action of noradrenaline in all three experiments (Table I3). In one experiment constriction of the artery was too great at 20 ng/ml serotonin and the concentration was reduced to 15 ng/ml in order to obtain a dose response curve for noradrenaline. The magnitude of the sensitivity changes are of the same order as in untreated arteries. It was concluded that treatment with reserpine had no effect on the action of serotonin.

60a

TABLE 13

Expt No.	Serotonin S.F. For reserpinized arteries
I	9.1
2	4.2
3	8.0 (approx)

Discussion

Waterson and Smale (1967) found that arteries taken from rabbits treated with reserpine did not exhibit noradrenaline fluorescence. The lack of fluorescence in arteries treated in this way was taken to mean that noradrenaline had been released from the storage sites at the sympathetic nerve terminals. Arteries taken from rabbits treated with reserpine and tested for sensitization to noradrenaline by serotonin showed no major difference in their response to noradrenaline but were more sensitive to the constrictor action of serotonin. Sensitization to noradrenaline by serotonin was of the same order as that in control arteries and it can thus be concluded that the sensitizing action of serotonin does not depend on the presence of noradrenaline in the nerve endings. Neither does it depend on the binding of serotonin itself to storage structures in the artery as reserpine depletes serotonin from such structures. (See review by Shore 1962).

Although reserpine depleted the noradrenaline content of the sympathetic noradrenaline stores, the actual structure of these stores was left intact as infusion of noradrenaline into reserpine treated animals restored the ability of nerve stimulation or tyramine to elicit an adrenergic response. (Reviewed by Shore 1962). In the experiments of de la Lande,

Frewin, Waterson and Cannell (1967) when one superior cervical ganglion was removed and time allowed for degeneration of the nerve, in contrast to the contra-lateral artery the sympathectomised vessel did not show fluorescence staining for noradrenaline. They suggest that as there is evidence in heart and other smooth muscle i.e. the cat nictitating membrane that degeneration is associated with loss of the uptake mechanism (Trendelenburg 1966) this might be the case in the artery. In the experiments described in this section when arteries were perfused by the method described on page 15, serotonin still sensitized these arteries to noradrenaline to the same degree as arteries obtained from control rabbit ears, although the chronically denervated arteries were more sensitive to noradrenaline. It was concluded from this evidence that the sympathetic nerve terminals in the artery are unlikely to play a part in the mechanism of serotonin sensitization.

de la Lande et al (1966) proposed that the low sensitivity to extraluminal noradrenaline reflected the relative positions of uptake and receptor sites, the former acting as a sink for noradrenaline at the outer boundary^a of the smooth muscle layer of the artery. On this basis the sites of uptake act as a sink for noradrenaline applied intraluminally when it diffuses from the smooth muscle into the adventitia after it has exerted its physiological action. However when noradrenaline is applied

extraluminally it must pass these sites of uptake before exerting its physiological action and thus the noradrenaline free to act on the smooth muscle is depleted before it reaches the receptors.

The assumptions made in putting forward this hypothesis are that the sites of concentration of noradrenaline in the artery are also sites of uptake and that cocaine which lowers the ratio of sensitivity between extraluminally and intraluminally applied noradrenaline does so by inhibiting the process of uptake as it does in heart and other smooth muscle. When the actions of serotonin and cocaine were compared by perfusing them alone and together through the artery in the present study it was found that serotonin sensitized the artery to extraluminally and intraluminally applied noradrenaline to approximately the same degree and that when cocaine was perfused through the artery together with serotonin the selective action of cocaine was not altered.

The new information gained in this study was:-

- 1) neither the noradrenaline in the sympathetic nerve storage sites nor bound serotonin appear to play any part in the sensitizing action of serotonin.
- 2) degeneration of the sympathetic nerve terminals does not alter the sensitizing action of serotonin.
- 3) serotonin is equally active in sensitizing the artery to noradrenaline applied intraluminally and extraluminally and

does not affect the selectively sensitizing action of cocaine
i.e. the actions of serotonin and cocaine perfused together seem
to be additive.

CHAPTER 5THE ACTION OF SEROTONIN ON THE CENTRAL ARTERY OF THE RABBIT EARIN VIVO

The experiments described in the previous chapters of this thesis were concerned with the phenomenon of serotonin sensitization of the isolated and perfused central artery of the rabbit ear to vasoconstrictor drugs by perfusion of serotonin, and other indole derivatives. The experiments in this chapter were performed in order to determine whether the phenomenon was confined to the isolated preparation or whether it occurred in vivo. For this purpose a technique for recording arterial diameter changes developed by Lippay (1956) was modified for use on the ear of the anaesthetized rabbit.

METHODS

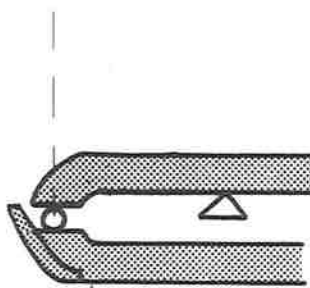
The principle of Lippay's method is that the artery is placed between light plastic calipers, of which one arm is fixed and the second arm rests lightly on the surface of the artery.

Movement of the second arm interrupts a light beam trained on a photo-cell. The method was modified by amplifying the output of the photo-cell which was then recorded on a Grass polygraph Model 5D. The recording system was calibrated at the conclusion of each experiment by inserting fine rods of known diameter between the arms of the calipers. Fig. 10 shows the recording system.

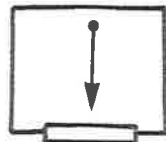
In preliminary experiments, it was apparent that exposure of the central artery, followed by contact with the calipers led to a spasm of the artery which frequently prevented satisfactory recording. However in a number of rabbits it was possible to demonstrate pulsations of the artery and a decrease in diameter in response to either sympathetic nerve stimulation or to infusion or external application of serotonin. The method of preparing the ear for recording and a summary of these results are given in appendix 3. The limited success of the direct measurement of arterial diameter was taken as proof that it was possible to measure changes in arterial diameter in the ear and hence attempts to record the diameter changes directly were abandoned and the calipers were modified to record changes in the thickness of the ear over the region of the central artery where it is separated from the vein, as an indirect measure of changes in arterial diameter.

Fig. 10. Diagram showing the recording system of the arterial diameter recorder (Lippay 1956).

