FACTORS INFLUENCING THE RESPONSE OF THE RAT TAIL ARTERY TO SYMPATHOMIMETIC AMINES

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Doctor of Philosophy

by

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S U M M A R Y

1. The aim of this study was to characterize the roles of neuronal and extraneuronal mechanisms in determining the sensitivity of the rat tail artery to catecholamines and to ascertain whether these roles changed in experimentally induced hypertension.

2. The isolated artery was perfused in vitro in such a way that drugs could be applied separately to the intima (intraluminally, IL) or the adventitia (extraluminally, EL). This permitted the influence of the surface of entry of the drug into the artery wall on its vascular action to be assessed.

3. The artery was ten times more sensitive to intraluminal than to extraluminal noradrenaline. The difference is explained in terms of the location of the sympathetic nerves at the medial-adventitial border, plus a declining concentration of noradrenaline within the wall from its surface of entry to the opposite surface.

4. In support of 3., cocaine selectively enhanced sensitivity to extraluminal noradrenaline, so that the difference between this sensitivity and that to intraluminal noradrenaline was virtually eliminated. Further support is provided by evidence (a) that the difference between the sensitivities to intraluminal and extraluminal adrenaline is less marked than in the case of noradrenaline; (b) methoxamine, an amine with little affinity for the neuronal uptake process, is equipotent by the intraluminal and extraluminal routes; and (c) cocaine had less effect on the sensitivity to extraluminal adrenaline than to extraluminal noradrenaline, and was without effect on the sensitivity to methoxamine.

5. In view of the similar sensitivities of the cocaine treated vessel to the intraluminally and extraluminally applied amines, it is suggested that there is little difference between the sensitivities to noradrenaline of the smooth muscle cells in the inner and outer regions

of the media.

6. In reserpine pretreated vessels, methoxamine was more sensitive by the extraluminal route. It is suggested that this reflects post-junctional supersensitivity of the smooth muscle cells close to the nerve terminals.

7. Extraneuronal uptake and O-methylation of noradrenaline and adrenaline appeared to have little effect on the sensitivities to these amines. The evidence is based on the small magnitudes of the increases in sensitivities to noradrenaline and adrenaline produced by an inhibitor of extraneuronal uptake (DOCA) and an inhibitor of COMT (U0521).

8. Evidence is presented that extraneuronal inactivation makes little, if any, contribution to the gradient of concentration of intraluminal adrenaline across the artery wall. The technique of immersing the adventitial surface in oil during a steady state response to intraluminal adrenaline was used to produce an indirect measure of the magnitude of the concentration gradient.

9. Vessels from three week DOCA/salt hypertensive rats were 2-4 times more sensitive to noradrenaline than those from normotensive rats. This occurred irrespective of the surface of entry of the amine into the vessel wall, implying that the greater sensitivity applied equally to the inner and outer smooth muscle cells of the media. These changes were relatively specific for exogenous noradrenaline, since changes in sensitivity to K^+, and low frequency nerve stimulation were much smaller. The changes appeared to be unrelated to neuronal uptake, since they occurred in cocaine treated arteries.

10. The experimental hypertension was not associated with changes in K^+ induced release of noradrenaline.

11. In view of the evidence in 9. and 10., it is considered that

the increases in sensitivity to exogenous noradrenaline in DOCA/salt induced hypertension are post-synaptic in nature.

12. To explain the apparently greater changes in sensitivity to exogenous than to endogenous noradrenaline, the hypothesis is considered that the properties of the alpha adrenoceptors on the smooth muscle cells adjacent to the nerve terminals may differ from those on more distant smooth muscle cells. However, analysis of the effects of prazosin and yohimbine on the responses to exogenous noradrenaline and to electrical stimulation of arteries from normotensive rats provided no support for this hypothesis.

D E C L A R A T I O N

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 contains no material previously published by another person, except where due reference is made in the text.

Michael Gerard Venning

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P U B L I C A T I O N S

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Clin. Exp. Pharmac. Physiol. 7:654 (1980).

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C H A P T E R 1

GENERAL INTRODUCTION

The studies in this thesis describe mainly the role of neuronal and extraneuronal factors in the control of the sensitivity of the rat tail artery to catecholamines. The aim was to characterize these roles in normotensive vessels, then ascertain whether these roles changed in experimentally induced hypertension. Unfortunately, time did not permit completion of the hypertension study; nevertheless, significant data was obtained and is described in Chapter 6. This introduction will describe the relevant features of the autonomic pharmacology of small muscular arteries, such as the rat tail artery, which prompted this study.

The ventral artery of the rat tail, a small muscular artery with an outside diameter of approximately 600 μ (Hinke and Wilson, 1962a), has a media about seven smooth muscle cells thick. In terms of Burton's (1965) arterial classification, it lies between a medium and a small muscular artery. Table 1 summarizes morphological and endogenous noradrenaline content of the vessel based on the author's observations and measurements.

Hodge and Robinson (1972) showed, histochemically, that the rat tail artery had a rich sympathetic innervation and that the sympathetic nerve terminals were confined to the border of the media and the adventitia. Sections of the rat tail artery, either stained with haematoxylin and eosin, or treated with formaldehyde vapour to demonstrate the adrenergic nerve endings, are shown in Fig. 1.1.

Before the commencement of this study there was little information available on the autonomic pharmacology of the rat tail artery. Using the whole perfused rat tail, Wadè and Beilin (1970) observed constrictor responses to noradrenaline (NA), adrenaline (A), serotonin (5-HT), vasopressin, angiotensin II, high K^+ concentration and sympathetic nerve stimulation. They also concluded that, due to the small effect of

Table 1.1

Data on rat tail artery perfused at 2 $ml.min^{-1}$ (n=4).

External diameter	0.7 mm	
Wall thickness:		
relaxed	0.032 mm	resting perfusion pressure = 10 mm Hg
contracted	0.42 mm	perfusion pressure = 67 mm Hg
Number of smooth muscle cells = 7		
NA content	28.1 $nmol.g^{-1}$ (n=17)	

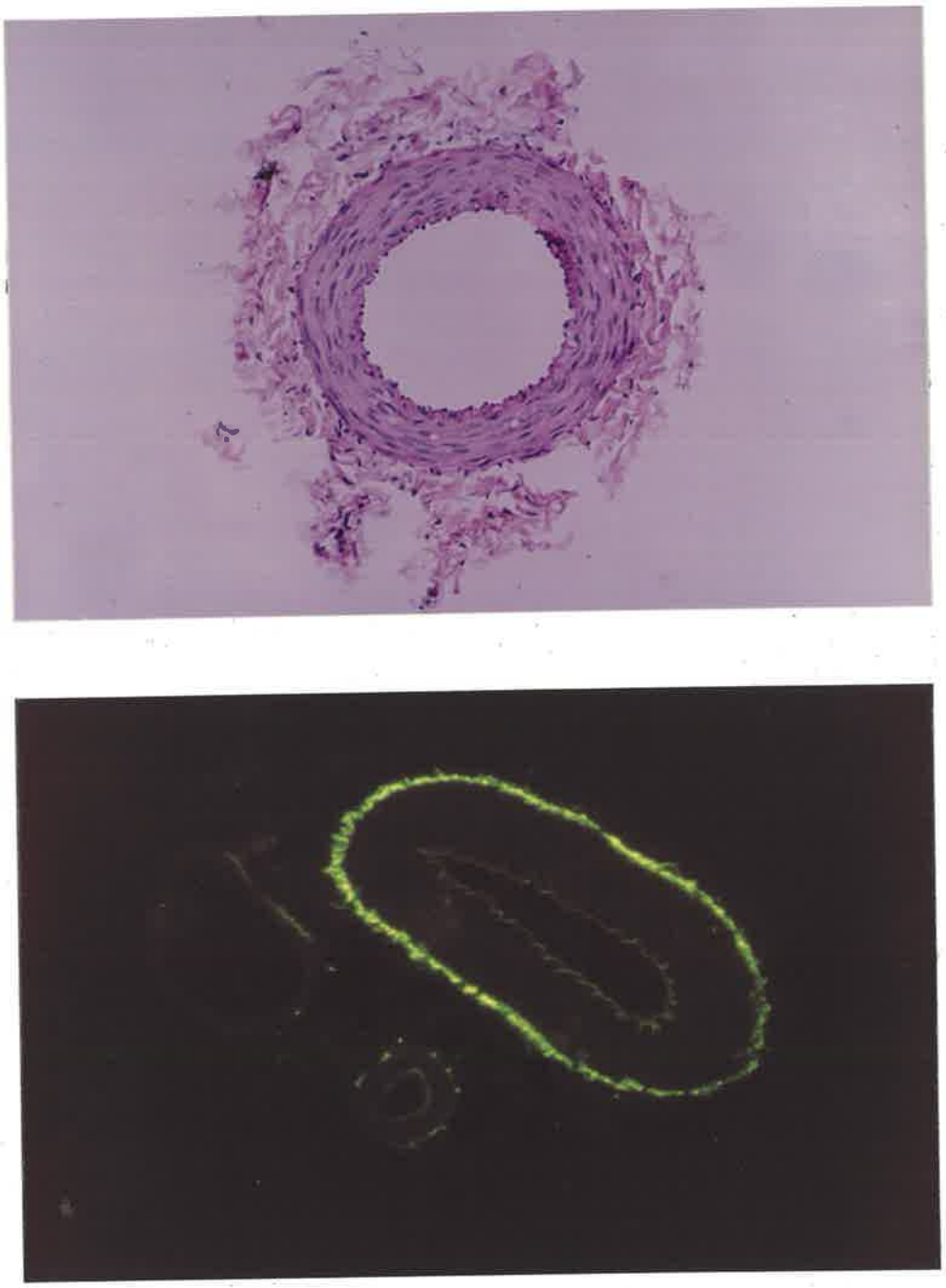

Figure 1.1

The upper photograph is of a transverse section of the rat tail artery stained with haematoxylin and eosin and shows the intima, muscular media and adventitial tissue.

The lower photograph is of a transverse section of rat tail artery that had been exposed to formaldehyde vapour. The autofluorescence of the intima and the fluorescent noradrenergic nerve terminals at the border of the media and the adventitia are clearly visible.

propranolol in increasing the pressor response to A and NA, the smooth muscle adrenoceptors were predominantly of the alpha type.

Hinke and Wilson (1962a, 1962b) characterized the elastic properties of the isolated rat tail artery alone, and determined that it contracted in response to A, NA, angiotensin II and Pitressin, that a high K^+ concentration potentiated the responses to all four constrictor agents as well as leading to constriction itself, and that a low Na^+ concentration reduced the sensitivity to the two peptides. Later, Hinke (1965, 1966) reported changes in sensitivity to NA in arteries from hypertensive rats (discussed further in Chapter 6) and suggested that these may be related to an increased efficiency in Ca^{++} utilization.

Nicholas (1969, 1970) appears to be the first worker to have used the isolated segment cannulated at both ends so that the effects of drugs added separately to the media or adventitia could be studied. Although he did not study the responses to NA other than by intraluminal injection (i.e., applied to the intima), he showed that cocaine potentiated responses of the vessel to both NA and electrical nerve stimulation and phentolamine antagonized the responses. The constrictor responses to nerve stimulation (elicited by periarterial electrodes) was inhibited by extraluminal guanethidine (i.e., applied to the adventitia). Amphetamine reversed this inhibition. The above effects did not indicate any important differences between the influence of neuronal uptake on NA sensitivity in this tissue and in other peripheral organs. He also showed that the vessel displayed tachyphylaxis to angiotensin, vasopressin and bradykinin, and that angiotensin potentiated the responses of the artery to NA and electrical stimulation. He proposed that the potentiation was via a cocaine insensitive mechanism related to the role of Ca^{++} in the contractile process.

Bonnaccorsi, Jespersen and Garrantini (1970) demonstrated that desipramine selectively enhanced the response of the perfused artery to externally applied NA compared with internally applied amine, implying that neuronal uptake was an important determinant of this response.

Wyse (1973, 1976) studied the inactivation of exogenous and neural NA by helical strips of rat tail artery. He used the oil immersion technique. This involves eliciting a steady state contractile response to an agonist, and then replacing the aqueous (Krebs) solution bathing the isolated tissue with agonist free paraffin oil. Since the agonist cannot diffuse from the tissue, the rate of relaxation of the preparation to the pre-agonist level is used as a measure of the rate of inactivation of the agonist within the tissue. The major known processes for inactivation of NA were inhibited by pharmacological agents (neuronal uptake by cocaine, extraneuronal uptake by corticosterone, COMT by tropolone, and MAO by iproniazid). Cocaine was the only agent which alone caused marked delay in relaxation. Small and equivocal effects were caused by inhibition of extraneuronal uptake, COMT and MAO, and these only occurred when high NA concentrations had been used in combination with the inhibitors, and when neuronal uptake was simultaneously blocked. Wyse concluded that low concentrations of NA, both endogenous and exogenous, were preferentially inactivated in the rat tail artery by neuronal uptake and storage.

Aprigliano and Hermsmeyer (1976), during the course of the present study, reported that the perfused rat tail artery was more sensitive to intraluminally (IL) applied NA than to extraluminally (EL) applied NA and that this sensitivity difference was abolished by pretreatment, in vitro, with 6-hydroxydopamine. The EL sensitivity was increased more than the IL sensitivity, implying that neuronal uptake played a

more important role in the response to NA when it entered the vessel wall via the adventitia.

Van Houtte and Webb (1979) showed that whereas cocaine potentiated responses to low frequency electrical stimulation, it had no such effect on higher frequencies. They concluded that contraction responses to cocaine resulted from inhibition of neuronal uptake, that neuronal uptake was not operative during the nerve impulse but that it was an important disposition mechanism in the rat tail artery.

The Rabbit Ear Artery

The relationship between inactivation and response to NA has been studied much more extensively in the rabbit ear artery than in the rat tail artery (de la Lande, 1975; Johnson and de la Lande, 1978; Head, de la Lande, Irvine and Johnson, 1980). Some aspects of this relationship will be considered here, as the findings on the rabbit ear artery provided the basis for the approach used by the author to examine the nature of the relationship in the rat tail artery.

The double cannulated perfused artery preparation (de la Lande, Cannell and Waterson, 1966) has the advantage over arterial strips or ring preparations in the study of drug actions, that drugs can be applied directly to either the adventitia or the intima of the artery. Hence, the morphology of the artery can be used to interpret the roles which neuronal and extraneuronal mechanisms play in the responses of the artery to agonists and their interactions with other drugs (de la Lande and Venning, 1980). Using this technique, Kalsner (1972) showed that when an agonist is applied to only one surface of the vessel to produce a steady state response, it is distributed non-uniformly in the media. He reasoned that, if when applied to one surface, the agonist was distributed uniformly (i.e., the concentration was the same

throughout the media), then the response to the agonist applied to one surface should not differ from the response to the agonist applied to both surfaces simultaneously. However, he showed that when an agonist (NA, K^+ or methoxamine) which constricted the artery was applied to both surfaces, the response was greater than when the agonist was applied to one surface only. He calculated that the augmented response was equivalent to increasing the concentration of agonist applied to one surface by 1.3-5.4 fold, depending on the agonist and the route of application. When NA was used, cocaine was present to block neuronal uptake. He concluded that IL and EL administered NA preferentially activated the inner and outer muscle cell layers of the media, respectively. Kalsner's study highlighted the fact that the technique of applying agonists separately to the adventitia and media permitted an analysis of the differential activation of the inner and outer smooth muscle cell layer of an artery.

The subsequent studies of de la Lande et al. (1980) have provided further information on the magnitude of the gradient of the concentration of IL applied NA within the vessel wall, between the intima and the region of the nerve terminals. They estimated that the concentration declined by about 90% on the basis that the rate of formation of a neuronally-formed metabolite of NA (dihydroxy phenylethylene glycol or DOPEG) was ten fold greater when the NA was applied to the adventitia than when it was applied to the intima. Their result adds further weight to the argument that the vasoconstrictor response to NA, when it is applied to one surface, is mediated largely by the smooth muscle cells closest to that surface.

Neuronal Uptake

The rabbit ear artery is more sensitive to NA applied to the intima

than to the adventitia (de la Lande et al., 1966) by a factor of 10-20 fold. Cocaine selectively enhances the sensitivity of the artery to EL NA so that it approaches the IL sensitivity (de la Lande, Frewin and Waterson, 1967). These workers proposed that the differential sensitivity of the artery to EL and IL NA was related to the uptake of NA by the adrenergic nerve terminals at the border of the media and the adventitia, as illustrated by the model in Fig. 1.2. de la Lande et al. (1970) reported that methoxamine, which is not a substrate for the neuronal uptake process (Iversen, 1967; Trendelenburg et al., 1970) was equipotent in the rabbit ear artery by both EL and IL routes of application. However, Yong and Chen (1975) reported it to be 3.4 times more potent by the EL route in the same artery. This apparent discrepancy is considered in relation to results on the rat tail artery in Chapter 4.

Extraneuronal Uptake

Iversen and Salt (1970) showed that corticosteroids, including deoxycorticosterone, are inhibitors of extraneuronal uptake. Johnson and de la Lande (1978), using deoxycorticosterone acetate (DOCA), demonstrated that it increased the sensitivities of the rabbit ear artery to both EL and IL A, each approximately three fold. The effect of EL A, but not IL A, was increased in cocaine treated or chronically denervated arteries. The fact that inhibition of extraneuronal uptake increased the sensitivities to both EL and IL A is in accord with the model in Fig. 1.2, and it appears that there is competition between neuronal and extraneuronal uptake systems for A. Subsequently, Head et al. (1980) confirmed, biochemically, that in the rabbit ear artery, DOCA inhibited the uptake of isoprenaline into a compartment possessing O-methylating activity.

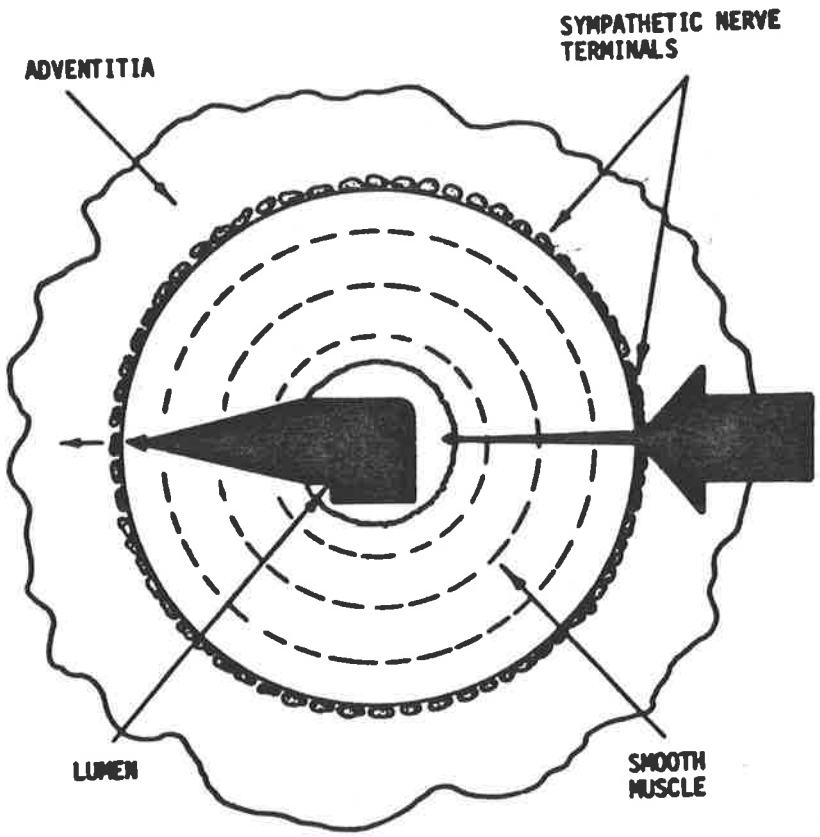

Figure 1.2

A diagrammatic representation of the influence of uptake by the sympathetic nerve terminals on the concentration of noradrenaline in the smooth muscle of the artery. The direction of the arrows represents the direction of diffusion of noradrenaline. The thickness of the arrows represents concentrations of noradrenaline. The model implies free penetration of both extraluminal noradrenaline (through the adventitia) and intraluminal noradrenaline (through the media) to the sympathetic nerve terminals.

Monoamine Oxidase

There is evidence, from studies in the rabbit ear artery, that MAO occurs both intra- and extraneuronally (de la Lande, Hill, Jellett and McNeil, 1970; de la Lande and Jellett, 1972). Head, Stitzel, de la Lande and Johnson (1977) showed that chronic sympathetic denervation decreased the activity of MAO of ear artery homogenates, using tyramine as a substrate, by a small (9%) but significant amount. Although this showed that most of the MAO was extraneuronal, the results of pharmacological experiments had shown the small neuronal component was functionally important.

It was earlier shown that inhibition of MAO, by nialamide, augmented the response of the artery to EL NA (Jellett and de la Lande, 1969), but not to IL NA (de la Lande and Jellett, 1972). The failure of nialamide to increase the sensitivity to IL NA indicated that extraneuronal MAO did not play a significant role in the inactivation of the catecholamine. Since the inhibition of MAO had no effect on the sensitivity to EL or IL NA in sympathetically denervated arteries, the increased response of the innervated artery to EL NA was attributed to the inhibition of intraneuronal MAO. This result also suggested that the deamination process in this vessel comprised uptake of NA by the nerve terminals followed by deamination by MAO present in the axoplasm of the nerve terminals. The failure of nialamide to increase the sensitivity to IL NA was attributed to the relative failure of IL NA, compared with EL NA, to penetrate the sympathetic nerves of the artery located near the medial-adventitial border. Histochemical studies (de la Lande et al., 1974) suggested that a contributing factor was loss of IL NA in the media resulting from the uptake and inactivation of the amine by the smooth muscle. The evidence was that inhibition of COMT by U0521, an inhibitor of extraneuronal uptake, enhanced the ability

of nerves to accumulate NA, as demonstrated histochemically.

Catechol-O-Methyl Transferase

In the rabbit ear artery, COMT activity appears to be entirely extraneuronal. Head et al. (1977) reported that chronic sympathetic denervation caused no change in COMT activity in homogenates of the rabbit ear artery, and concluded that the major portion of, if not all, COMT was located extraneuronally. Their conclusion was in accord with the results of an earlier pharmacological study, where it was found that inhibition of COMT by UO521 increased the sensitivity of the rabbit ear artery, to both EL and IL NA, by approximately four fold (Johnson, 1975; Head, Johnson, Berry and de la Lande, 1975). As cocaine did not alter the potentiating effects of UO521, the COMT was not considered to be neural in location. Head et al. (1975), and also Johnson and de la Lande (1978) further demonstrated that the sensitizing effect of UO521 on the vasoconstrictor response to A was markedly reduced in the presence of a steroid inhibitor of extraneuronal uptake. From this study, and the biochemical study of Head, de la Lande, Irvine and Johnson (1980) it was concluded that O-methylation occurred in an extraneuronal compartment to which NA gained access by a steroid sensitive extraneuronal uptake process. In this respect, the rabbit ear artery resembled the rat heart in its pattern of extraneuronal uptake and O-methylation (Trendelenburg, 1977).

In summary, these studies showed that the uptake and inactivation of NA in a small muscular artery conformed to a pattern which now appears fairly general for peripheral tissues, and which, as defined by Trendelenburg (1977), occurs via two main metabolizing systems. The first is a neuronal deaminating system, which comprises neuronal uptake followed by deamination via MAO in the nerve cytoplasm; the

second is an extraneuronal O-methylating system, consisting of extraneuronal uptake followed by O-methylation. From the work of Wyse (1973, 1976) it would seem that the first, the neuronal deaminating system, is the major system in the rat tail artery.

The Present Study

The aim of the first experiments described in this thesis was to assess whether the hypothesis of the roles of neuronal and extraneuronal in the vascular responses to catecholamines which had been deduced from studies on the rabbit ear artery were also applicable to the rat tail artery. In this sense, the study represented a test of the generality of the model shown in Fig. 1.2.

Initially, the effects of a neuronal uptake inhibitor on the sensitivity of the perfused rat tail artery to catecholamines (NA and A) and to methoxamine were investigated. These amines were selected on the basis of their differing affinities for the neuronal and extraneuronal uptake systems (Iversen, 1967). The amines were applied separately to the intimal and adventitial surfaces so that the inter-relationships between neuronal uptake, sensitivity and vessel morphology could be studied.

The influence of inhibition of extraneuronal uptake and O-methylation of the catecholamines were next investigated. This study included comparisons between perfused segments in which the Krebs solution bathing the adventitia was replaced with oil in an attempt to define further the influence of the extraneuronal uptake process on the gradient of concentration of the catecholamine within the vessel wall.

Following these studies, the sensitivities of perfused arteries to NA, K^+ and sympathetic nerve stimulation from rats made hypertensive

with DOCA/salt treatment were investigated. This study revealed significant effects of hypertension on exogenous NA sensitivity. K^+ was included as an agonist in an attempt to define whether the effects were specific for NA, while the effects of nerve stimulation were included to assess whether the differences between normotensive and hypertensive vessels extended their responses to endogenously released NA.

The results of the studies on hypertensive rats prompted a speculative hypothesis that hypertension may have been associated with a change in the types of alpha adrenoceptors mediating vasoconstriction in the rat tail artery. A preliminary investigation of this hypothesis is presented in Chapter 7.

CHAPTER 2

GENERAL METHODS

The procedures described in this chapter include the removal and preparation of tail segments and arteries for perfusion, perfusion techniques and apparatus, and data processing common to many of the experiments reported in this study. Less commonly used techniques employed are described in the methods section of the relevant chapters.

A list of drugs used in the study and the methods of their preparation are to be found in Appendix I.

Animals

Male, albino rats of the Porton derived strain were used. Weights ranged between 220 and 450 gm. Animals were bred and raised in the University of Adelaide central animal house where they were supplied with tap water and Charlick's M+V mouse cubes ad libitum.

Animals were stunned by a blow to the head and killed by cervical section.

Tail Segments

The tail was amputated from the animal at its base by a clean incision passing through an intervertebral space. Two segments, each 3 cm in length, were then cut from the proximal end of the tail and put into a petri dish containing warm Krebs-bicarbonate solution gassed with 95% O_2 and 5% CO_2. A strip of skin, 1 cm in length, covering the ventral groove, was removed from the proximal end of each segment, exposing the fibrous tunnel containing the ileo-lumbar artery. A one cm incision was made on the ventral surface of this tunnel, exposing the artery, which was then cut away from its fascial tissue. A polythene cannula, drawn from Dural medical grade tubing, 1 mm I.D., 2 mm O.D., was inserted into the artery and tied in place with cotton thread. The cannulated segment was placed on top of a brass tank,

through which warm distilled water was circulated (at 37° C), and connected to the perfusion system, which is described subsequently. The brass warming tank and tail segment were both enclosed in a perspex box which had a suction drain outlet so that Krebs and drug solutions, which had passed through the tail segment, were removed from the box (Fig. 2.1). Prior to the administration of any drugs, artery segments were perfused with Krebs solution for at least one hour to allow for equilibration.

Perfusion

Tail segments perfused at a constant rate, 4-4.5 $ml.min^{-1}$ with Krebs-bicarbonate solution by means of a roller pump (design: O. Saxby, Department of Pharmacology, Oxford University). The Krebs solution was maintained at 37° C and bubbled with 95% O_2 : 5% CO_2. The perfusate passed through a warming coil (37° C) immediately before the perfusion chamber. After passing through the tail segment, the solution flowed on to the brass warming tank and then into the floor of the perspex perfusion chamber, where it was removed by suction. Pressure changes in the perfusion system were measured by a Statham pressure transducer (P23AC or P23DC) inserted into the perfusion line between the pump and the tissue. These changes were recorded on a Rikadenki double channel chart recorder. Drugs were added to the Krebs solution in the reservoir. An increase in resistance to perfusion produced by the drug was recorded by the increase in perfusion pressure. Responses were measured as the steady state level of perfusion pressure produced by the drug while it was perfused through the artery, minus the perfusion pressure in the absence of the drug. In most experiments, cumulative concentration response curves to the constrictor amine were obtained by increasing the concentration of the drug after steady state

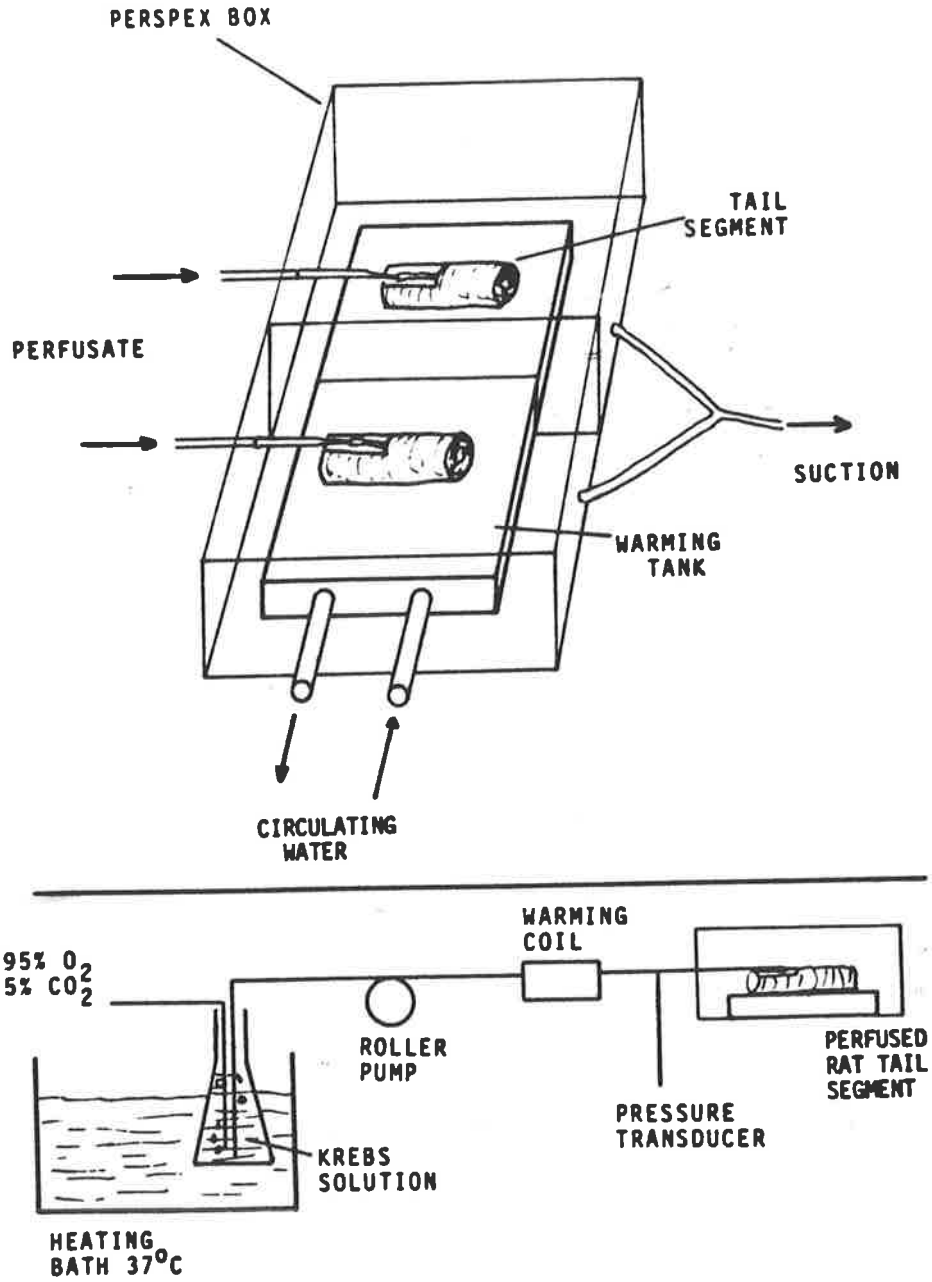

Figure 2.1

Diagrams of the perspex box and warming tank, and perfusion apparatus used for the perfusion of rat tail segments.

responses had been elicited.

Isolation and Perfusion of Tail Arteries

Tails were amputated by a clean incision passing through an inter-vertebral space at the junction of the body and tail. The distal portion of the tail was removed similarly, leaving a piece of tail about 4 cm in length. This was placed in a petri dish containing warmed ($37^{o}C$) Krebs solution bubbled with carbogen (95% O_2 + 5% CO_2). A scalpel was used to make two longitudinal incisions through the skin of the tail, one on each side of the ventral groove containing the ileo-lumbar artery. The skin covering the groove was cut away using fine scissors. A pair of de Weisser scissors was then used to cut through the tissue on the ventral surface of the groove, exposing the artery. Starting at the proximal end, the artery was carefully dissected free of fascial tissue, removed from the tail and bathed in Krebs solution.

A polythene cannula (drawn from Dural medical grade tubing, 1 mm I.D., 2 mm O.D.) was inserted into the proximal end of the artery and tied in place with cotton thread, using two pairs of No. 5 stainless steel jewellers' forceps under a X10 dissecting microscope. A 2.5 ml syringe was attached to the end of the cannula and Krebs solution was gently flushed through the artery to clear it of blood and to visualize any leaks, due to side branches, on the artery. The vessel was cut at the distal end so that it was now 1.5 cm in length and free of side branches. A second polythene cannula, about 20 cm long and U-shaped, was inserted and tied into the distal end of the artery so that the length of vessel between the two cannula tips was 1 cm. The double cannulated artery was mounted in an organ bath for perfusion.

In some experiments arteries were cannulated at the proximal end

only. In this case they were cut so that the length of the artery from cannula tip to distal end was 1 cm.

The perfusion apparatus is illustrated in Fig. 2.2 and is basically that described by de la Lande et al. (1966). Krebs solution, bubbled with carbogen, was warmed in a water bath (37^{o}C) and pumped through polythene tubing, by means of a Saxby roller pump, at a constant rate of 2 or 4 ml.min^{-1}, and through the artery which was mounted in a double jacketed, heated organ bath. The distal cannula was supported on a pivoted hook under a tension of 1 gm-wt. The external surface of the artery was bathed in warmed Krebs solution contained in the organ bath, so that there was no mixing of solutions in contact with the intraluminal (IL) and extraluminal (EL) surfaces of the arteries. This meant that drugs could be selectively applied to either surface of the artery. Pressure changes in the perfusion system were monitored by a Statham pressure transducer (P23AC or P23DC) connected to the perfusion line between the pump and the organ bath, and recorded on a Rikadenki pen recorder.

Drug solutions were applied to the EL surface of the artery by adding them to the bathing solution in the organ bath, and to the IL surface of the artery by adding them to the perfusate.

In the case of single cannulated arteries, the internal and external bathing solutions were allowed to mix in the organ bath. Drug solutions were applied to both artery surfaces simultaneously so that the same concentration of drug was in contact with both EL and IL artery surfaces. Drug solutions were removed from the perfusion system by replacing them with fresh Krebs solution.