ARTERY

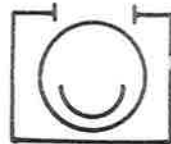


**CALIPER:
FIXED**



LIGHT SOURCE

**CALIPER:
MOVABLE**



**PHOTO
CELL**

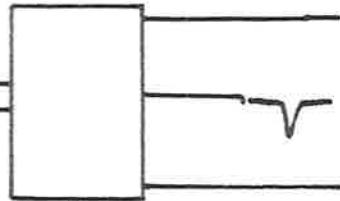


CHART RECORDER

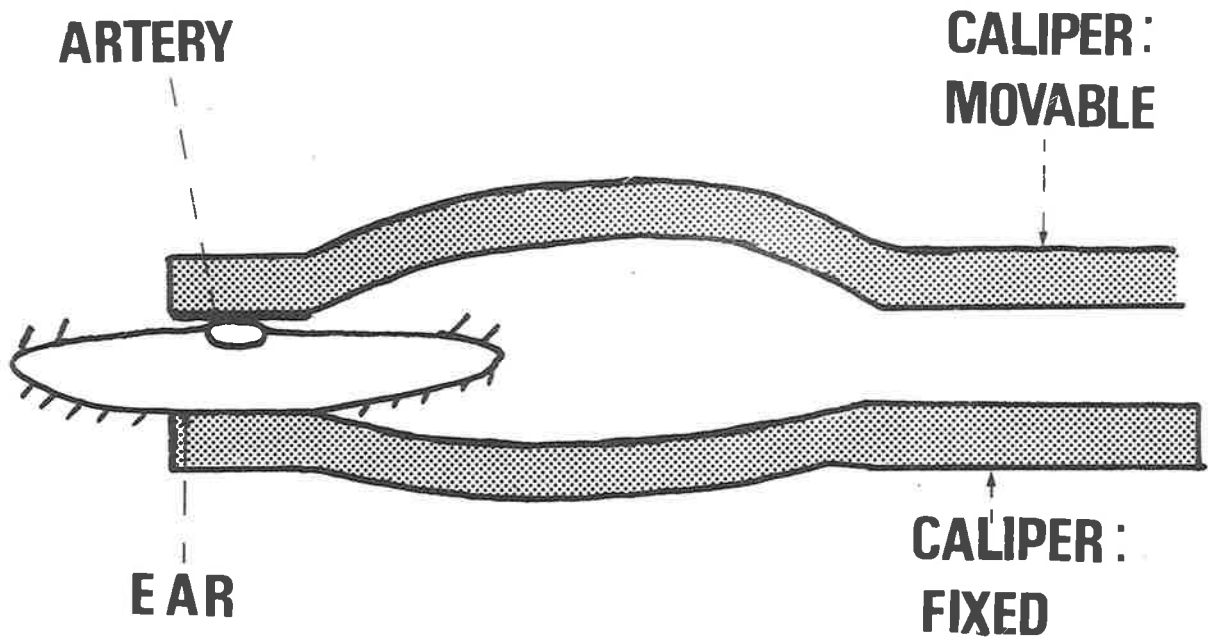
Preparation of the rabbit

Rabbits were anaesthetized by the following procedure:- after atropine 5 mg intraperitoneally ether was administered to a stage of light anaesthesia. Paraldehyde 2 - 2.2 ml/Kg was administered intramuscularly and after 45 minutes, pentobarbitone 6 mg/ml was infused intravenously until surgical anaesthesia was attained. The foregoing procedure was employed following a number of unsatisfactory attempts to achieve long lasting anaesthesia with each of paraldehyde, urethane or pentobarbitone, or with combinations of these other than the above. In a few animals, it was necessary to maintain anaesthesia with an occasional intravenous dose of pentobarbitone (approximately 5 - 10 mg).

Where ear thickness was recorded, cannulae were placed in each of the femoral artery (for recording blood pressure), the femoral vein (for injecting drugs) and in the jugular vein (for infusing drugs). Shielded electrodes were placed around the pre-ganglionic cervical sympathetic nerve in the neck and sewn into place. The animal was then placed on its stomach, and the fixed arm of the calipers sewn into place on the undersurface of the ear immediately under the central artery. The moveable arm of the calipers rested lightly on the upper surface of the ear over the central artery. Fig. II shows the calipers in place around the ear.

Fig. II. Diagram showing the calipers of the arterial diameter recorder in place around the ear.

The upper caliper rested lightly on the upper ear surface and the fixed lower caliper was sewn into place under the ear.



In a number of experiments, contraction of the nictitating membrane was recorded simultaneously with changes in ear thickness. A thread was attached to the membrane at the central border of the eye, and connected to a Grass force-displacement transducer model FT03B, the output being recorded on the Grass polygraph.

Nerve stimulation

The sympathetic nerve was stimulated by means of a Grass model S4G stimulator. At the beginning of an experiment, the effect on ear thickness of pulses of 1 msec duration applied for ten seconds at low frequency (usually 0.5 - 2 pulses per sec) and at low voltage (commencing at 1-2 volts) was examined. The voltage was then increased until it was supramaximal (usually between 5 and 10 volts) for the elicited response.

RESULTS

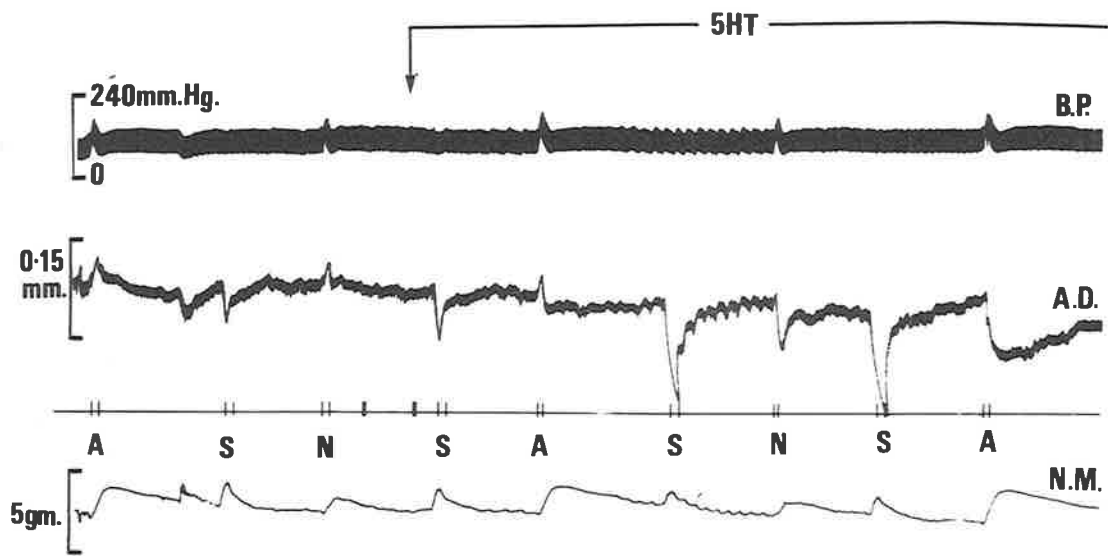
In two of fourteen rabbits noradrenaline injected intravenously in doses ranging from 0.4 ug to 5 ug caused a rise in blood pressure which was associated with a reduction in ear thickness. In the remaining twelve rabbits the rise in blood pressure was associated with either no change or an increase in ear thick-

ness. The responses to adrenaline were examined in eight of the above rabbits. At the same dose the pressor effect of adrenaline was less marked than that of noradrenaline, but the effects on ear thickness of the two amines were similar in that a rise in blood pressure was sometimes associated with a reduction in ear thickness, but more commonly was associated with no change or an increase in ear thickness. The responses of any one ear were reproducible and were characterised by their rapid onsets, and somewhat slower offsets, examples are shown in Fig. 12 .

Twelve rabbits failed to show a reduction in ear thickness at all dose levels of the amines whereas two rabbits differed at different dose levels. An increase in ear thickness was observed at doses of 1 μg in one and 2 μg in the second while at 2 μg and 4 μg respectively a biphasic response was observed. The first phase was an increase corresponding in time to the rise in blood pressure and the second phase was a decrease in ear thickness corresponding in time to the return of the blood pressure to normal. The response of the artery was the result of two opposing tendencies, i.e. the rise in systemic blood pressure and arterial constriction due to catecholamines.

Fig. 13 shows a biphasic recording of an arterial response.

Fig. 12. Portion of a Grass polygraph recording showing responses of the rabbit blood pressure, ear thickness and nictitating membrane to intravenous doses of adrenaline and noradrenaline and stimulation of the cervical sympathetic nerve, before and during intravenous infusion of serotonin 10 ug/ml.



S: STIM. FREQ. 3/ SEC.
FOR 10 SEC.

N: NORAD. 1 μ g

A: ADREN. 2 μ g

H: 5HT. 10 μ g/min.

Fig. 13. Portion of a Grass polygraph recording showing biphasic responses of the rabbit ear thickness to catecholamines.