Responses to Constrictor Amines

Responses to vasoconstrictor agents were measured when they had

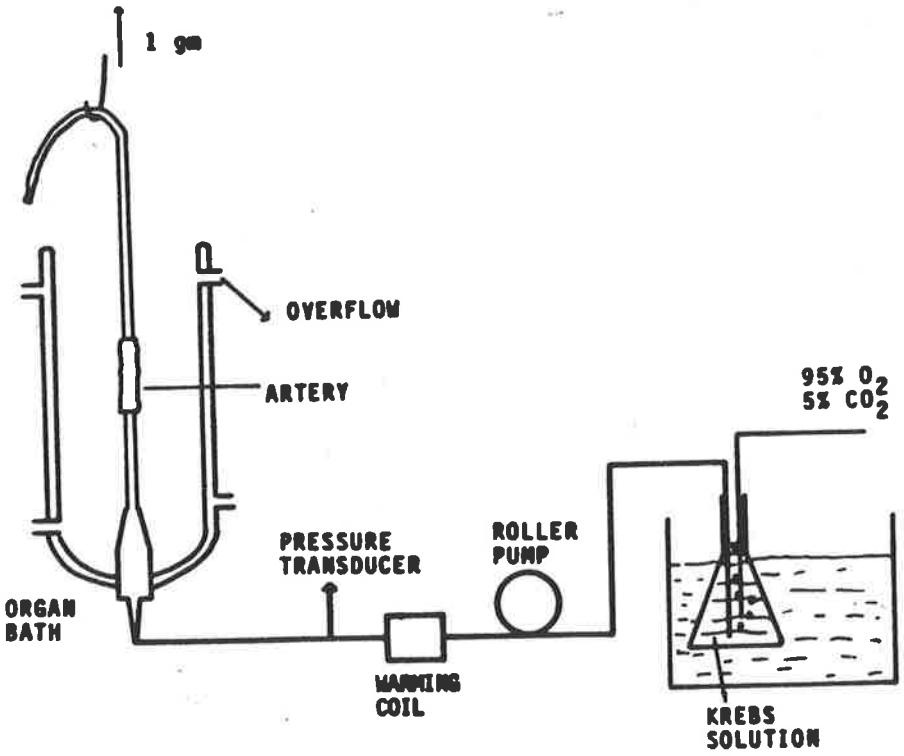

Figure 2.2

Diagram of the apparatus used for perfusing arterial segments and monitoring changes in perfusion pressure.

achieved a steady state level of response. Generally, a series of responses to cumulated concentrations of drug were measured, rather than responses to single concentrations. Responses, ΔP, were measured as the steady state level of perfusion pressure produced while the drug was in contact with the artery, minus the perfusion pressure in the absence of the drug.

Log concentration response curves (i.e., log concentration of agonist on the abscissa, and change in perfusion pressure on the ordinate) were plotted, and the concentrations of drug producing a ΔP of 5, 10, 20, 50, 100, and 150 mm Hg were read from the curves. Sensitivity ratios were calculated. These were the ratios of equi-effective concentrations of a drug applied EL and IL to the artery, or equieffective concentrations of a drug in the presence and absence of antagonists or potentiating substances. The ratios were determined at the response levels previously mentioned.

Electrical Stimulation of Sympathetic Nerves

The sympathetic nerves in the artery segments were stimulated by short square wave pulses (0.3 msec duration) delivered through platinum wire field electrodes placed on either side of the artery. Each electrode was about 5 mm from the artery. The electrodes were covered with polypropylene tubing except for 5 mm at the tip. The top electrode was positioned next to the distal end of the artery and the bottom electrode adjacent to the proximal end of the artery. Trains of pulses, 10 sec in duration, were delivered from a Grass model S48 stimulator at supramaximal voltage (70 V) and the frequency was varied from 0.1-80 Hz. In this way, constrictor responses of up to about 150 mm Hg, to a variety of frequencies, were measured. Log frequency-response curves were plotted and dealt with in a similar way to the log

concentration-response curves mentioned previously.

At the end of many of the experiments, tetrodotoxin (10^{-6} mol. l^{-1}) was added to the organ bath to check that it abolished responses to the highest frequency used in the experiment, thus confirming that the constrictor responses observed were a result of nerve stimulation and not direct stimulation of the smooth muscle of the artery (Duckles, 1980).

Routine Histology

For routine microscopic analysis of arterial structure or measurement of arterial dimensions, standard methods of tissue fixation, staining and mounting were employed. Arterial segments were fixed in 10% formal saline, dehydrated in ethanol, and cleared before blocking in paraffin wax. Sections were cut at 5-7 μm thickness on a microtome, stained with haematoxalin and eosin, and examined under a normal light microscope.

C H A P T E R 3

THE INFLUENCE OF NEURONAL UPTAKE ON SENSITIVITY TO NORADRENALINE AND ADRENALINE

INTRODUCTION

There have been many studies of in vivo and in vitro preparations of small muscular arteries. These utilize both perfused artery and helical strip preparations. However, many of these methods are unable to demonstrate whether vasoactive compounds are exerting their effects on the innermost or outermost smooth muscle cells of the artery adjacent to the lumen or adventitia (termed inner and outer cells subsequently) and whether there is any difference between the responses of the inner and outer cells to these compounds.

de la Lande, Cannell and Waterson (1966) used a double cannulated rabbit ear artery preparation that enabled them to selectively apply drugs to either the extraluminal (EL) or intraluminal (IL) artery surfaces. They observed that the perfused arteries were more sensitive to noradrenaline (NA) applied to the IL artery surface than to the EL surface. Later, de la Lande, Frewin and Waterson (1967) hypothesized that the difference in sensitivity of the artery to IL and EL NA was related to the uptake of NA into the noradrenergic nerve terminals which were situated at the border of the media and the adventitia of the artery. Cocaine, by selectively enhancing the sensitivity to EL NA, reduced or abolished the sensitivity difference (de la Lande and Waterson, 1967). Histochemical studies of the rabbit ear artery and the rat tail artery (Waterson and Smale, 1967; Bevan, Bevan, Purdy, Robinson, Su and Waterson, 1972; Hodge and Robinson, 1972) show that both of these small muscular vessels have a rich sympathetic nerve plexus at the border of the media and adventitia.

Cocaine has been shown to potentiate responses of rat tail artery preparations to NA (Nicholas, 1969; Aprigliano and Hermsmeyer, 1976) and to increase the relaxation time of rat tail artery strips exposed to NA and allowed to relax in oil (Wyse, 1976).

One of the aims of the present study was to ascertain whether neuronal uptake exerted a similar influence on the IL and EL sensitivities to NA in the rat tail artery as it did in the rabbit ear artery, and whether the influence on sensitivity to NA differed from that to adrenaline (A). Adrenaline was included in view of evidence that its affinity for the neuronal uptake system is less than that of NA in the rat heart (Iversen, 1967). It was reasoned that, if this difference applied to the nerves in the blood vessel, the relative sensitivities to NA and A should differ according to the route of application of these amines to the vessel wall. A second aim was to establish whether effects of neuronal uptake on sensitivities to the IL amines in the isolated vessel extended to their sensitivities in the vessel in situ. In the in vitro situation, the extravascular (i.e., intracellular) compartment is a stirred aqueous medium in which the amine is freely diffusible, and whose volume (10 ml) is sufficiently high to ensure that the concentration which IL applied amine achieves in this compartment remains negligible. These situations do not apply in situ where it is probable that the extracellular compartment is sufficiently small to enable appreciable accumulation of the IL applied amine. In situ, it is also possible that, in the connective tissue matrix in which the vessel is located, the diffusion of NA differs from that in aqueous media, a factor which would influence the concentration which an IL applied amine could achieve in the vessel wall.

In view of these considerations, the relative sensitivities to NA and A, both in the presence and absence of cocaine, have been compared in isolated perfused vessels, and in segments of the whole tail in which the drugs were perfused intra-arterially.

METHODS

The isolation and perfusion of tissues referred to in this chapter are detailed in Chapter 2, General Methods.

When cocaine was used to block neuronal uptake, it was present in a concentration of 2.9×10^{-5} $mol.l^{-1}$, both in the bathing solution and perfusate. This concentration of cocaine was selected in view of Iversen's (1967) report that 10^{-5} $mol.l^{-1}$ cocaine inhibited neuronal uptake of NA in the isolated rat heart by 95%.

The perfusion flow rate was 4 ml/minute for both tail segments and isolated perfused arteries.

Experimental Design

(a) Perfused Tail Segments

Adjacent proximal and distal segments, each 3 cm in length, were taken from the base of the tail and perfused intra-arterially with Krebs solution. Firstly, both were given increasing doses of agonist, NA or A. Cocaine (2.9×10^{-5} $mol.l^{-1}$) was added to the solution perfusing the proximal segment and again the two segments were exposed to increasing doses of agonist. This procedure allowed a comparison of any sensitivity change occurring with repeated exposure to the agonist.

(b) Perfused Isolated Rat Tail Arteries

Segments of artery 1 cm in length were perfused in separate organ baths. Each segment came from a different tail. Initially, each segment was exposed to increasing doses of agonist (NA or A) EL and IL, and then, after recovery, when the perfusion pressure returned to base-line resting level, cocaine (2.9×10^{-5} $mol.l^{-1}$) was added to both the perfusate and the bathing solution, and, ten minutes later, the artery was once again exposed to increasing concentrations of agonist EL and

IL. Control segments were not used since it was found that the sensitivity of the perfused artery to NA and A did not alter with successive exposure to these amines over a period of 5-6 hours.

RESULTS

Perfused Artery

(a) Sensitivity of the Perfused Artery to NA and A

Arteries were significantly more sensitive, by about 10 fold, to NA applied IL as compared with NA applied EL. A comparison of the dose ratios EL/IL at the four response levels studied (Fig. 3.2, Table 3.4) indicates that the ratios are similar at response levels from 20 mm Hg up to 150 mm Hg.

When A was used as the constrictor agent, a similar pattern to that with NA emerged (Fig. 3.3, Table 3.5). The arteries were significantly more sensitive to A applied IL compared with EL application. The EL/IL dose ratios tended to be lower than those for NA, ranging from 7.5-8.5 (Fig. 3.5). However, this trend was only statistically significant at the 100 mm Hg response level ($p<0.05$, 19 d.f., unpaired t-test).

On a molar basis the arteries were 2.0-2.2 times more sensitive to A than to NA (Fig. 3.4) when the amines were applied to the EL surface of the vessel (Table 3.3). In the case of IL application of the amines, examination of Fig. 3.4 and Table 3.3 suggests that the vessels were 1.5 times more sensitive to A than to NA, but this was significant only at the 20 mm Hg response level.

(b) Effect of Cocaine

Cocaine sensitized the arteries to both NA and A, whether they were applied EL or IL. However, this effect was much greater for the

EL application of the amines, 12-18 fold for A and 19-21 fold for NA (Tables 3.4 and 3.5). Although the cocaine sensitization appeared greater for NA compared with A, the difference was not statistically significant (Table 3.3).

Cocaine also increased the sensitivities of the arteries to IL applied A and NA. These changes were significant except for NA at the 20 and 50 mm Hg response levels; however, the potentiating effects of cocaine on NA were not significantly different from those on A (Table 3.3).

Although arteries were initially more sensitive to both NA and A when they were applied IL, and in general more sensitive to A, compared with NA, in the presence of cocaine, sensitivity to both NA and A was the same, irrespective of whether the amines were applied EL or IL (Tables 3.3, 3.4 and 3.5); that is, in the presence of cocaine, NA and A became equipotent constrictor agents regardless of whether they were applied to the inner or outer surfaces of the artery.

Tail Segments

Table 3.3 shows the ratios of molar concentrations of NA and A producing the same increases in perfusion pressure in the presence and absence of cocaine. The untreated tail segments were 3.4-4.3 times more sensitive to A than to NA (Fig. 3.1). There was no significant difference, at the response levels examined, between the sensitivities of the tail segments to two successive exposures to either of the constrictor catecholamines used in these experiments. Thus, when cocaine was added to the perfusate to inhibit the neuronal uptake system, the sensitivity changes observed (Tables 3.1 and 3.2, Fig. 3.1) to NA were attributable to the cocaine. Cocaine caused a significant increase in the sensitivity of the perfused tail to the constrictor

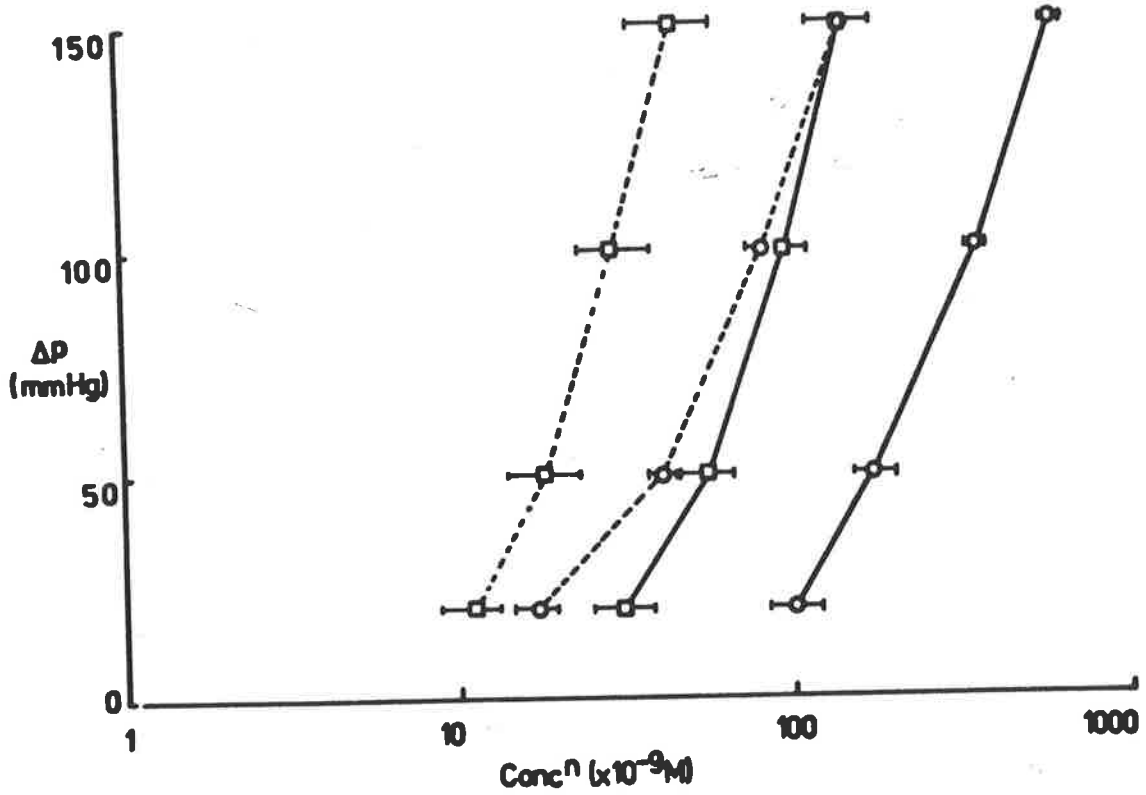

Figure 3.1

Concentration response curves of the perfused rat tail segment to noradrenaline (n=8) and adrenaline (n=10) in the presence and absence of cocaine (2.9×10^{-5} $mol.l^{-1}$). Each point represents the geometric mean and standard error of n observations. The vessel is more responsive to A than NA at all response levels (t-test, $p<0.001$).

Noradrenaline O—— Adrenaline □—— Cocaine - - - -

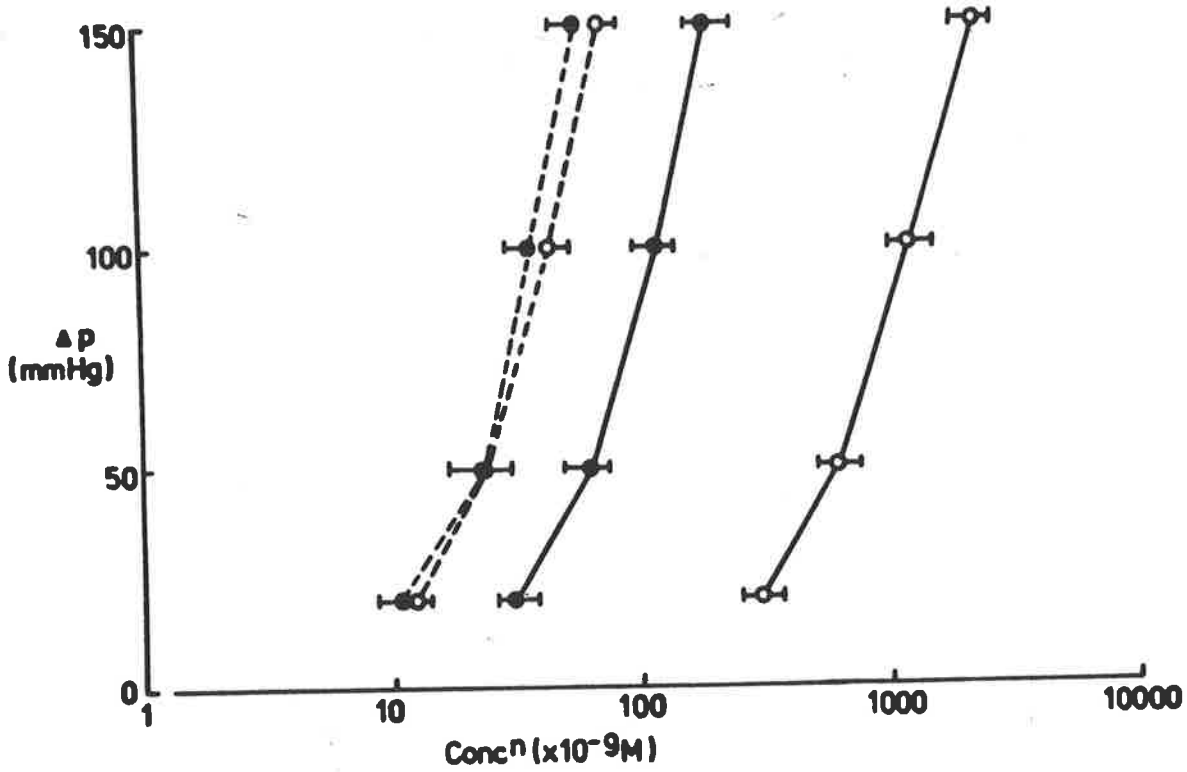

Figure 3.2

Concentration response curves of the perfused isolated rat tail artery to extraluminal and intraluminal noradrenaline in the presence and absence of cocaine (2.9×10^{-5} $mol.l^{-1}$). Each point represents the geometric mean and standard error of 5 to 10 observations.