N



N. Noradrenaliné 2 μ g

Phentolamine

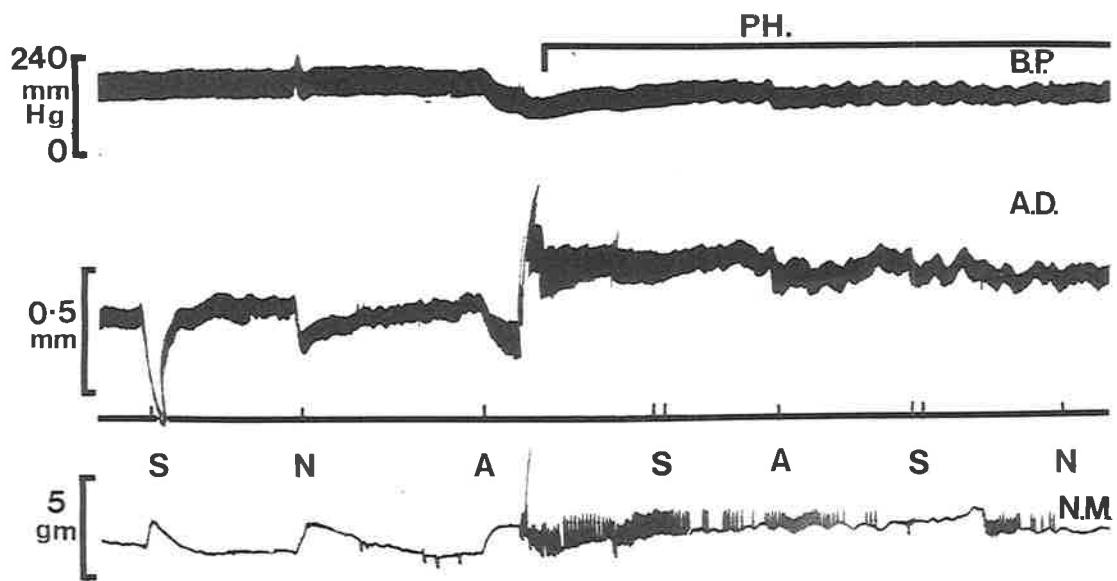
In each of two rabbits, phentolamine mesylate when injected intravenously in a dose of 2 mg/Kg abolished the constrictor response of the ear to noradrenaline and to nerve stimulation. The contractile responses of the nictitating membrane to noradrenaline and to nerve stimulation were also abolished.

Fig. 11 shows a recording of the action of phentolamine mesylate on the responses to noradrenaline, adrenaline and nerve stimulation. The effects of this drug are consistent with an assumption that the reduction in ear thickness caused by nerve stimulation is mediated by noradrenaline released from the post-ganglionic nerve terminal.

Sympathetic nerve stimulation

When the sympathetic nerve supplying the artery was stimulated the artery constricted. The fall in pressure recorded on the polygraph was taken to be a constriction as there was either no effect on the blood pressure recording or a small rise. Therefore the fall was not a passive collapse due to fall in blood pressure. Constriction and recovery were rapid. Fig. 12 shows a recording of a response to nerve stimulation.

Fig. 14. Portion of a Grass polygraph recording showing the responses of the rabbit blood pressure, ear thickness and nictitating membrane to intravenous catecholamines and stimulation of the cervical sympathetic nerve before and after intravenous phentolamine 5 mg.



S: STIM. FREQ. 3/SEC. N. NORAD. $1\mu\text{g}$ A. ADREN. $2\mu\text{g}$
 FOR 10 SEC. PH. PHENTOLAMINE 5mg

Effect of serotonin

Serotonin was infused intravenously into twelve rabbits and its effect on responses of the ear and the nictitating membrane and/or noradrenaline examined. The rate of infusion of serotonin was initially of the order of 10 $\mu\text{g}/\text{minute}$ but in a number of experiments was increased step-wise to a maximum of 30 $\mu\text{g}/\text{minute}$, or decreased to 5 $\mu\text{g}/\text{minute}$. The only change was a small decrease in ear thickness which occurred in five rabbits with concentrations of 5-30 $\mu\text{g}/\text{minute}$ of serotonin.

Catecholamines

In six rabbits, the responses to noradrenaline were unaffected by serotonin. In another six rabbits serotonin enhanced the constrictor effect of noradrenaline on the ear vessels as indicated by either a greater decrease in ear thickness, or less pronounced increases in ear thickness. In three of these rabbits serotonin altered the response to noradrenaline from an increase to a decrease in ear thickness. In two the increase was less and in one the increase was more. However, there was no obvious change in the nature or magnitude of the responses of the nictitating membrane.

Fig. 12 shows the responses to catecholamines and nerve

stimulation in the presence of serotonin.

Sympathetic nerve stimulation

The decrease in ear thickness caused by nerve stimulation was markedly enhanced by serotonin in seven out of ten rabbits, whereas in three rabbits the responses to nerve stimulation were not effected. When the infusion of serotonin was terminated the responses to nerve stimulation returned to their former levels.

In one of the experiments where serotonin failed to alter the responses to nerve stimulation the artery was removed subsequently and perfused in vitro with Krebs bicarbonate under the conditions described by de la Lande and Harvey (1965). When serotonin was added to the perfusate at the same rate as that employed in vivo it caused a marked potentiation of the constrictor response to noradrenaline. No attempt was made, however, to ensure that the perfusion rate approximated to blood flow through the artery.

Effect of cocaine

In nine experiments cocaine was infused at the same rate as serotonin and the reduction in ear thickness caused by stimulation was increased in five rabbits and by noradrenaline was increased in three rabbits.

Nictitating membrane

The nictitating membrane responses tended to be erratic, unlike those of the cat. The membrane usually contracted in response to doses of catecholamines and to stimulation of the sympathetic nerve but in a few experiments it relaxed to these stimuli. There was no evidence of a potentiation of the response although this has been demonstrated in the cat (Lecomte 1953).

Fig. 12 shows responses of the nictitating membrane to catecholamines and nerve stimulation. Table I4 summarizes the results of the recording of ear thickness.

TABLE I4Summary of the results of *in vivo* recording

	SEROTONIN		COCAINE	
	Noradrenaline	Nerve Stimulat ⁿ	Noradrenaline	Nerve Stimulat ⁿ
No. of rabbits	12	10	9	9
No. of rabbits showing sensitization	6	7	5	3

Discussion

The results indicate that thickness of the ear over the central artery provides a simple and useful guide to changes in tone of this vessel. The effect of preganglionic nerve stimulation on the exposed artery and on ear thickness were similar and comprised a rapid reduction followed by return to the resting level on cessation of stimulation. In the majority of experiments these changes were not accompanied by lowering of blood pressure indicating that they are direct effects of stimulation and are not mediated indirectly by changes in blood flow. Their sympathetic origin was shown by blockade and potentiation of constriction by phentolamine and cocaine respectively. The changes following intravenous administration of catecholamines are best interpreted in terms of two opposing tendencies, the increase in blood flow through the artery due to rise in blood pressure and a direct constrictor effect on the artery itself. The latter action was clearly demonstrated when an increase in blood pressure was associated with reduction in ear thickness. The effects of the catecholamines were modified by phentolamine and cocaine in a similar fashion to nerve stimulation.

The effects of serotonin on the artery in vivo were more variable than in vitro in that, in the isolated and perfused

artery serotonin consistently enhances the vasoconstrictor effects of noradrenaline and nerve stimulation whereas the drug produced clear-cut potentiation of the response to nerve stimulation and catecholamine induced constriction in only one half of the preparations examined. In the experiments where there was no potentiation there was no obvious reason why these animals should have behaved differently and in one of the experiments the artery when removed and perfused in vitro subsequently with Krebs solution showed the usual sensitizing action of serotonin.

There are many factors which may conceivably have contributed to the failure of potentiation. These include:-

1) failure of serotonin to achieve a sensitizing concentration before its own constrictor action supervened.

2) interference by plasma factors which themselves sensitize blood vessels to constrictor agents. These factors are unlikely to include serotonin itself, since plasma levels in the rabbit are quoted as less than 2 ng/ml and probably include serotonin derived from platelet breakdown in the process of collection. (Humphrey and Jaques, 1954). Hence the normal level is threshold for sensitization of the isolated artery, unless significant amounts of serotonin were released by surgical trauma.