Extraluminal O—— Intraluminal ●—— Cocaine - - - -

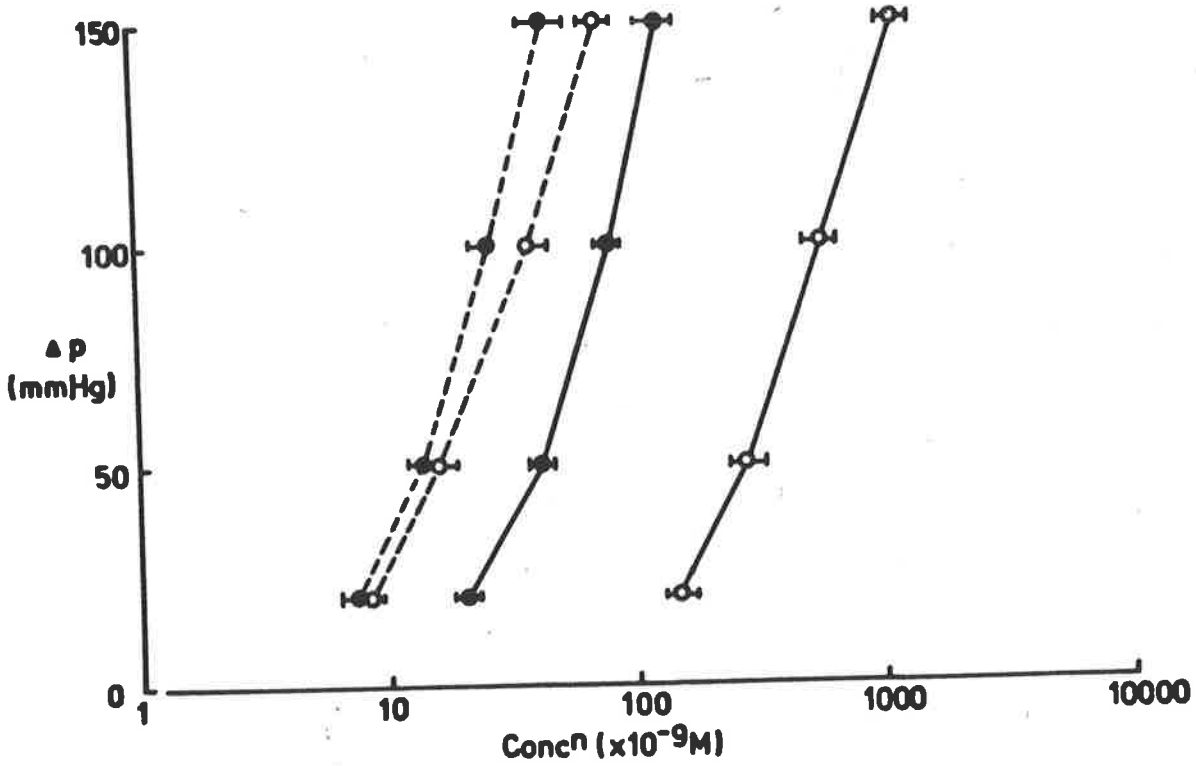

Figure 3.3

Concentration response curves of the perfused isolated rat tail artery to extraluminal and intraluminal adrenaline in the presence and absence of cocaine (2.9×10^{-5} $mol.l^{-1}$). Each point represents the geometric mean and standard error of 6 to 11 observations.

Extraluminal O—— Intraluminal ●—— Cocaine- - - -

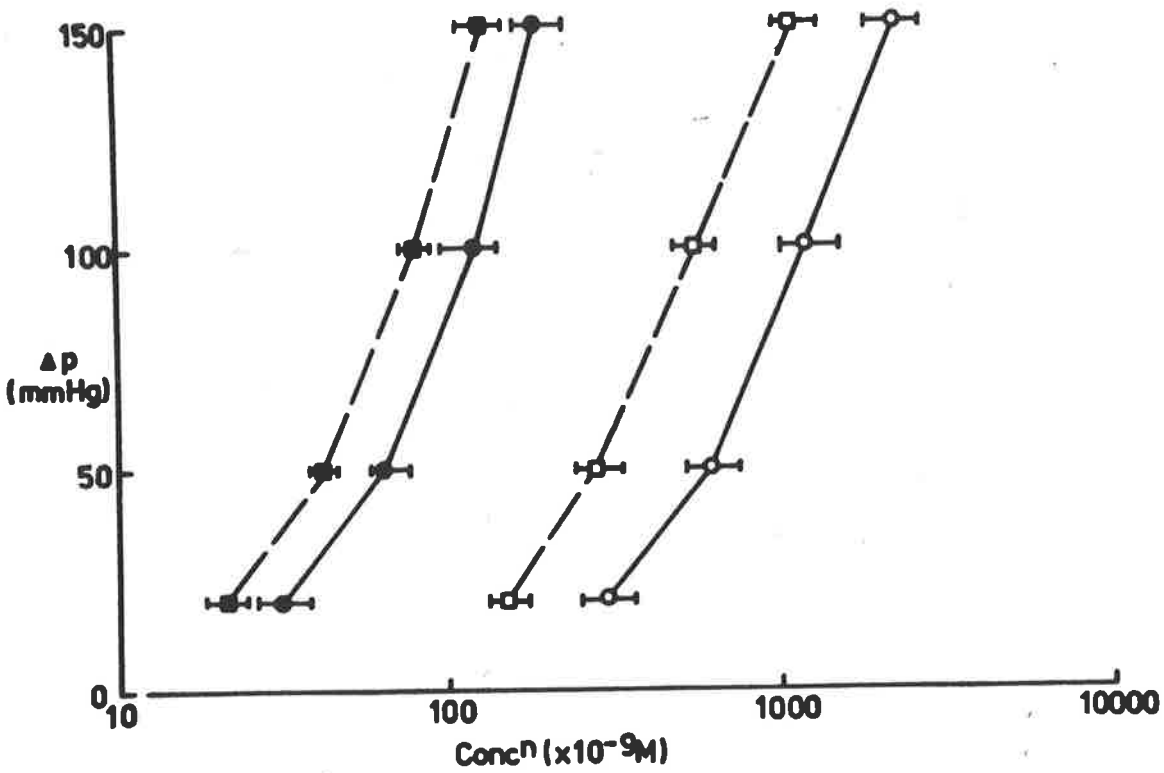

Figure 3.4

Concentration response curves of the perfused isolated rat tail artery to extraluminal and intraluminal adrenaline and noradrenaline. Each point represents the geometric mean and standard error of 10 or 11 observations.

Extraluminal noradrenaline ○ Extraluminal adrenaline □

Intraluminal noradrenaline ● Intraluminal adrenaline ■

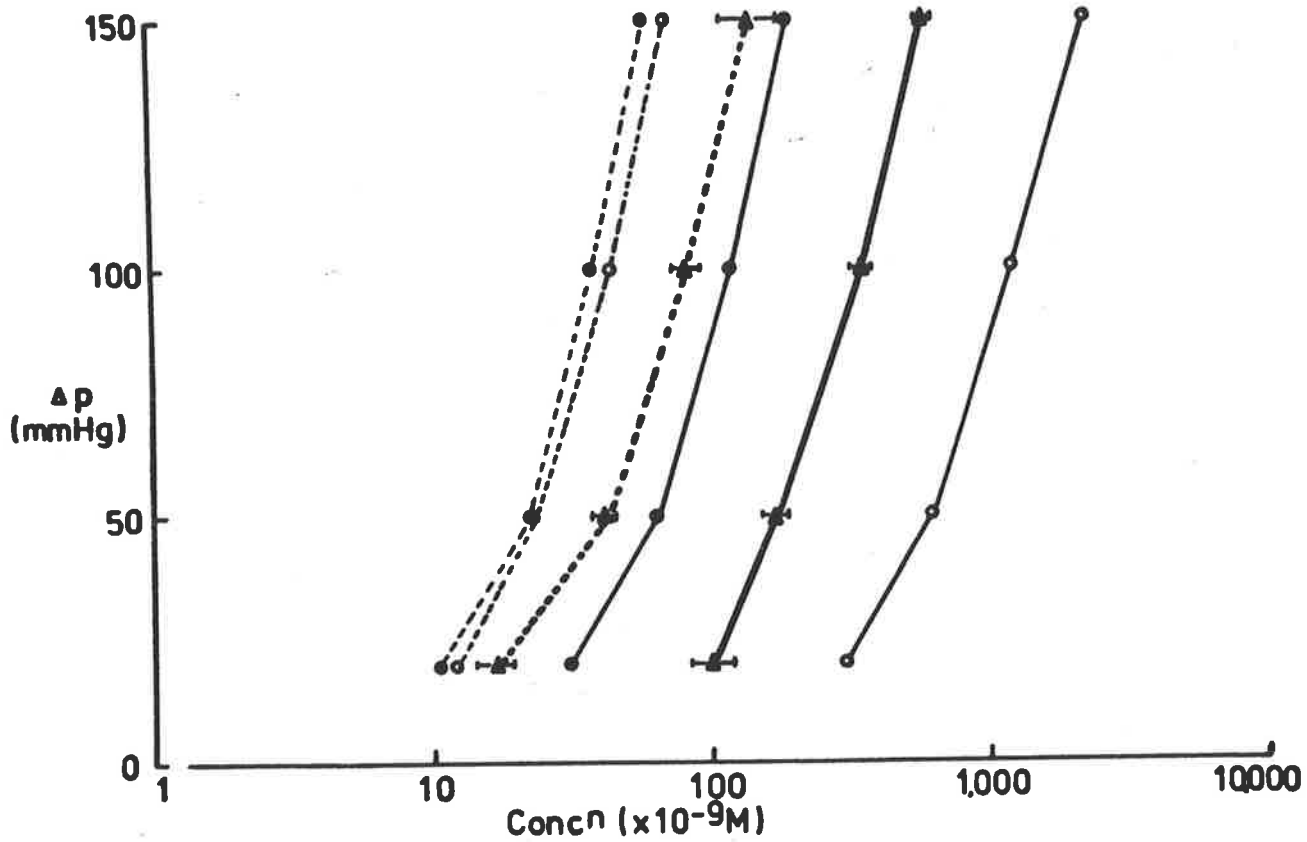

Figure 3.5

Concentration response curves of the perfused isolated rat tail artery and the perfused rat tail segment to noradrenaline in the presence and absence of cocaine (2.9×10^{-5} $mol.l^{-1}$).

Tail segment ——— Extraluminal noradrenaline ———

Intraluminal noradrenaline ——— Cocaine present - - - -

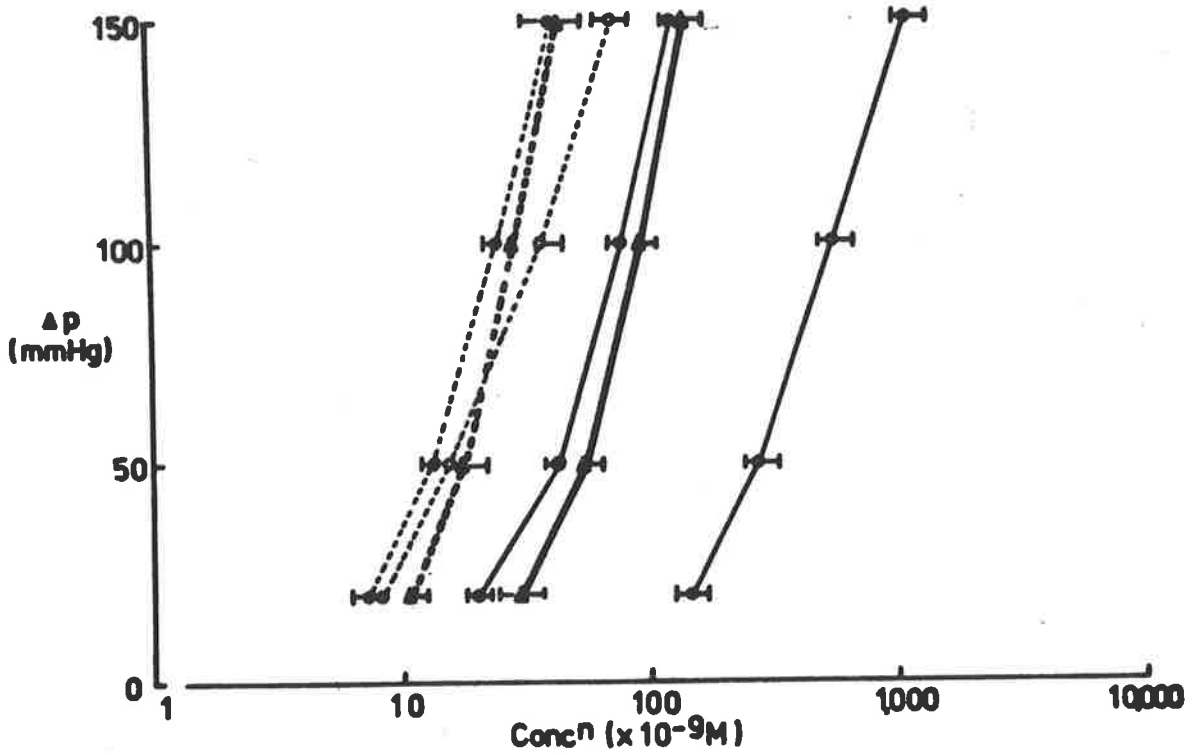

Figure 3.6

Concentration response curves of the perfused isolated rat tail artery and the perfused rat tail segment to adrenaline in the presence and absence of cocaine (2.9×10^{-5} mol.l^{-1}).

Tail segment ▲—— Extraluminal adrenaline O——

Intraluminal adrenaline ●—— Cocaine present- - - -

Table 3.1

Geometric means and standard errors of dose ratios for noradrenaline on the perfused rat tail segment.

Response Level mm Hg	NAI/NAII	n	NAI/NA Coc	n
150	1.3		4.1 *	
	1.0-1.6	4	3.2-5.3	4
100	1.1		4.6 *	
	0.9-1.4	4	4.0-5.4	4
50	1.0		5.1 *	
	0.9-1.2	4	4.7-5.5	4
20	0.9		7.2 *	
	0.7-1.1	4	7.0-7.4	4

NAI/NAII unpaired t-test.

NA/NA Coc paired t-test.

* $p<0.05$.

n = number of arteries.

Table 3.2

Geometric means and standard errors of dose ratios for adrenaline on the perfused rat tail segment.

Response Level mm Hg	AI/AII	n	AI/A Coc	n
150	1.3 1.0-1.6	4	4.1 * 3.2-5.3	4
100	1.2 1.0-1.4	5	3.5 * 3.1-3.8	5
50	1.1 1.0-1.3	5	3.6 * 3.4-3.9	5
20	1.2 1.1-1.4	5	3.4 * 3.1-3.8	5

AI/AII unpaired t-test.

AI/A Coc paired t-test.

* $p<0.05$

n = number of arteries.

Table 3.3

Ratios of mean concentrations of NA and A producing changes in perfusion pressure of 150, 100, 50 and 20 mm Hg in perfused artery and tail segments.

				Cocaine 2.9×10^{-5} mol.l^{-1}		
Response Level mm Hg	Artery		Perfused Tail	Artery		Perfused Tail
	NA/A		NA/A	NA/A		NA/A
	IL	EL		IL	EL	
150	1.5 a	2.0 *	4.3 *	1.4	1.0	3.2 *
100	1.5 a	2.3 *	3.8 *	1.5	1.2	2.9 *
50	1.6 a	2.3 *	3.3 *	1.7	1.5	2.4 *
20	1.6 *	2.1 *	3.4 *	1.5	1.5	1.6 a
n=	10, 11	10, 11	8, 11	5, 6	5, 6	4, 5

n = number of arteries: NA, A.

* $p<0.05$)
) unpaired t-tests
a $0.1>p>0.05$)

Table 3.4

Geometric means and standard errors of dose ratios for noradrenaline on the perfused rat tail artery. Numbers in parentheses refer to the number of arteries from which the ratios were derived.