Another factor which may influence the interactions observed in the present study is that all drugs were given intravenously, and the nerve was stimulated preganglionically. These conditions were selected on the basis of preliminary experiments where it was apparent that the additional trauma to the animal associated with intracarotid arterial cannulation and injection, or dissection of post ganglionic nerve fibres greatly reduced survival time. Hence sensitization might be explained in terms of facilitation by serotonin of ganglionic transmission or in view of its well known ability to modify cardiovascular reflexes (Page et al 1962). Neither explanation can be reconciled with the following observations:

I. Serotonin when enhancing arterial constriction caused by nerve stimulation failed to enhance the associated contractions of the nictitating membrane.

2. The sympathetic nerve was always cut preganglionically so that reflex changes could not be mediated by this nerve. The sympathetic innervation of the central artery in the proximal part of the ear is derived from the superior cervical ganglion.

Hence the simplest interpretation of these findings is that the potentiation of vasoconstriction by serotonin is a direct effect of serotonin in the central artery. The interaction between

serotonin and noradrenaline which has been observed in vitro
also occurs in vivo.

CHAPTER 6

DISCUSSION

Discussion

This thesis is an attempt to elucidate some of the conditions under which serotonin sensitizes the isolated and perfused central artery of the rabbit ear, to vasoconstriction. This phenomenon does not appear to be a general one as there are very few descriptions of its occurrence in the voluminous literature dealing with the action of serotonin on smooth muscle. However experiments expressly designed to show sensitization were not included in most studies. Preparations in which the phenomenon has been demonstrated include:-

- 1) the isolated and perfused rabbit ear (Ginzel and Kottegoda 1953).
- 2) the isolated and perfused central artery of the rabbit ear (de la Lande and Rand 1965).
- 3) the central artery of the rabbit ear in vivo (Chapter 5 of this thesis).
- 4) the spiral strip of the rabbit ear artery (Appendix I of this thesis).
- 5) the human digital artery isolated and perfused in a similar fashion to the rabbit artery (de la Lande et al 1966).

In muscle other than ^{arterial} smooth muscle, Bulbring and Burnstock reported that serotonin potentiated the effects of histamine on the guinea-pig ileum (Bulbring and Burnstock 1960), and Twarog and her colleagues have observed that serotonin sensitized the anterior byssus retractor muscle of the common mussel Mytilus edulis L. to the effects of stimulation. and also exhibits an unusual effect on the contracted muscle leading to relaxation (Hidaka, Osa and Twarog 1967, Twarog 1967). The last three reports will be discussed in more detail subsequently.

However, before doing so one point deserves emphasis, namely that the phenomenon of sensitization is not simply a result of the techniques used. This might be considered to be the case since the perfused digital artery of the human displays marked sensitization although the vessels of the intact human hand and forearm display only a small degree of sensitization (Walsh 1967). However the phenomenon occurs not only in the isolated and perfused arterial segment of the rabbit ear, but also in the spiral strip preparation of this vessel, in the whole perfused ear in vitro, and in the intact ear in vivo. Serotonin induced sensitization is not observed in at least two other types of artery preparations namely the femoral artery of the duck and segments of artery from the duck foot. ^{These} displays only constriction despite the fact that the

segments were perfused in an identical fashion to the rabbit ear vessel. Thus sensitization seems to be specific for the particular type of smooth muscle and does not depend on the techniques employed. The significance of this specificity is impossible to say at this stage without a wider survey of vascular tissues. One possibility is that sensitization is specific for blood vessels involved in temperature control since this is a function of the rabbit ear. However vessels of the human forearm and probably those of the duck foot are involved in temperature regulation and neither of these types of vascular beds display sensitization.

The most significant contribution made by the studies described in this thesis is to delineate the properties and characteristics of the sensitization phenomenon.

At this stage of the study it is possible to draw the following conclusions about the nature of the action of serotonin in the central artery of the rabbit ear:-

1) sensitization is not specific for noradrenaline but extends to a variety of procedures or substances causing vasoconstriction.

2) it is not influenced by the integrity of the sympathetic nerve supply as the sensitization was observed in chronically sympathectomised arteries, arteries depleted of both noradrenaline and serotonin by reserpine and also arteries ex-

posed to cocaine both before, during and after perfusion with serotonin. Neither was the presence of monoamine oxidase necessary for sensitization, as techniques shown to inhibit this enzyme had little or no effect on the action of serotonin.

3) it was mediated by receptors which differed in chemical requirements from those in other vascular smooth muscle such as the rabbit aortic strip (Furchgott 1960) where the action of serotonin is mainly constrictor.

4) sensitization did not occur in the depolarized vessel (de la Lande et al 1966).

These findings suggest that serotonin acted through a mechanism common to all substances or procedures which constricted the artery. A likely point of action is the muscle membrane. One possible explanation then would be that serotonin reduces the resting membrane potential of the arterial smooth muscle cell and thus lowers the threshold for excitation by other vasoconstrictors. This would be consistent with the lack of sensitization in the depolarised artery observed by de la Lande et al (1966). However such a mechanism does not appear to apply to another type of smooth muscle, namely guinea-pig ileum. Bülbbring and Burnstock (1960) attempted to relate the changes in membrane potential in the guinea-pig ileum to the magnitude of the contractile response. They observed that acetylcholine or histamine pro-

duced changes in the resting membrane potential which outlasted the period of application of the drug, and that when the change was in the direction of depolarization the effects of a second dose of the excitatory agent were potentiated. Serotonin did not conform to this pattern in that although it was able to potentiate the effects of another stimulant namely histamine, this effect was not consistently associated with a reduction in membrane potential.

Another muscle sensitized to the effects of stimulation by serotonin in the absence of any effect on the resting membrane potential is the anterior byssus retractor muscle of Mytilus edulis L. Twarog and her colleagues found that serotonin had a dual effect on the dissected muscle bundles, in that it not only relaxed the catch mechanism, that is the tension persisting in the muscle after the active state has ceased but serotonin also lowered the threshold for spike discharge and contraction (as measured by micro electrodes) (Twarog 1967, Hidaka, Osa and Twarog 1967). These effects were brought about in the absence of any effect on the resting membrane potential although the resting membrane resistance was lowered.

The above studies suggested that in at least two other tissues the action of serotonin was unlikely to be mediated by changes in the resting membrane potential. The answer to

this question in the rabbit ear artery can only come from direct measurement of the effects of serotonin on membrane potential using micro electrodes.

As serotonin has a dual action on the tissues which it sensitizes namely one of constriction or relaxation an alternative explanation for its action in sensitizing smooth muscle may be sought in applying some of the suggestions on its mode of action in constriction to that in sensitization. The role of Ca^{++} in the constrictor action of serotonin on smooth muscle was investigated by Woolley (1958) who has proposed that serotonin facilitates transport of Ca^{++} across the cell membrane. This theory takes into account the considerable body of evidence that intracellular Ca^{++} is intimately associated with muscle contraction (Physiology and Biophysics, Ruch and Patton. W.B. Saunders and Company 1966).

Woolley and Campbell (1960) proposed that a specific lipid in the cell membrane combines with serotonin and Ca^{++} to form a complex which transports Ca^{++} into the cell. Such a mechanism relies on the presence of extracellular Ca^{++} . Since sensitization no longer occurs in the depolarized artery, despite the fact that extracellular Ca^{++} is present, and the muscle still contracts to noradrenaline it becomes unlikely that Ca^{++} participates in the sensitizing action of serotonin along the lines proposed by these two authors. The ability of

adrenaline to contract depolarized arteries has also been described in the dog mesenteric artery (Waugh 1962b), he has also presented evidence that the constriction due to adrenaline is mediated by an influx of Ca^{++} into the smooth muscle cell (Waugh 1962a). If, as seems likely, Ca^{++} plays an equally important role in the response of the depolarized ear artery to noradrenaline, failure of serotonin to potentiate the effects of noradrenaline under these conditions is further evidence that Ca^{++} is not intimately associated with the sensitizing action of serotonin. Nevertheless, the observations on the depolarized artery imply that at least an intact membrane potential is essential for serotonin's sensitizing action.