Response Level mm Hg	EL/IL	EL/EL Coc	IL/IL Coc	EL Coc/ IL Coc
150	11.1 *	21.9 *	2.7 *	1.2
	9.0–13.6	16.1–30.0	2.0–3.5	1.0–1.5
	(10)	(5)	(5)	(5)
100	11.8 *	19.0 *	2.6 *	1.2
	10.0–13.8	14.8–24.3	2.1–3.2	0.9–1.5
	(10)	(5)	(5)	(5)
50	10.1 *	19.2 *	2.2 a	1.0
	8.2–12.4	14.7–25.1	1.8–2.8	0.8–1.3
	(10)	(5)	(5)	(5)
20	9.7 *	19.0 *	2.3 a	1.0
	8.0–11.7	15.4–23.4	1.9–2.9	0.8–1.4
	(10)	(5)	(5)	(5)

* $p<0.05$)
) paired t-tests
a $0.1>p>0.05$)

Table 3.5

Geometric means and standard errors of dose ratios for adrenaline on the perfused rat tail artery. Numbers in parentheses refer to the number of arteries from which the ratios were derived.

Response Level mm Hg	EL/IL	EL/EL Coc	IL/IL Coc	EL Coc/ IL Coc
150	8.5 *	12.0 *	3.2 *	1.8
	7.2-10.0	10.2-14.2	2.6-3.9	1.3-2.3
	(11)	(6)	(6)	(5)
100	7.1 *	13.5 *	3.5 *	1.4
	6.2- 8.1	11.5-16.0	2.8-4.3	1.1-1.9
	(11)	(6)	(6)	(5)
50	6.8 *	16.1 *	3.5 *	1.2
	5.8- 7.8	13.3-19.3	2.7-4.5	1.0-1.4
	(11)	(6)	(6)	(5)
20	7.5 *	18.1 *	3.1 *	0.97
	6.6- 8.4	14.9-22.0	2.4-3.9	0.9-1.04
	(11)	(6)	(6)	(5)

* $p<0.05$ (paired t-tests)

effects of both NA (4-7 fold) and A (3-4 fold). Cocaine caused a larger sensitivity increase at lower response levels, e.g., 7.2 times at 20 mm Hg, than at higher response levels, 4.1 times at 150 mm Hg, but the reverse trend was observed with A. The difference in sensitivity change between low and high response levels was smaller with A than with NA (0.7 compared with 3.1). At the 150 mm Hg response level, the degree of cocaine sensitization was the same for NA and A, i.e., 4.1 fold. Cocaine treated tail segments remained more sensitive to A than to NA although the difference in sensitivity was less than when cocaine was not present (1.6-3.2). In both cocaine treated and untreated segments the sensitivity differences between NA and A were greater at higher response levels.

DISCUSSION

The effects of the amines on the isolated vessel will be discussed first to enable the results on the isolated segment to be interpreted.

The ability of cocaine to eliminate both the differing sensitivities of the vessel to NA and A, and the influences of the different routes of application to the artery, implies that these differences are due entirely to the influence of neuronal uptake on the concentrations which these amines achieve at the adrenergic receptor sites in the smooth muscle. The influence of neuronal uptake on the sensitivities to the EL amines can be explained simply in terms of these nerves representing a site of loss as the amines diffuse from the adventitia into the smooth muscle, as shown by the model in Fig. 3.7. This model is a modification of the original one proposed by de la Lande et al. (1967) to take into account pharmacological and biochemical evidence that the concentration of NA declines in the artery wall as it diffuses from one surface to the other (de la Lande et al., 1980). The lower

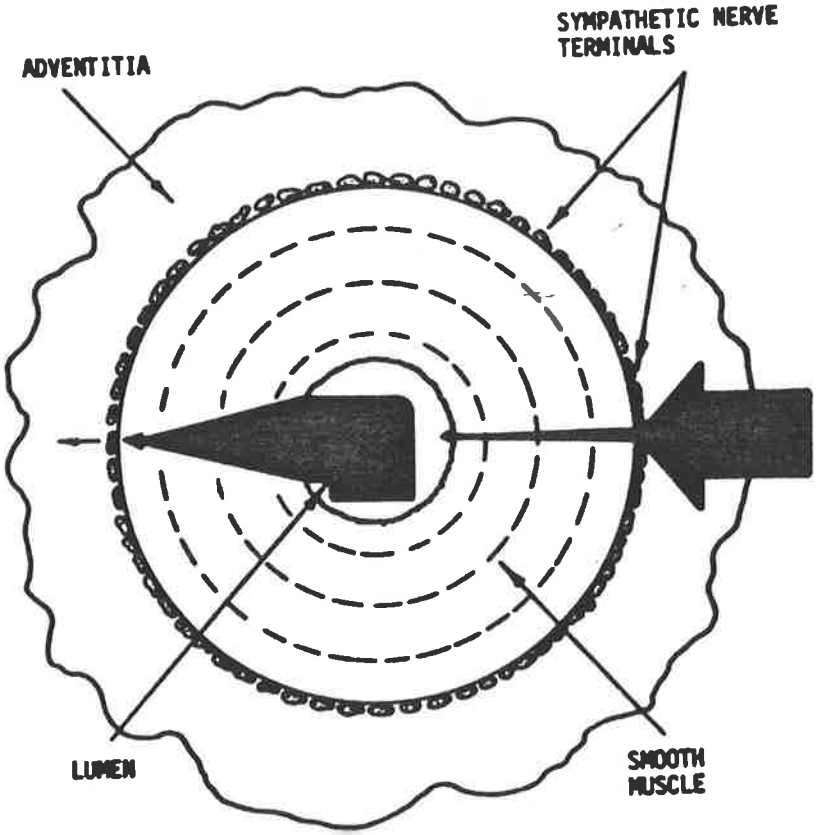

Figure 3.7

A diagrammatic representation of the influence of uptake by the sympathetic nerve terminals on the concentration of noradrenaline in the smooth muscle of the artery. The direction of the arrows represents the direction of diffusion of noradrenaline. The thickness of the arrows represents concentrations of noradrenaline. The model implies free penetration of both extraluminal noradrenaline (through the adventitia) and intraluminal noradrenaline (through the media) to the sympathetic nerve terminals.

sensitivity of the artery to EL A, as compared with EL NA, and the smaller potentiating effect of cocaine on this sensitivity are in accord with the lower affinity of the neuronal uptake system for A than for NA (Burgen and Iversen, 1965). The smaller effects of cocaine on the sensitivities to IL A and NA (compared with EL A and NA) are in general accord with the model insofar as the location of the nerve terminals at the adventitial border of the media means that neuronal uptake will not represent a site of loss of the amines as they enter the smooth muscle from the intima, while the decreasing gradient implies that the vasoconstrictor response will be mediated primarily by smooth muscle cells adjacent to the intima, i.e., by the inner rather than the outer layer of smooth muscle cells. However, the greater sensitizing effect of cocaine on IL A cannot be interpreted in this way without invoking an additional sensitizing effect of cocaine which is not mediated by inhibition of neuronal uptake. Such an action may conceivably arise from the weak inhibitory effect of cocaine on extraneuronal uptake (Iversen, 1967; Kalsner, 1964), an increase in cellular mobilization of Ca^{++} (Greenburg and Innes, 1976), or a post-junctional sensitization (Kalsner and Nickerson, 1969). It is conceivable also that the endothelial cells possess a cocaine-sensitive amine transporting system similar to that of the endothelial cells of the lung (Gillis, 1976). The results of Chapter 4 provide more information on these possibilities.

Wyse (1976) found that neuronal uptake was the major process involved in the inactivation of small concentrations of exogenous and neural NA in rat tail artery strips. This is in accord with the findings presented in this chapter.

The effects of A on the perfused segment appear to be entirely consistent with its effects on the perfused artery insofar as the

sensitivity of the segment is identical with that of the artery, as are also the potentiating effects of cocaine on these sensitivities. These findings suggest that the same factors which influence the sensitivity to IL A in the isolated artery apply when the artery is in the tissue. However, the same conclusion does not apply to NA, as both the sensitivity of the segment to NA and the potentiating effect of cocaine are intermediate in value between the corresponding values for IL and EL NA. This result indicates that neuronal uptake exerts a greater influence on the response of the artery to NA in the whole segment than it does to IL NA in the isolated artery. The difference between the effects of NA and A in the two types of preparation can be explained in two ways, although neither explanation is satisfactory. One possibility is that less A than NA penetrates to the nerve terminal due to greater extraneuronal uptake of the former amine in the media. However, a pharmacological study (Venning and de la Lande, 1979) suggests that extraneuronal uptake exerts little influence on the sensitivity of the artery to A. This will be discussed more fully in Chapter 5. A second possibility arises from that the fact that, in the segment, the extracellular compartment outside the adventitia will fill much more rapidly, due to its small size, than the equivalent compartment around the isolated artery, the 10 ml extraluminal bath volume. As a result, the gradient of concentration across the wall of the artery in the segment, during IL perfusion of the amine, will tend to disappear, so that the outer cells will become exposed to a higher concentration of amine and contribute more to the vascular response. Since these cells are close to the nerve terminals, the influence of neuronal uptake will be greater. The difficulty with this explanation is that it implies that the sensitivity to IL NA in the segment should exceed that to IL NA in the artery, but, in fact, the opposite

situation applies. Hence, the only conclusion which can be drawn from these results at present is that neuronal uptake exerts a greater influence on the response of the IL perfused vessel in situ to NA than it does to A, but the relationship of this difference to the different morphological environments of the vessel is not known.

C H A P T E R 4

METHOXAMINE

INTRODUCTION

In the preceding chapter the effect of cocaine on the sensitivities of the rat tail artery to IL and EL NA was used as a test of the hypothesis that the low sensitivity to EL NA was due to neuronal uptake of the amine. As a further test of this hypothesis, the response of the artery to methoxamine was examined.

Methoxamine is an $alpha_1$ receptor agonist which undergoes little, if any, neuronal or extraneuronal uptake (Burgen and Iversen, 1965; Trendelenburg, Maxwell and Pluchino, 1970). It is neither a substrate for MAO or COMT. It lacks beta adrenoreceptor action on smooth muscle (Goodman and Gilman, 1980). Consequently, it appeared to be a suitable agonist with which to further test the above hypothesis. Furthermore, it offered the opportunity to test more critically the argument that the sensitizing effect of cocaine on blood vessels was partly, if not entirely, due to a direct effect not related to its effects on neuronal uptake. It has been reported that cocaine has a weak inhibitory effect on extraneuronal uptake (Iversen, 1967; Kalsner, 1969).

Accordingly, it was anticipated that, if cocaine does sensitize the contractile response of the vessel to NA directly, it would have the same effect on the contractile response to methoxamine. The possible modification of the response to methoxamine by pretreatment with reserpine has also been studied. This was done in part as a precautionary measure in case its action on the rat tail artery was not entirely direct and did include an indirect component.

METHODS

Double cannulated rat tail arteries were perfused at a rate of 2 $ml.min^{-1}$ as described in Chapter 2.

Dose response curves to EL and IL methoxamine were obtained in the

presence and absence of cocaine. The cocaine was present in the IL perfusing solution and in the EL bathing solution. Arteries from normal rats and from reserpine pretreated rats were used. Reserpine (4.1×10^{-6} mol.Kg) was administered 24 hours prior to the perfusion experiments. Portions of each reserpine pretreated artery were analyzed for NA fluorimetric assay by the method of Head, Crabb, de la Lande and Frewin (1977) to ascertain the extent of depletion of NA. The mean ($\pm$ sem) NA level in arteries from normal rats was 5.3 ± 0.3 $\mu g.g^{-1}$ of tissue weight. Only rats with a NA level less than 1 $\mu g.g^{-1}$ after reserpine pretreatment were used.

The action of cocaine was also studied by adding it EL during the course of a steady state response to EL methoxamine. An increase in response, if it occurred, was translated into a dose ratio by relating the increase in response to the concentration response curve for methoxamine.

RESULTS

The steady state responses to methoxamine in both normal and reserpine pretreated arteries were slower in onset than responses to NA or A, particularly when the response was close to threshold. An example is illustrated in Fig. 4.1. However, the dose response curve was steeper.

Dose response curves to EL and IL methoxamine are shown in Fig. 4.2 (normal arteries) and Fig. 4.3 (reserpine pretreated arteries). It will be seen that, in the normal artery, the sensitivities to EL and IL methoxamine were not significantly different. However, in the reserpine pretreated arteries (Fig. 4.3), the sensitivity to EL methoxamine was 1.5-1.7 fold greater than the sensitivity to IL methoxamine. This was due to a shift to the left in the dose response curve to EL methoxamine

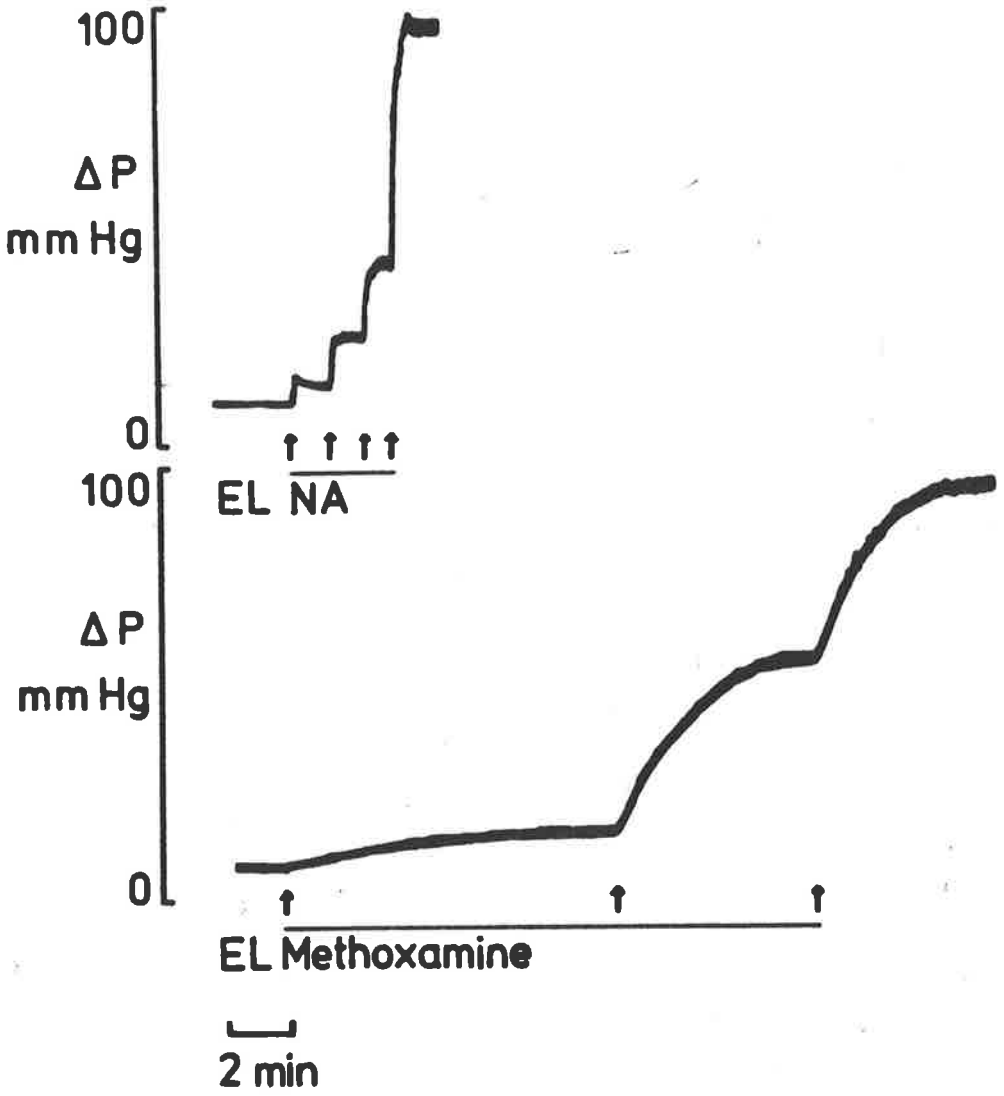

Figure 4.1

Photographs of responses of the perfused isolated rat tail artery to concentrations of noradrenaline and methoxamine showing the difference in the onset of action of each amine.

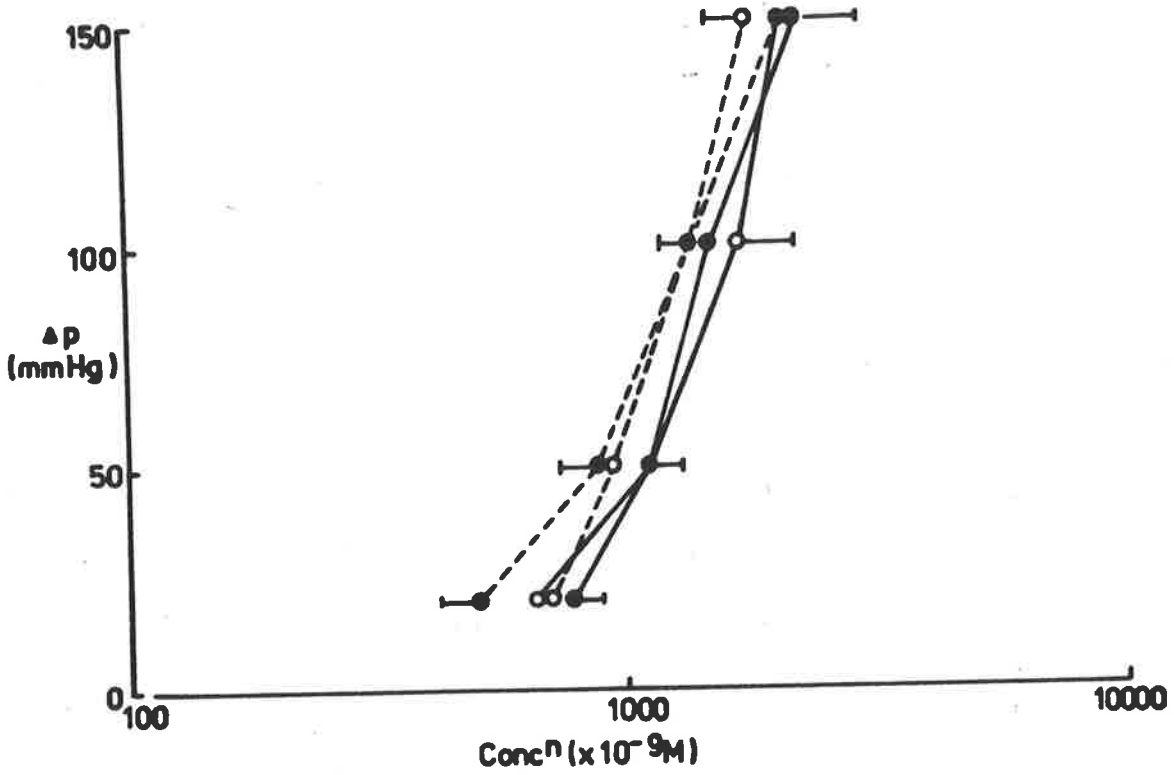

Figure 4.2

Log concentration response curves of the perfused rat tail artery to methoxamine in the presence and absence of cocaine (2.9×10^{-5} mol.l^{-1}). Open circles, EL methoxamine; closed circles, IL methoxamine; broken lines, methoxamine in the presence of cocaine. Each point represents the geometric mean and standard error of between 6 and 8 observations.