Twarog has also discussed the importance of Ca^{++} in the action of serotonin on the mollusc muscle. She found that serotonin releases catch in the presence of EDTA (Twarog, 1967b) and she has suggested that serotonin enters cells (as shown by Born 1962) and there binds intracellular Ca^{++} . As it appears that all factors which bring about release of the catch mechanism also increase excitability of the muscle, then it is probable that the same or a similar mechanism is involved in the release of catch and in the increase in excitability. It is difficult to account for the sensitizing action of serotonin on the smooth muscle of the rabbit ear artery in these terms as it would appear that in most muscle the reduction

of intracellular free Ca^{++} leads to relaxation. (Physiology Symposium; The relaxing factor of muscle, 1964). If however serotonin does sensitize rabbit artery muscle cells by an action similar to that proposed by Twarog then it is necessary to assume that serotonin does not enter cells through a depolarized muscle membrane, a point on which there does not appear to be any evidence as yet. The analogy between the action of serotonin on the molluscan muscle and the rabbit ear may be extended a little further. Twarog (1968) describes experiments by Henkart which suggest that although the receptors mediating release of catch and increase in excitability are similar, they are not identical in their chemical requirements. Henkart compared the actions of serotonin and methysergide. Serotonin as already stated releases catch and increases the excitability of the muscle, methysergide also increases the excitability of the muscle, but does not release the catch. The dual action of serotonin on the molluscan muscle is reminiscent of the dual action of serotonin on the rabbit ear artery where it sensitizes to vasoconstriction at low concentrations and constricts at higher concentrations. In this artery as in the mollusc muscle the receptors mediating the two effects of serotonin are similar but probably not identical as methysergide sensitizes the artery to noradrenaline but is approximately four times less constrictor than

serotonin in this preparation.

A possible physiological implication of serotonin sensitization;
aetiology in migraine headache

Curran and Lance (1964) have summarized evidence implicating serotonin as a factor in the aetiology of migraine headache. Among the symptoms which may be observed during an attack of this type of headache are constriction and dilatation of extra-cranial blood vessels, oliguria, nausea, vomiting and diarrhoea. Serotonin qualifies as a humoral agent capable of producing a number of these rather diverse symptoms. They have pointed out that excretion of the chief breakdown product of serotonin, 5-hydroxyindoleacetic acid, is increased and that administration of reserpine which is known to deplete platelets of serotonin precipitates a migraine-type headache in susceptible subjects. These headaches are considered to be relieved by injection of serotonin or its precursor 5-hydroxytryptophan. Although scant this type of evidence prompted the successful application of a serotonin antagonist, methysergide, in the prophylaxis of migraine.

A possible mode of action for serotonin in the aetiology of migraine headache has been put forward by Lance, Anthony and Hinterberger (1967). They observed that serotonin constricted extra-cranial arteries in man when injected

into the common carotid artery, and proposed that it may normally exert a tonic vasoconstrictor effect. Although the effect of serotonin on small arteries of the scalp has not been reported, intravenous injection causes facial flushing. Lance et al (1967) suggested that it was reasonable to assume that serotonin dilates the latter smaller vessels, and the sudden withdrawal of circulating serotonin would lead to dilatation of extra-cranial arteries and constriction of smaller vessels. The effects would be to increase intra-arterial pressure with consequent distension of the arterial wall. The resulting oedema formation might then lead to the accumulation of pain producing substances. The mean total plasma serotonin level does fall at the onset of a migraine headache in most subjects (Lance et al 1967). A further piece of evidence which supports this hypothesis has been reported. Lance et al (1967) found that 'headache free' platelets incubated with 'migraine plasma' released some serotonin. They postulate the presence of a serotonin releasing substance present in the plasma at the onset of a migraine headache.

The present study provides evidence which is consistent with the theory of Lance et al (1967). Serotonin sensitizes the rabbit ear artery to noradrenaline both in vivo and in vitro and methysergide also sensitizes the artery in vitro to the constrictor action of noradrenaline.

Lance et al (1967) suggested that serotonin exerted a tonic effect on extra-cranial vessels. If this effect is indirect, that is by sensitization of the arteries to the constrictor action of circulating noradrenaline, then methysergide might mimic this action and prophylactic treatment with methysergide would replace serotonin when for some reason, as yet unknown, it is suddenly lowered at the onset of a migraine attack.

In view of this hypothesis Lance and Anthony (1968) have suggested that new 'antiserotonin' agents should be tested for activity in potentiating humoral vasoconstriction as a means of predicting their possible value in the control of migraine headache.

The above relationships are admittedly speculative, but might prove to have some value in the treatment of a severe affliction. Future studies have been proposed involving investigation of these relationships in isolated cranial blood vessels.

Appendix ISensitization of the spiral strip of the central artery of the rabbit ear to noradrenaline by serotonin

It was not possible to obtain a maximum response of the isolated and perfused artery to noradrenaline as the perfusion system did not permit a maximum response. An attempt to obtain a maximum response was made by cutting a spiral strip of the artery and measuring contraction of this strip in response to noradrenaline. Dissection of the artery was similar to that described on page I4 but the artery was removed without cannulation. The isolated artery was threaded onto an inert metal rod which had a diameter which allowed the artery to slide around it. Spiral strips were cut by hand under a dissecting lens.

Arterial strips were suspended in Krebs bicarbonate solution which was bubbled with 95% oxygen and 5% carbon dioxide (Carbogen). The organ bath had a capacity of approximately 20 ml and was heated by means of a jacket to 37°C. The lower end of the artery was attached to a tissue holder clamped into the organ bath and the upper end was attached to the lever of a Harvard strain gauge recording on a Grass polygraph. The arteries were stretched by a one gram tension.

The two arteries from a single rabbit were set up and dosed simultaneously. Right and left arteries were not noted after removal from the rabbit.

Drug administration

Doses of noradrenaline were placed in the organ bath cumulatively (arithmetically) until a maximum response was obtained. The drug was then washed out and the tissue allowed to relax. This procedure was repeated once and then the dose needed to obtain a half maximal response was calculated and two single doses were given at this level. Serotonin at 10 ng/ml was placed in the Krebs bicarbonate in one of the organ baths, and the procedure above was repeated. The serotonin was then washed out for thirty minutes and the procedure repeated once more.

A cumulative dose response curve was obtained for serotonin, on the artery not previously treated with serotonin.

Results

The artery constricted rapidly after the first dose and fairly rapidly after the second dose but subsequent contractions for each dose became smaller until a maximum was reached. When the tissue was washed by upward displacement it returned fairly rapidly to the resting level.

The two arteries obtained from the same animal were very

often of different sensitivities, one was up to a hundred times more sensitive than the other. Results obtained were not due to this difference in sensitivity as the artery which was to be exposed to serotonin was chosen before the noradrenaline sensitivity was determined.

Serotonin accelerated the contraction towards a maximum but this was never more than in the normal untreated arterial strip. Table I5 shows the height of the maximum response to noradrenaline before addition of serotonin, and the dose needed to obtain a response approximately half this height. This is compared with the dose of noradrenaline needed to obtain a half maximal contraction in the presence of serotonin. All maximum responses were steady for at least two additions of drug in one of the cumulative response curves.

Serotonin itself gave rise to a very small contraction, a dose of 5 μ g eliciting a response of about 2mm which appeared to be a maximum response of the artery to this substance.

Pieces of arterial strip were removed prior to the experiment and were compared for fluorescence staining of noradrenaline (by the method of Falck (1962) as modified by Waterson and Smale (1967)) with other portions of arterial strip removed after the experiment. The noradrenaline storage sites, at the periphery of the smooth muscle showed fluorescence in each case. Fig. I5 is a photograph of these strips obtained from one artery.

Fig. 15. Fluorescence staining for noradrenaline of spiral arterial strips prepared from arteries before being placed under 1 gram tension and after recording maximum responses of the strips to noradrenaline, (under 1 gram tension), before and in the presence of serotonin 10 ng/ml.

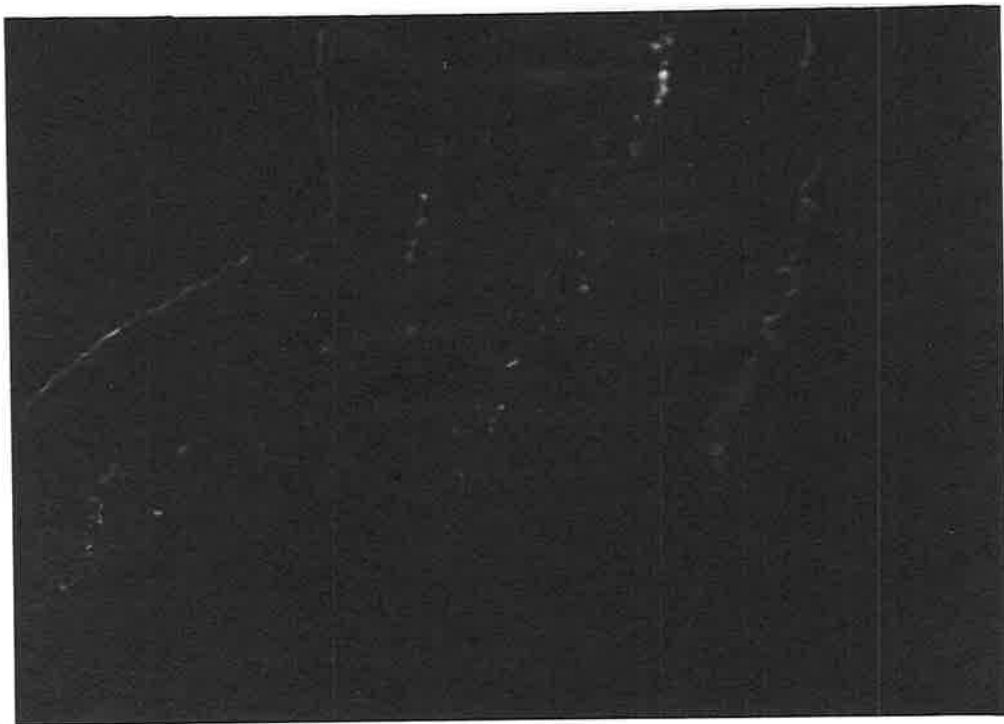
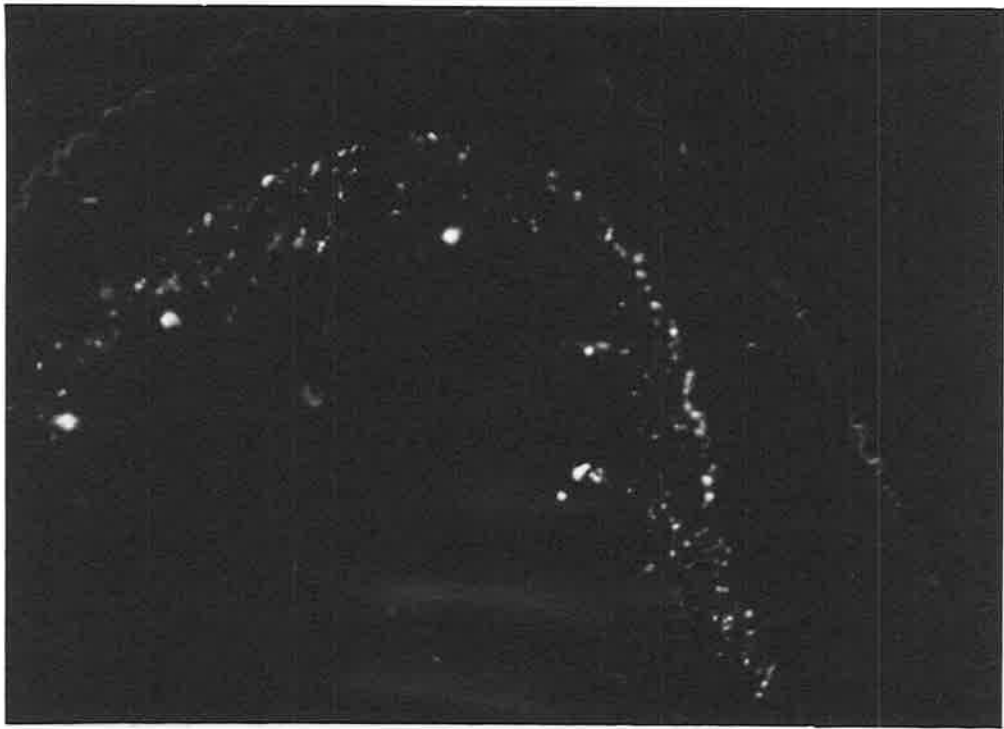


Table I5. The approximate sensitization (7th column of table) was calculated from the reduction (in the presence of serotonin) in the dose necessary to obtain a response approximately half the maximum response. These figures are shown in the third column of the table. The fourth column lists the responses obtained at these doses. The fifth and sixth columns list the procedure used i.e. which artery was treated with serotonin.

TABLE 15

Expt No.	Maximum response mms	Dose for half maximum response	Resp. at half maximum dose	Procedure		Sensitization (approx)
				Artery No.	5HT 10ng/ml	
1	10.0	4.0	5.0	1	-	
	9.7	4.0	4.5	2	-	
1	9.7	4.0	5.0	1	-	0 2.0
	8.7	2.0	5.2	2	+	
2	24.0	2.0	8.7	1	-	
	19.0	2.0	8.2	2	-	
2	22.2	3.0	12.0	1	-	0 1.5
	14.5	1.0	6.2	2	+	
3	28.5	4.0	16.5	1	-	
	43.2	3.5	30.0	2	-	
3	28.7	4.0	14.2	1	-	0 2
	47.7	2.0	26.2	2	+	
4	28.2	5.0	19.7	1	-	
	34.0	7.5	21.5	2	-	
4	26.5	2.5	13.7	1	-	1.5 2.0
	27.0	2.5	14.7	2	+	

Discussion

The spiral strip cut from the central artery of the rabbit ear was a convenient and fairly easy preparation to set up and examine. The initial contractions to a cumulative dose were rapid and although the later responses were much slower, recovery was rapid when the artery was maintained under tension.

It was possible to obtain a maximum response for this tissue to noradrenaline and serotonin. Table I5 shows that the maximum response to noradrenaline was not increased by serotonin added to the fluid bathing the artery. The sub-maximal responses were increased. The action of serotonin in this case then, was the attainment of a maximum response more rapidly, by increasing the contractions caused by sub-maximal doses of noradrenaline.

In some experiments an attempt was made to obtain a dose response curve for serotonin. This proved impossible as the tissue was almost completely insensitive to serotonin when recordings were made under the conditions outlined previously.

A much greater concentration of serotonin was needed to contract the arterial strip than to sensitize to the constrictor action of noradrenaline. The situation is similar to that in the perfused artery in that sensitization and constriction can apparently be separated by concentration of serotonin.

However, although it is not possible to state in either case that there was no constriction or contraction respectively when the sensitization was demonstrated, it is reasonable to state that in the case of the arterial strip any contraction would have been very small at sensitizing concentrations.

The very small contraction due to serotonin suggests several possibilities:-

1) the spiral cutting of the artery may have destroyed or separated the muscle fibres which constrict in response to serotonin in the intact artery.

2) there may be fewer receptors for serotonin than for noradrenaline in the artery.

Appendix 2Perfusion of the isolated central artery of the rabbit ear at constant pressure

The rabbit ear artery preparation of Gaddum and Kwiatowski (1938) which was used in a modified form by several groups of workers was perfused at constant pressure and room temperature. The isolated artery preparation of de la Lande and Rand (1965) was perfused at constant rate and 37°C. Perfusion at constant pressure more nearly approaches a physiological situation than perfusion at constant rate as the blood pressure of the whole animal remained constant, within limits, due to interplay of various pressor and depressor reflexes. In a closed system such as the isolated artery or ear the pressure responses cannot be directly compared to those of the artery or ear in the intact animal where there is a balance of dilatation and constriction not confined to one artery but spread over a plexus of vessels. However it was of interest to ascertain whether the phenomenon of serotonin sensitization could be demonstrated in an artery perfused at constant pressure.