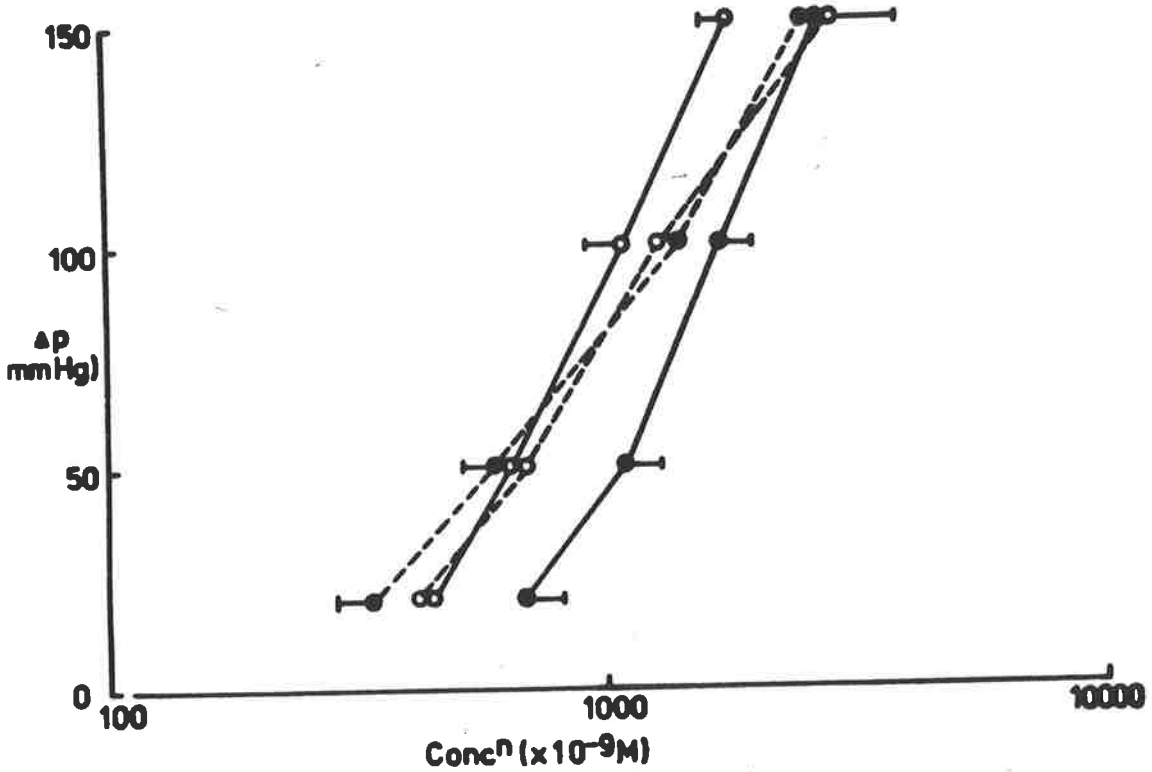

Figure 4.3

Log concentration response curves of perfused tail arteries from rats pretreated with reserpine (4.1×10^{-6} $mol.Kg^{-1}$) to methoxamine in the presence and absence of cocaine (2.9×10^{-5} $mol.l^{-1}$). Open circles, EL methoxamine; closed circles, IL methoxamine; broken lines, methoxamine in the presence of cocaine. Each point represents the geometric mean and standard error of between 4 and 7 observations.

Table 4.1

Geometric means and standard errors of dose ratios of methoxamine from normal arteries and arteries from rats pretreated with reserpine ($4.1x10^{-6}$ $mol.Kg^{-1}$) 24 hours prior to the experiments. Cocaine, when used, was present in a concentration of $2.9x10^{-5}$ $mol.l^{-1}$.

Response Level mm Hg	EL/IL	IL/IL Coc	EL/EL Coc	EL Coc/IL Coc
		Normal Arteries		
150	0.90 0.71-1.14 n=6	1.14 0.77-1.67 n=6	0.99 0.76-1.30 n=5	1.17 0.93-1.48 n=6
100	1.10 0.91-1.32 n=8	1.13 0.77-1.65 n=6	1.18 0.95-1.48 n=8	1.08 0.88-1.34 n=6
50	0.98 0.84-1.15 n=8	1.25 0.86-1.81 n=6	1.17 0.97-1.41 n=8	1.15 0.92-1.42 n=6
20	0.87 0.78-0.98 n=8	1.51 1.12-1.51 n=6	1.03 0.90-1.19 n=6	0.87 0.78-0.98 n=8
		Reserpine Pretreated Arteries		
150	0.68 * 0.62-0.73 n=7	0.94 0.82-1.07 n=4	0.68 0.52-0.89 n=4	1.14 0.94-1.38 n=3
100	0.62 * 0.58-0.67 n=7	1.24 1.04-1.47 n=5	0.89 0.73-1.09 n=4	1.12 0.97-1.30 n=3
50	0.58 * 0.55-0.62 n=7	1.82 * 1.26-2.03 n=5	0.98 0.84-1.14 n=4	1.24 0.93-1.63 n=3
20	0.64 * 0.60-0.64 n=7	2.01 1.17-2.36 n=5	1.12 0.92-1.36 n=4	1.36 1.02-1.81 n=3

* $p<0.05$ (paired t-test).

in the reserpinized artery. The effects of cocaine on these sensitivities is included in Table 4.1. In the normal artery the dose response curves to methoxamine in the presence of cocaine are slightly to the left of those in the absence of cocaine; however, the difference between the two sets of curves was not significant at any level of response. However, cocaine did have a significant effect on the sensitivities to methoxamine in the reserpinized arteries. It can be seen in Fig. 4.3 that the dose response curves to methoxamine, both EL and IL, in these vessels were less steep than those in the absence of cocaine, so that the sensitivity to low concentrations of methoxamine was significantly increased (by a factor of 1.82 times at 50 mm Hg), whereas the sensitivity to EL methoxamine tended to be decreased, although not significantly, at the maximum level of response recorded. However, there was no significant difference between the dose response curves to EL and IL methoxamine in the presence of cocaine. Although not significant in the data in Table 4.1, a small but definite sensitizing effect of cocaine on EL methoxamine was confirmed by showing that, in seven of ten reserpinized arteries tested, it augmented the steady state response to EL methoxamine when added after the response was established. It was calculated that the sensitivity to EL methoxamine was increased by a factor of 1.07 (sem 1.04-1.10) in the ten arteries (when the mean was calculated from only seven arteries that showed an augmented response, the mean sensitivity increase was 1.11, sem 1.09-1.15). This is considerably less than the increase in sensitivity to IL methoxamine produced by cocaine in reserpinized arteries (1.82 times at the 50 mm Hg level of response).

DISCUSSION

The identical potencies of IL and EL methoxamine in the normal

artery, and the failure of cocaine to significantly influence these potencies, provides strong support for the argument that neuronal uptake is the major factor responsible for the increased sensitivity to EL NA observed earlier. However, the results from the reserpinized arteries require explanation in the light of the early evidence (Burgen and Iversen, 1965; Trendelenburg et al., 1970) that methoxamine acts as a pure receptor agonist and its action is not influenced by the presence of sympathetic nerves. The selective increase in sensitivity to EL methoxamine by reserpine appears at first sight to be explicable in terms of post-junctional, or non-deviation, supersensitivity (as defined by Fleming, 1975) resulting from the interruption of the normal tone stimulus to the outer smooth muscle cells from the sympathetic nerves. The failure of IL methoxamine to display the same phenomenon may simply reflect the greater distance of the inner smooth muscle cells from the nerve terminals, so that these cells would be less influenced by the level of sympathetic transmitter output from the nerve terminals. However, if this is the case, it is difficult to explain why cocaine abolishes this difference in sensitivity unless the two drugs act via a common mechanism. Such a mechanism may involve Ca^{++} since there is indirect evidence that reserpine may alter the binding or movement of Ca^{++} (Carrier, 1975; Carrier and Hester, 1976), and that cocaine may make bound stores of Ca^{++} more available to promote NA induced contraction in the isolated cat spleen strip (Greenberg and Innes, 1976). The possibility that it involves the effects of cocaine on neuronal uptake seems excluded by the fact that cocaine enhances responses to IL methoxamine more than to EL methoxamine. Indeed, the potentiating effect on EL methoxamine was so small that it could only be detected by the small increment it produced in the steady state response to methoxamine when added after the response was established. Furchgott (1978)

suggested that this effect, more pronounced on the rabbit aorta, could be due to cocaine displacing tissue bound methoxamine, temporarily giving rise to a higher free concentration of methoxamine in the vicinity of the receptors. However, direct evidence of such displacement is lacking.

Studying responses to EL and IL methoxamine in the rabbit ear artery, de la Lande et al. (1970) reported that it was equipotent by both routes. Although this was based on comparisons on only two arteries, the equipotency has been subsequently confirmed on a further seven arteries (de la Lande, private communication). However, Yong and Chen (1975) have reported methoxamine to be 3.4 times more potent by the EL route in the same artery, and neither EL or IL sensitivities were altered by cocaine. The cause of the discrepancy remains to be explained.

Although the present study has drawn attention to some puzzling features of the interactive effects of reserpine and cocaine on the response to methoxamine, these effects are minor in magnitude compared with the magnitude of cocaine's effect on the responses to EL NA and appear to be smaller, even, than its effects on IL NA. Hence the results add to, rather than detract from, the hypothesis which relates the differences in EL and IL sensitivities to NA, and the modification of these sensitivities by cocaine, to the location of the sympathetic nerves of the medial-adventitial border, and their ability to function as a major site of loss of NA, differing from the adventitia to the media.

C H A P T E R 5

EXTRANEURONAL UPTAKE

INTRODUCTION

It was shown in the previous chapters that inhibition of neuronal uptake caused a greater sensitivity increase to EL NA and A than to IL NA and A, but had no effect on the sensitivity to methoxamine. This route dependent sensitizing effect of cocaine was explained on the basis of the localization of the sympathetic nerve terminals at the medial adventitial border of the artery. In this chapter the effects of inhibition of extraneuronal uptake and metabolism by COMT have been studied. DOCA was used to inhibit extraneuronal uptake and UO521 to inhibit COMT metabolism.

Iversen and Salt (1970) reported that a number of steroids, including deoxycorticosterone (DOC) inhibited the extraneuronal uptake of catecholamines and their subsequent metabolism by COMT. In the rabbit ear artery, Johnson and de la Lande (1978) provided pharmacological evidence that DOCA inhibited extraneuronal uptake. They reported that DOCA increased the sensitivity of that vessel to NA by 1.5-2 fold, and to A by 3-5 fold. Head et al. (1980) provided biochemical evidence that DOCA inhibited the extraneuronal uptake system and that it was linked to the O-methylating system to provide a high affinity, low capacity mechanism for removing a catecholamine, isoprenaline, from its receptor sites.

In the present study shifts in dose response curves to EL and IL A produced by DOCA were investigated. In addition, the effects of both DOCA and UO521 on steady state responses to A were investigated. A was used as the amine of choice as it has been shown to have a higher affinity than NA for extraneuronal uptake (Iversen, 1967) and it was thought that any sensitizing effect of inhibition of neuronal uptake would be more apparent with A. In many experiments cocaine was present to eliminate any effect of neuronal uptake. In addition to the use of

an aqueous bathed preparation, an oil bathed preparation was also utilized. This was done to assess whether, as in the rabbit ear artery, there was a declining, non-uniform gradient of concentration of amine across the artery wall when the amine was applied to one surface. If there was such a gradient, then it seemed likely that removal of amine by extraneuronal uptake would accentuate the declining gradient. To test these possibilities, the response to IL A was compared with the response to A applied to both surfaces in cocaine treated arteries, by achieving steady state response to IL A and then applying EL A. After wash-out of the EL A the response to IL A was then compared with the response to IL A when the aqueous bathing solution was replaced with oil. The effect of both these procedures should be to render the gradient uniform across the artery wall, and hence responses to IL amine and EL amine should be identical to responses to IL amine plus oil. If, however, extraneuronal uptake contributed substantially to the gradient, then the response to IL amine plus EL amine should exceed that to IL amine plus oil, but the two responses should become equal in the presence of an extraneuronal uptake inhibitor.

METHODS

The isolation and perfusion of the rat tail artery is detailed in Chapter 2.

DOCA

The effect of DOCA on the sensitivity of the artery to A was tested in identical fashion to that of cocaine (Chapter 3). However, because the DOCA stock solution was in ethanol, it was necessary to test also the same concentration of ethanol alone on artery responses. Concentrations of DOCA and ethanol used in the bathing solutions were

$2.7x10^{-5}$ and $6.9x10^{-3}$ $mol.l^{-1}$, respectively. Cocaine, $2.9x10^{-5}$ $mol.l^{-1}$ was used in some of the experiments with DOCA.

U0521

Concentrations of A that produced increases in perfusion pressure of about 100 mm Hg were used. Cocaine ($2.9x10^{-5}$ $mol.l^{-1}$) was present to enable the same concentration of EL and IL A to be used. Once a steady state response to A had been obtained, U0521 was added by the same route as the A. The % change in steady state response was measured.

DOCA and U0521

The influence of DOCA on the effect of U0521 was determined by adding DOCA to both sides of the artery before responses to A and U0521 were obtained.

Diffusion Studies

After eliciting dose response curves in the usual fashion, concentrations of A or NA were added IL to the artery to produce a sustained steady state response within the range of 30-60 mm Hg increase in perfusion pressure. The Krebs solution bathing the outside of the artery was then replaced with the same solution containing A or NA that was perfusing the artery, and allowed to remain until a new, augmented, steady state response was achieved. The EL solution was then replaced with amine free Krebs solution. Approximately 5 minutes after the response had returned to the steady state level produced by IL amine alone, the EL solution was replaced with warmed paraffin oil bubbled with gas mixture (95% O_2, 5% CO_2). The oil was allowed to remain until a new steady state level of response had been achieved, after which it was washed out and replaced with amine free Krebs solution. This

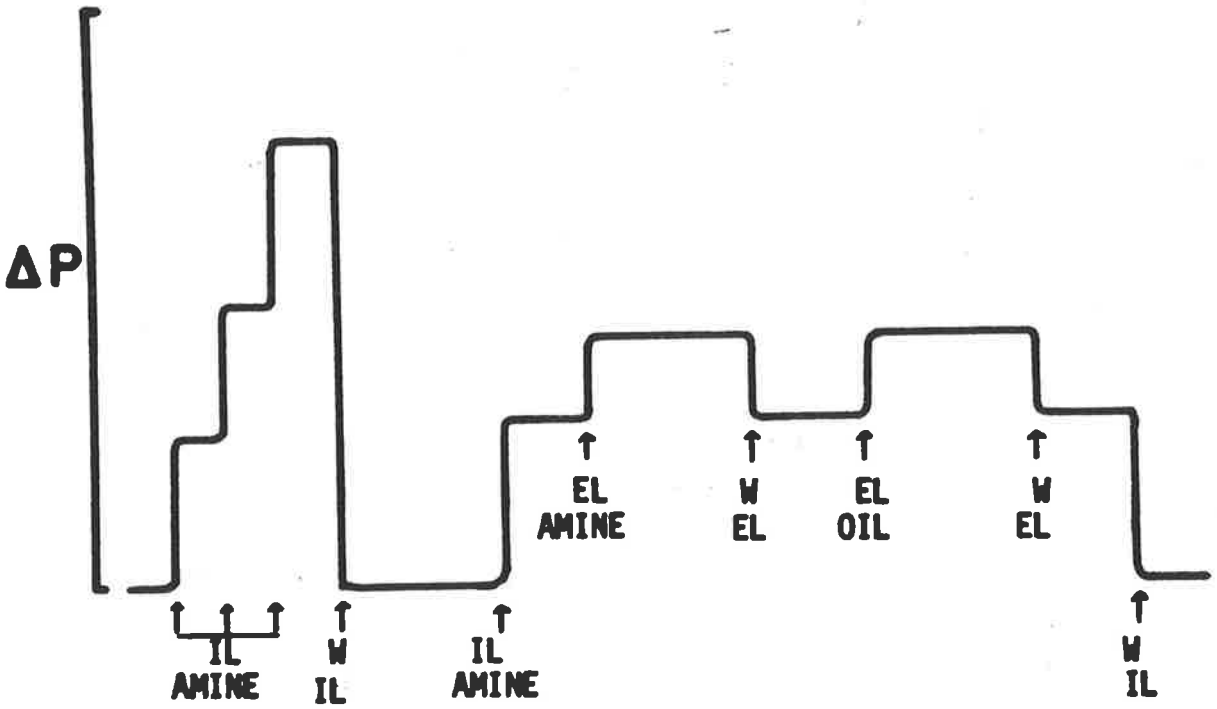

Figure 5.1

Diagram of the procedures used in the diffusion studies. After eliciting a dose response curve to IL amine, a steady state response to IL amine was produced. The aqueous bathing solution outside the artery was then replaced with external amine solution and then oil.

procedure is illustrated in Fig. 5.1. This procedure was repeated with cocaine (2.9×10^{-5} $mol.l^{-1}$), or cocaine and DOCA (2.7×10^{-5} $mol.l^{-1}$) present in the Krebs solution. The potentiation (increase in response) produced by EL amine or oil was estimated from the IL concentration response curve in terms of the increase in concentration of IL amine required to produce the same increase in response.

MAO and COMT Activities

MAO and COMT activities of rat tail artery homogenates were determined using the methods of Jarrott (1971a and 1971b).

RESULTS

DOCA

Arteries were about six times more sensitive to IL A than EL A. DOCA had no effect on the sensitivity of the artery to EL A, but it produced a small but not significant sensitization (1.2-1.5 times) to IL A (Fig. 5.2, Table 5.1). This sensitization tended to be greater at lower levels of response.

Cocaine and DOCA

When cocaine was present, the artery was about two times more sensitive to EL A at the two highest response levels, and DOCA now tended to shift both the EL and IL dose response curves to A to the left (Fig. 5.3, Table 5.2). However, these shifts failed to reach significance. Although the dose response curves were not significantly different for EL A, this is probably a reflection of the small magnitude of the change, since in each of the arteries, DOCA, when perfused IL during a steady state response to EL A, caused a small but clear cut

augmentation of the response. This amounted to a mean increase of 1.42 (sem 1.33-1.52) in sensitivity (Fig. 5.4). The effect of IL DOCA, without cocaine present, on the steady state response to NA, was to cause a small, clear cut augmentation in response of 1.19 (sem 1.09-1.30, n=4).

Ethanol

Ethanol, in the concentration used as the DOCA vehicle, had no effect on the dose response curves to A (Fig. 5.5).

Diffusion Studies

The effects of applying A EL, and also of replacing the amine free EL bathing solution with oil, during a sustained steady state response to IL A are shown in Fig. 5.6 and Table 5.3.

In the untreated artery, the effects of applying either EL A, or external oil varied from no effect to a small transient increase in the response which was sometimes sustained during the period of application. However, both EL A and EL oil gave well defined further increases in the responses to IL A elicited in the presence of cocaine. Following EL A, the response increased rapidly before falling to its new steady state level, and returned rapidly to a level close to the pre-existing level when the EL A was washed out. Following application of EL oil, the new steady state level was significantly less, and it was achieved more slowly than in the case with EL A. However, recovery from oil was as rapid as that from EL A. When the above procedure was repeated, on separate arteries, but with DOCA present as well as cocaine, the effect of adding EL A was slightly less, but not significantly so, from its effect when cocaine alone was present. However, the effect of oil tended to be greater, so that now the steady state level achieved by the

oil was equal to that produced by the EL A.

When NA was used in place of A, the onset time of response to EL NA was biphasic, and faster than that with oil, as with A. In addition, responses to EL NA and oil were larger in the presence of cocaine, and cocaine and DOCA. However, there was no significant difference between the sizes of the responses produced by the EL NA and oil in the presence of the uptake inhibitors, and the response to oil was significantly less when cocaine and DOCA were present than with cocaine alone. In addition, in the untreated arteries, oil produced a significantly larger response than EL NA, although both these responses were small and transient.

U0521

U0521 caused an increase in steady state response to A whether it was applied EL or IL. The % increase was larger for IL than for EL. However, when cocaine was present, this situation was reversed, and the % increase was larger for EL than for IL. DOCA, when present prior to the U0521, largely eliminated the effect of U0521 on both EL and IL A in the presence of cocaine (Table 5.4).

The incremental responses that U0521 alone produced on steady state responses to A, expressed as potentiations, were: IL 1.52 (1.51-1.54, n=2); EL 1.22 (1.16-1.29, n=4).

MAO and COMT

The mean enzyme activities determined in the rat tail artery homogenates were: MAO 67.6$\pm$7.5 (n=10) n.mol of tyramine oxidised.mg protein^{-1}.hour^{-1}; COMT 2.47$\pm$0.34 (n=10) n.mol ^{14}C S adenosyl methionine. mg protein.hour^{-1}.

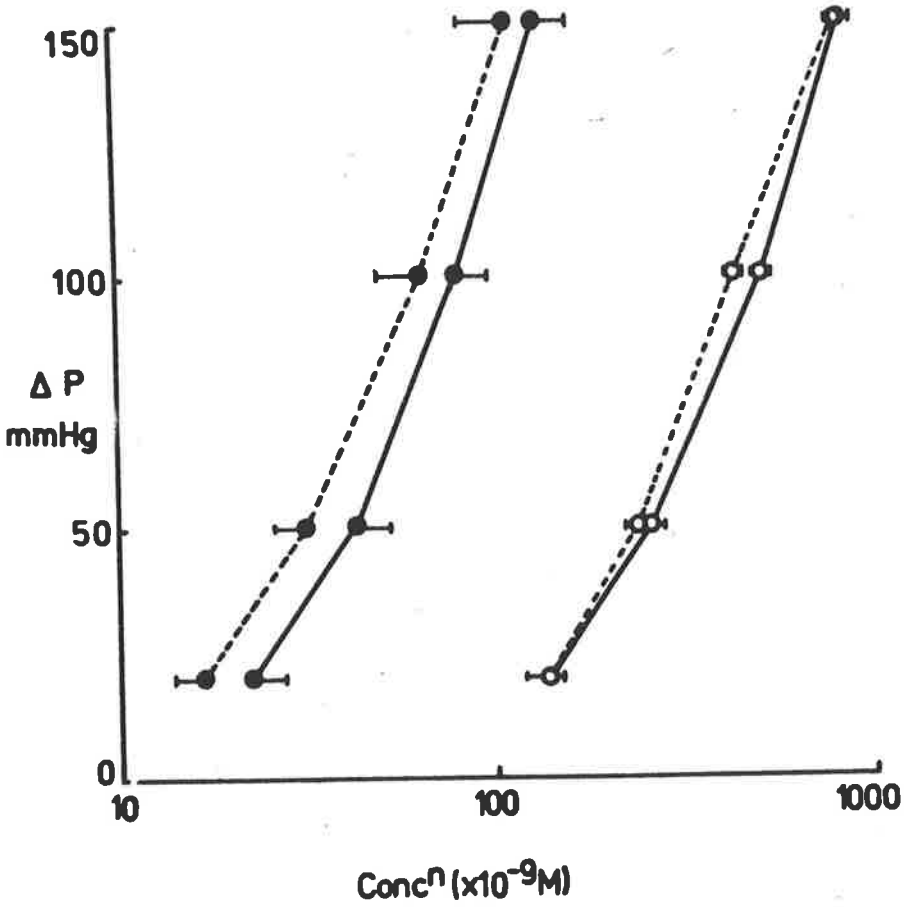

Figure 5.2

Concentration response curves for EL and IL A in the presence and absence of DOCA (2.7×10^{-5} $mol.l^{-1}$). The value at each point represents the geometric mean and standard error of 5 or 6 observations.

EL A O—— IL A ●—— DOCA- - - -

Table 5.1

Geometric means and standard errors of dose ratios of adrenaline and the effect of DOCA (2.7×10^{-5} $mol.l^{-1}$). (n) = number of arteries from which means were derived.

Response Level mm Hg	EL/IL	EL/EL DOCA	IL/IL DOCA	EL DOCA/ IL DOCA
150	6.3 * 5.0-7.9 (6)	0.97 0.9 -1.05 (6)	1.2 a 1.19-1.25 (5)	7.4 * 5.9-9.2 (5)
100	6.4 * 5.1 8.0 (6)	1.07 0.96-1.19 (6)	1.3 a 1.21-1.40 (5)	7.0 * 5.8-8.5 (5)
50	6.1 * 4.8-7.7 (6)	1.07 0.96-1.20 (6)	1.47 a 1.32-1.63 (5)	7.1 * 5.8-8.7 (5)
20	6.1 * 4.8-7.8 (6)	1.00 0.90-1.11 (6)	1.46 a 1.30-1.64 (5)	7.9 * 6.4-9.9 (5)

* $p<0.05$ (paired t-test).

a $0.1>p>0.05$ (paired t-test).

Table 5.2

Geometric means and standard errors of dose ratios of adrenaline and the effect of DOCA (2.7×10^{-5} mol.l^{-1}). (n) = number of arteries from which means were derived. Cocaine present throughout (2.9×10^{-5} mol.l^{-1}).

Response Level mm Hg	EL/IL	EL/EL DOCA	IL/IL DOCA	EL DOCA/ IL DOCA
150	0.50 *	1.08	1.26	0.58
	0.46-0.54 (5)	0.87-1.35 (5)	1.12-1.42 (5)	0.49-0.67 (5)
100	0.48 *	1.30	1.34	0.49 *
	0.43-0.53 (5)	1.01-1.70 (5)	1.17-1.54 (5)	0.42-0.58 (5)
50	0.51 a	1.53	1.42 a	0.48 *
	0.43-0.60 (5)	1.13-2.08 (5)	1.24-1.62 (5)	0.41-0.56 (5)
20	0.63	1.59	1.29	0.51 *
	0.49-0.80 (5)	1.08-2.34 (5)	1.11-1.50 (5)	0.41-0.62 (5)

* $p<0.05$ (paired t-test).

a $0.1>p>0.05$ (paired t-test).

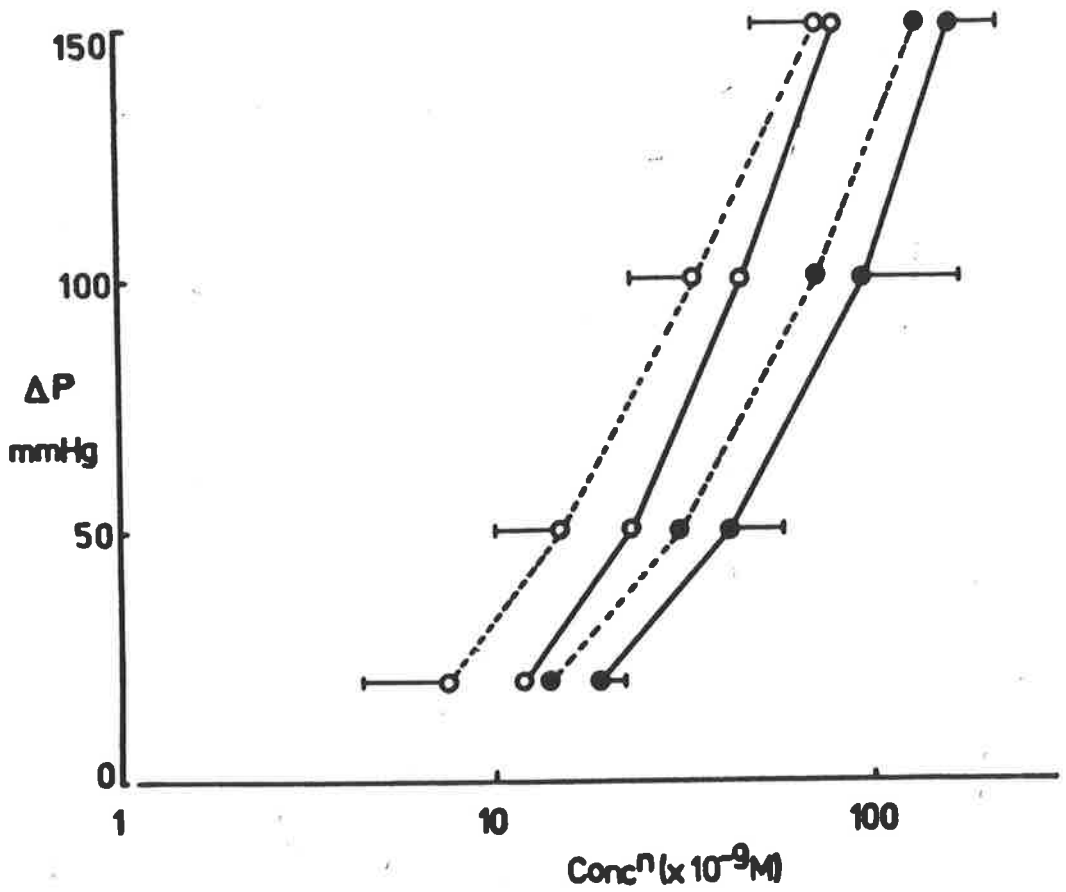

Figure 5.3

Concentration response curves for EL and IL A in the presence and absence of DOCA (2.7×10^{-5} $mol.l^{-1}$). Cocaine (2.9×10^{-5} $mol.l^{-1}$) was present throughout. The value at each point represents the geometric mean and standard error of 5 observations.

EL A O—— IL A ●—— DOCA ----

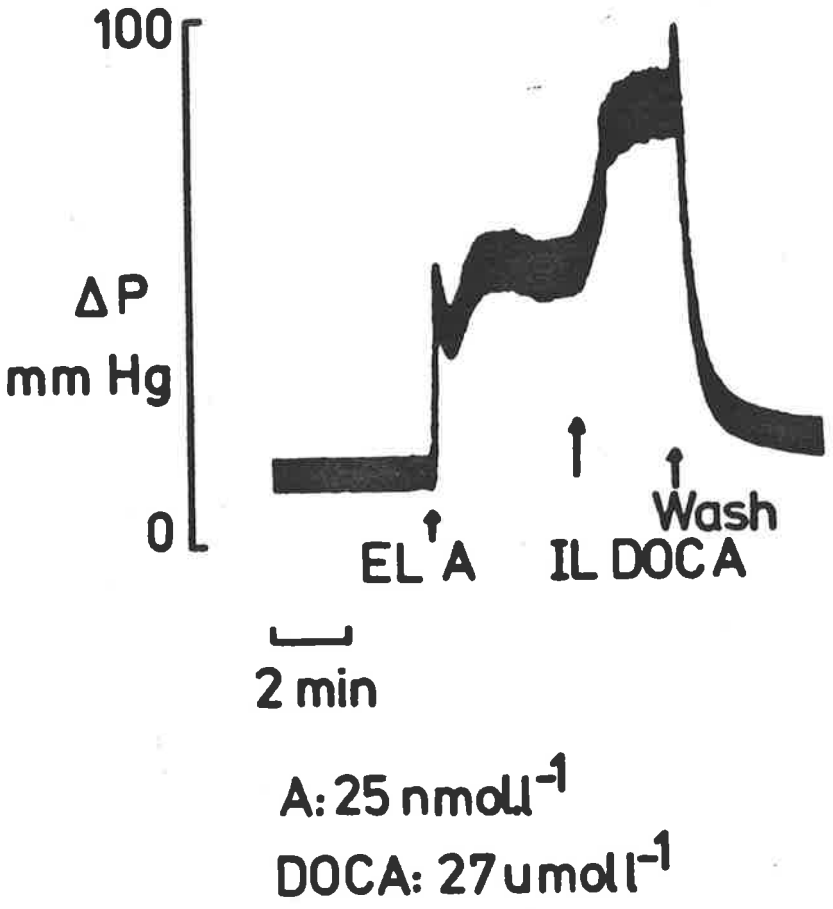

Figure 5.4

Photograph showing incremental response produced by IL DOCA on a steady state response to EL adrenaline.

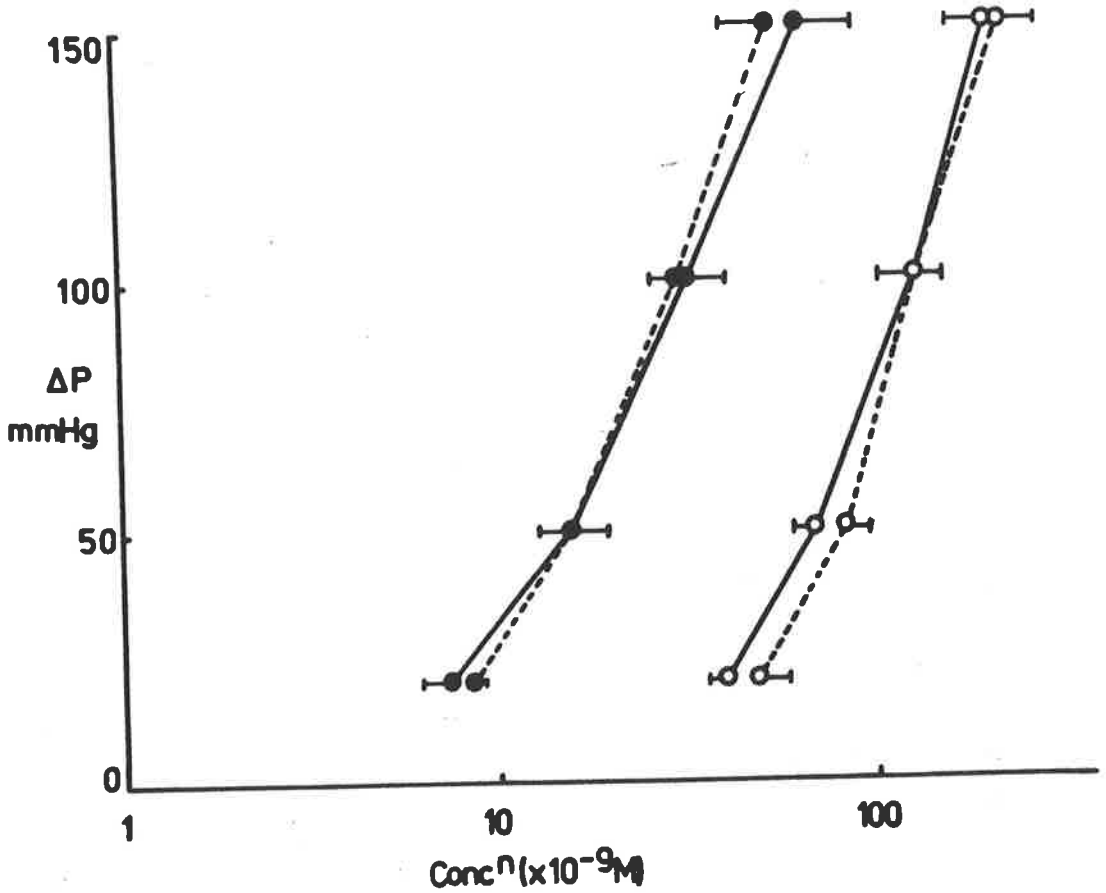

Figure 5.5

Concentration response curves for EL and IL A in the presence and absence of ethanol (6.9×10^{-3} mol.l^{-1}). Ethanol did not cause a shift in either of the curves; paired t-test. The value at each point represents the geometric mean and standard error of 5 observations.