Procedure

1) Perfusion at constant rate.

Dose response curves were obtained for intraluminal injections of noradrenaline in double cannulated arteries, (perfused at constant rate at 37°C) before, during and after perfusion of serotonin at 10 ng/ml. Control sensitization factors were calculated for serotonin.

2) Perfusion at constant pressure.

The pump was removed from the system and the Krebs bicarbonate reservoir raised so that approximately 100 drops per minute issued from the top cannula. Responses were measured by eye by counting the number of drops issuing from the top cannula in one minute. This was the shortest interval in which a complete response occurred. A typical experiment was carried out as follows:-

1) three control minutes were counted,

2) a dose of noradrenaline was injected and the drop rate was counted for one minute,

3) two control minutes were counted,

4) a second dose was injected and the drop rate was counted for one minute.

Responses were obtained at two dose levels, in the absence and in the presence of serotonin 10 ng/ml in the Krebs

bicarbonate solution.

The response was calculated as a percentage of the preceding control mean drop number. The values in **Table 16** are the means of several doses at each level.

TABLE 16

Expt. No.	Before serotonin		During serotonin	
	Dose	% reduction in drop rate	Dose	% reduction in drop rate
1	10 ng	24.1	5 ng	59
	20 ng	51.1	10 ng	74.2
2	12.5 ng	25.8	12.5 ng	64.8
	25 ng	52.5	25 ng	78.0
3	5 ng	22.1	5 ng	56.7
	10 ng	49.9	10 ng	72.5

Discussion

The perfused ear preparations used by most groups of workers before de la Lande and Rand (1965) were perfused at constant pressure and room temperature. However Ginzel and Kottogoda (1953) and Savini (1956) demonstrated the sensitization of these preparations to the constrictor action of noradrenaline and adrenaline by prior injection of serotonin. It was not surprising therefore that it was possible to demonstrate this action in the isolated artery perfused at constant pressure and approximately room temperature.

The perfusion at constant pressure was a non-pulsatile perfusion and in order to obtain a perfusion method which corresponded more closely to that in the body it would be necessary to devise a method of constant pressure, pulsatile flow, with a network of other vessels into which the blood could be shunted when the artery constricted. An attempt has been made to use a model like this in the intact rabbit, and the results are reported in chapter 5 of this thesis.

Appendix 3Diameter recording of the exposed central artery of the rabbit ear *in vivo*

Chapter 5 of this thesis describes an attempt to measure in vivo the changes in thickness of the rabbit ear in response to sympathetic nerve stimulation, catecholamines and serotonin. In a number of experiments direct recordings of arterial diameter were made using the diameter recorder already described (Lippay 1956) in Chapter 5. From these experiments it was possible to devise the method of measuring changes in ear thickness and be confident that it was an indirect measurement of changes in arterial diameter. Some of these experiments and the method used are briefly described in this Appendix.

Dissection of the artery for direct measurement of diameter

The skin over the central artery was excised and a length was freed from the surrounding connective tissue. This was done very carefully as the artery tended to constrict at the least possible trauma and in many cases did not dilate again. In the first series of experiments the large nerve which lies beside



the artery in the ear was left intact but any pressure on this nerve caused a sharp fall in blood pressure and the animals often died. In later experiments it was cut and the ends carefully parted. Recordings were made from the ear artery by sliding the fixed caliper under the artery and allowing the moveable caliper to rest lightly on top of the artery. The artery tended to dry and also to constrict with changes in temperature. To prevent this, a flap of skin was lifted up and two loops of cotton sewn through it, the cotton was attached to a bar and the skin was pulled up to form a cup around the artery. Krebs bicarbonate pre-heated to approximately 37° was run into the cup and drained off with a suction tube. The temperature was monitored by a thermistor recording on a galvanometer. By regulating the flow of Krebs bicarbonate into the cup the temperature was controlled at about 37° and a stable preparation lasting for several hours was the final result in a small number of experiments. Fig. 16 is a diagram showing the calipers in place around the artery.

Blood pressure

In a number of experiments a double ended cannula was inserted into the carotid artery. Two tubes cemented into the barrel of the cannula were used, one for blood pressure measurement by a pressure transducer recording on a Grass poly-

Fig. 16. Diagram showing calipers around the exposed central artery of the rabbit ear.

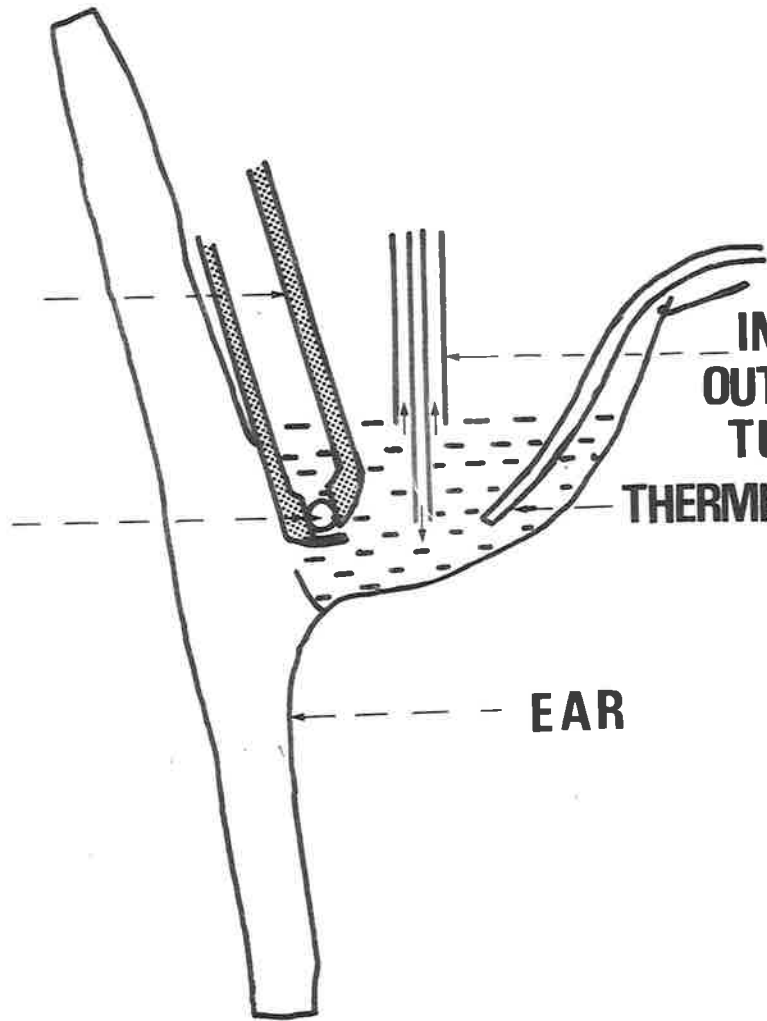
**CALIPER :
MOVABLE**

ARTERY

**INLET :
OUTLET
TUBE**

THERMISTOR

EAR



graph and the other for drug injection. A dose of heparin of approximately 2500 units was given into an ear vein just before cannulation and the cannula was filled with heparinised saline before insertion. However these cannulae tended to become twisted when the animal was moved to a prone position and were later abandoned in the experiments described in Chapter 5.

Sympathetic nerve stimulation

The method of sympathetic nerve stimulation was identical to that already described in Chapter 5.

RESULTS

Direct recording

Initial recording:-

1) It was possible to record systemic blood pressure and the pulse in the central artery of the ear, the recording was similar to that shown in Fig. I2 which was made in a non-exposed artery.

2) When the sympathetic nerve was stimulated the artery constricted. The response was similar to that recorded for ear thickness as shown in Fig. I2, and is illustrated in Fig. I7. This was a constriction of the artery and not due to a fall in blood pressure as the blood pressure recording either showed no change or a slight rise.

3) The response of the artery varied with the frequency and the voltage as shown in the experiment illustrated in

Table 17.

TABLE 17

Duration	Frequency	Voltage	Resp.
10 secs	5/sec	2.5	1.5
		5.0	1.5
		7.5	15.5
		10.0	38.5
5 secs	10/sec	5.0	12) 8.5)
		7.5	21) 23)
		10	28.5

Serotonin constriction

When serotonin was infused into the carotid artery or placed into the fluid bathing the artery in concentrations shown in Table 18, the artery constricted. It was not possible to show any definite effect of serotonin on the response to nerve stimulation.

The results of some of the direct reading experiments are shown in Table 18. Constriction of the artery to serotonin is shown in Fig. 17.

Fig. 17. Portion of a Grass polygraph recording showing responses to cervical sympathetic nerve stimulation and serotonin intravenously and external in an exposed artery

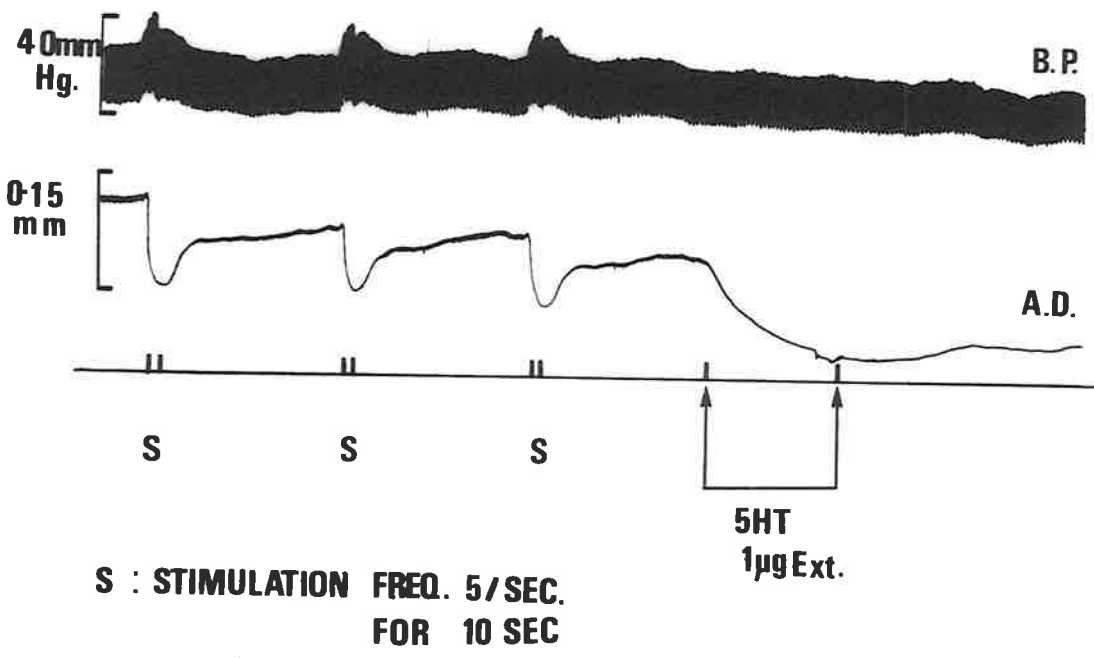


TABLE 18

Expt No.	5HT $\mu\text{g}/\text{min}$	Route of administration of serotonin	Constriction of ear artery
1	0.31 - 0.62	Carotid	-
	1.25 - 10.0	Carotid	+
2	0.47	Carotid	-
	0.09	Carotid	+
3	0.02	Carotid	-
	0.03	Carotid	+
	0.5 - 1.0	External	+
	0.25	External	+

Appendix 4Inhibition of monoamine oxidase

Noradrenaline and serotonin are substrates for the enzyme monoamine oxidase (MAO). However, the latter substance is a much better substrate and in view of the sensitizing action of serotonin on the noradrenaline response in the artery it seemed possible that MAO might play some part in this action. MAO was shown to be present in the central artery of the rabbit ear by de la Lande, Hill and Jellett (personal communication) using the staining method of Glenner, Burtner and Brown (1957).

Procedure

The arteries were single cannulated and dose response curves were obtained for intraluminally injected noradrenaline before, during and after simultaneous perfusion of serotonin at 20 ng/ml. Nialamidine or iproniazid 100 ug/ml was perfused for one hour, and the artery was then perfused with Krebs bicarbonate solution without addition for one and one half hours. After this treatment the stain specific for monoamine oxidase was no longer effective, (de la Lande et al pers. comm.) Dose

response curves were obtained before, during and after serotonin perfusion as before.

RESULTS

The results of the inhibition of monoamine oxidase are shown in Table I9. The inhibition had no significant effect on the sensitization of the artery to noradrenaline by serotonin, or on the sensitivity of the artery to noradrenaline.

TABLE I9

MAO inhibitor	5HT S.F. Int. Inj. NA.	MAO inhibitor S.F.	5HT in presence of MAO inhibitor
Nialamide	3.9	0.74	3.65
Nialamide	3.45	0.16	2.9
Nialamide	5.55	0.53	4.25
Nialamide	14.2	0 - 1.35	10
Iproniazid	5.2	1.15	6.25
Iproniazid	7.9	0.22	1.25
Iproniazid	2.39	1.6	6.1
Iproniazid	7.25	1.25	10
Iproniazid	4.7	0.37	19

Discussion

It does not appear from these experiments that monoamine oxidase inhibition had any effect on the sensitizing action of serotonin, and serotonin therefore does not sensitize the artery to noradrenaline by inhibition of monoamine oxidase for which they are both substrates.

Appendix 5Drugs and Chemicals

L-Adrenaline bitartrate	Sigma Chemical Company
Adrenochrome semicarbazone	Light and Co. Ltd.
Atropine sulphate	Macfarlan Smith
Bufotenine monooxalate hydrate	Sigma Chemical Company
Ergotamine tartrate	Sandoz Ltd.
Ethyl Carbamate (Urethane)	The British Drug Houses Ltd.
Heparin	Boots Pure Drug Company Ltd.
Histamine acid phosphate	Koch-Light Laboratories Ltd.
5-hydroxyindoleacetic acid	Sigma Chemical Company
5-hydroxytryptophan	Light Laboratories
Lignocaine	Astra
Methysergide bimaleate	Ciba Company Pty.Ltd.
L-Noradrenaline bitartrate	Sigma Chemical Company
Paraldehyde	The British Drug Houses Ltd.
Phenoxybenzamine (Dibenyline)	Smith, Kline and French Laboratories
Phentolamine (Regitin)	Ciba Company Pty. Ltd.
Reserpine	Ciba Company Pty.Ltd.

Sodium pentobarbitone (Sagatal)

May and Baker Ltd.

SQ I0643

E.R. Squib and Sons
Fty. Ltd.

Tryptamine

Koch-Light Laboratories
Ltd.

Drug Solutions

All drugs with the exception of adrenaline, noradrenaline and ethyl carbamate were dissolved in 0.9% w/v NaCl solution. (normal saline).

Ethyl carbamate was dissolved in distilled water.

Adrenaline and noradrenaline were dissolved in 0.02 NHCl to give a solution of 1 mg/ml. Dilutions were made in normal saline containing ascorbic acid (1 in 10,000) and subsequently adjusted to pH 5 to 6 with NaOH. In some experiments the two drugs were directly dissolved in normal saline containing ascorbic acid and diluted as above. Doses of noradrenaline and adrenaline are expressed as weight of free base.

Doses of all other drugs are expressed as weight of salt.

Physiological solutionsKrebs Bicarbonate Solution grams/litre

NaCl	6.9
KCl	0.35
KH_2PO_4	0.16
NaHCO_3	2.1
CaCl_2	0.28
MgCl_2	0.1
Glucose	1.0

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