EL A O—— IL A ●—— EtOH - - - -

Table 5.3

Geometric means (and sem) of potentiations of IL responses to noradrenaline or adrenaline by EL noradrenaline, or EL oil.

			Cocaine	Cocaine/DOCA	
NA	EX	1.10 1.06-1.14 n=6	2.36 2.10-2.65 n=5	2.25 2.13-2.37 n=5	
	OIL	1.13 1.07-1.19 n=6	2.65 2.36-2.97 n=5	1.98 1.82-2.15 n=5	*
A	EX	1.09 1.00-1.18 n=4	2.65 2.55-2.76 n=6	2.47 2.30-2.66 n=5	
	OIL	1.19 1.09-1.30 n=4	1.86 1.71-2.03 n=6	2.22 2.03-2.42 n=5	
			*	*	

1. * below column in table indicates that sensitivity changes in oil and in EL A are significantly different ($p<0.05$; paired t-test).
2. * on right hand side of column in table indicates that sensitivity in cocaine, and cocaine/DOCA treatments were significantly different ($p<0.05$; unpaired t-test).
3. The sensitivity ratios with oil and with EL A (or NA in the presence of cocaine and cocaine/DOCA were significantly greater in the untreated artery ($p<0.05$; unpaired t-test).

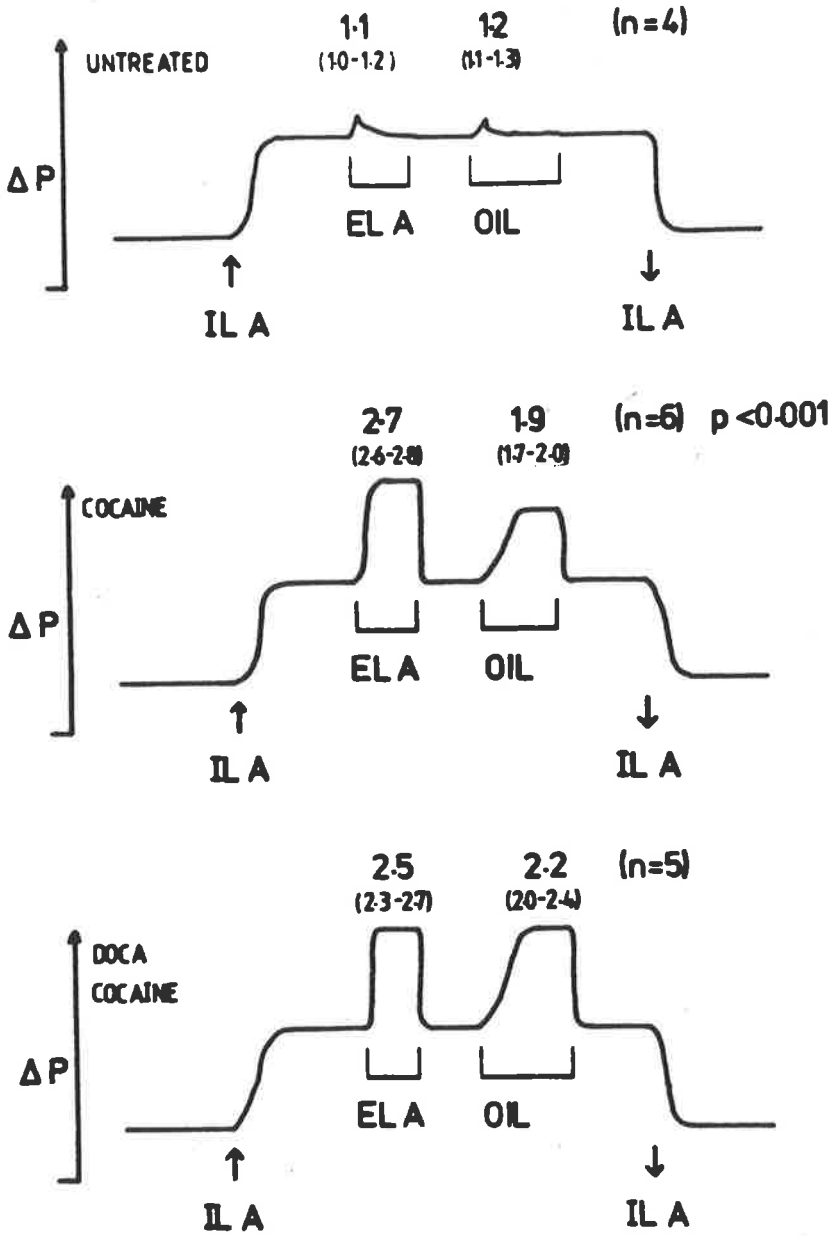

Figure 5.6

Drawings of traces showing the effect of extraluminal application of adrenaline, and of oil, on the steady state responses of the perfused rat tail artery to intraluminal adrenaline. Geometric means and standard errors of potentiations are shown above each response. Cocaine 2.9×10^{-5} mol.l^{-1} and DOCA 2.7×10^{-5} mol.l^{-1}.

Table 5.4

Percentage increases in steady state responses to adrenaline produced by U0521 (1.1×10^{-5} mol.l^{-1}) and the effect of DOCA (2.7×10^{-5} mol.l^{-1}) and cocaine (2.9×10^{-5} mol.l^{-1}). DOCA and cocaine were present on both sides of the artery. U0521 was present on the same side of the artery as the A producing the response, i.e., IL or EL. Initial steady state responses to A were in the order of 100 mm Hg.

		Cocaine	
	U0521	U0521	U0521/DOCA
EL	13 ± 4 n=4	27 ± 6 n=8	2 ± 1 n=4
IL	25 ± 4 n=4	14 ± 5 n=8	2 ± 1 n=4
	*	*	

* $p<0.05$ paired t-test: significant difference between % increase for EL and IL for treatment group.

DISCUSSION

In the absence of cocaine, DOCA caused a small but not quite significant increase in the sensitivity to IL A but appeared without effect on EL A. In the presence of cocaine, the effect of DOCA on IL A was unchanged (and perhaps slightly diminished), but now it produced an equivalent (at least) increase in sensitivity to EL A. That DOCA does have some sensitizing effect is evident, however, from the augmentation by IL DOCA of the steady state response to EL A. It seems that the effect of DOCA is so small as to not be particularly evident as a leftward shift of the dose response curves. These results imply that, when neuronal uptake is active, extraneuronal uptake contributes to the removal of IL, but not EL A, from the receptors in the smooth muscle.

This difference is readily explained on the basis that the neuronal and extraneuronal uptake systems represent alternative pathways for the removal of A from its receptors in the smooth muscle. Evidence that these two pathways do compete in this fashion has been presented in the early study of Hughes (1972) on the vas deferens. In the case of the rat tail artery, evidence has already been presented which indicates that neuronal uptake is much more active in removing NA from the receptors in the artery than in the inner smooth muscle cells. It is probable, therefore, that the contribution of extraneuronal uptake will be masked (i.e., proportionally smaller) in the case of EL than in the case of IL A.

The sensitizing effect of COMT inhibition was also small and, like that of DOCA, it tended to be greater for IL A than for EL A in the absence of cocaine. However, when cocaine was present, COMT inhibition had a greater sensitizing effect on EL A and a smaller effect on IL A, which is again similar to the effect of DOCA on A in the presence of cocaine but more significant.

Since the sensitizing effect was largely eliminated by DOCA, it would appear that, in this artery, as in the aorta and the rabbit ear artery, the O-methylation of A occurs after uptake by the extraneuronal system (Kalsner, 1975; Johnson and de la Lande, 1978; Head et al., 1980).

The results indicate that when A is applied EL, extraneuronal uptake has little influence on its concentration in the biophase of the receptors at the smooth muscle cells. Extraneuronal uptake appears somewhat more important when A is applied IL. The 1.3 fold increase in the sensitivity to IL A produced by DOCA can be interpreted to mean that normally, extraneuronal uptake removes about 25% of the A in the biophase of the receptors in the smooth muscle cells in the inner region of the vessel wall. By the same reasoning, the potentiation of the IL A removed from the outer region by neuronal uptake is about 66%. This influence of extraneuronal uptake is small compared with that on the rabbit ear artery, where DOCA increases the sensitivity of that artery to IL A 3-5 fold, compared with an increase of 1.8 fold produced by cocaine, implying that, in the rabbit ear artery, extraneuronal uptake is the major mechanism for removing IL A (Johnson and de la Lande, 1978). Although it is tempting to attribute the difference between the tissues to a greater activity of the extraneuronal uptake system in the rabbit ear artery, alternative explanations are possible. Guimaraes et al. (1975) showed that the pharmacological effects of inhibiting extraneuronal uptake in the isolated dog saphenous vein was more pronounced with increasing thickness of the tissue. He attributed the difference to the greater length of the diffusion pathway in the thicker tissues, as a result of which the amine was subjected to greater loss by extraneuronal uptake before penetrating into the inner region (i.e., the middle) of the tissue. This hypothesis may also explain the

reduced response of the rat tail artery to extraneuronal uptake inhibition compared with the rabbit ear artery since the wall thickness of the rat tail artery is only about 60% of that of the rabbit ear artery. In support of this explanation, a parallel study in the author's laboratory has shown that isoprenaline is O-methylated in the two vessels at comparable rates (Morris et al., 1977; Head et al., 1980).

Further evidence of the contribution of extraneuronal uptake to pharmacological responses to A and NA was sought by investigating whether extraneuronal uptake influenced the diffusion gradient of these two amines in the tissue. This was done by replacing the external Krebs bathing solution in the organ bath by paraffin oil or an A or NA solution during a steady state response to IL A or NA. During a steady state response in an artery with no uptake or metabolic removal systems functioning, the gradient of concentration of amine across the artery wall will be represented by the solid line in Fig. 5.7. The amine concentration at the outer surface of the media will be zero, as the external bathing solution can be regarded as a site of infinite dilution for the amine. If the external aqueous solution is replaced with oil, in which the amine is not soluble, there will be a diffusion barrier set up through which the amine will not be able to pass, so at steady state, there will be a uniform concentration across the artery wall. Similarly, if the same concentration of amine is present on both sides of the artery, at steady state the concentration of amine across the wall will also be uniform.

The broken line in Fig. 5.7 indicates how extraneuronal uptake and its associated metabolic inactivation processes might modify this model. The influence of neuronal uptake has been eliminated by cocaine. With EL Krebs solution there would be a steeper concentration gradient of amine across the wall because, as well as diffusion into the outer

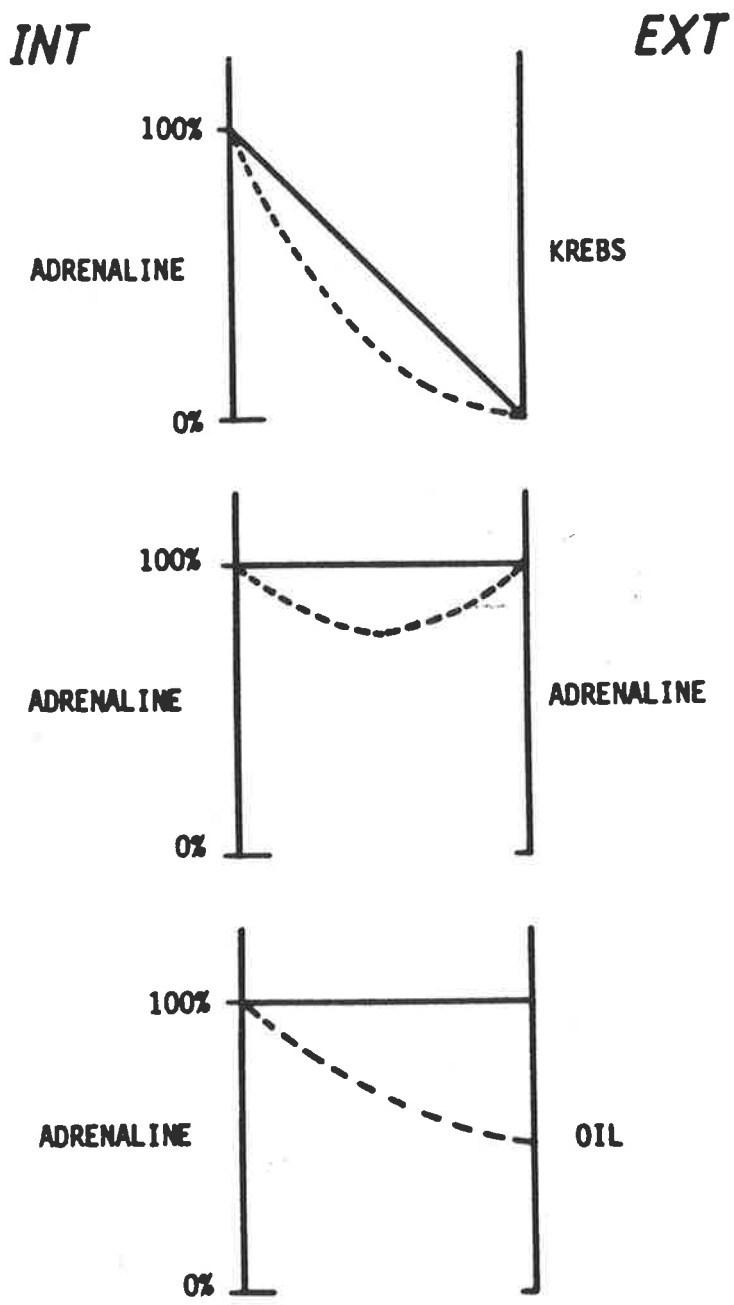

Figure 5.7

Models of adrenaline concentration gradients across the artery wall (considered as a plane) and the effects of the addition of EL A or oil, and the influence of extraneuronal inactivation. INT and EXT refer to the inner and outer surfaces of the artery. Solid lines represent the A concentration gradient in the absence of extraneuronal inactivation and broken lines represent the A concentration gradient in the presence of extraneuronal inactivation. % refers to the concentration of amine bathing the intimal surface of the vessel.

aqueous bathing solution, amine would also be removed by extraneuronal uptake. When oil is present, the gradient would also be reduced by extraneuronal uptake. When amine is present on both sides of the artery wall in the same concentration, it will diffuse across the wall in both directions and, as a result of extraneuronal uptake, the concentration of amine will be lower in the middle of the media, but there will be more amine in the wall than in the situation when oil is on the outside wall and amine only diffuses in from one side.

On the basis of this model, it can be predicted, firstly, that when extraluminal uptake is inhibited by DOCA, the addition of oil or amine solution externally should increase the steady state response of the artery to IL amine to the same extent; and, secondly, when the extraneuronal uptake system is functioning, external amine will increase the steady state response to IL amine more so than will oil, because there is a greater concentration of amine across the wall. These predictions describe exactly what occurred when the experiment was performed (Table 5.3).

When neuronal uptake was functioning, oil produced a small but greater increase in steady state response to IL A than did EL A presumably because the nerve terminals further reduced the concentration of A across the wall. The differences in potentiating effects seen with oil and EL A on IL A were not apparent when NA was used instead of A, possibly because of the lower affinity of NA for extraneuronal uptake.

Thus it appears that the contribution of COMT and extraneuronal uptake are minor compared with that of neuronal uptake in the inactivation of A and NA in the rat tail artery. This is in accord with the findings of Wyse (1976), who estimated the relative contributions of neuronal and extraneuronal inactivation in the artery indirectly from

the rates of relaxation of artery strips totally immersed in oil following prior exposure to NA, or following nerve stimulation. Of the agents he used (cocaine, corticosterone, tropolone and iproniazid), cocaine was the only agent that markedly prolonged relaxation. Hence, the present results confirm and extend the findings of Wyse, who concluded that, in the rat tail artery, neuronal uptake and storage take precedence over extraneuronal mechanisms in the inactivation of small concentrations of both exogenous and neuronal NA.

C H A P T E R 6

HYPERTENSION

INTRODUCTION

A number of studies have reported that vessels from spontaneously and DOCA/salt hypertensive rats show an increased responsiveness to NA and other constrictor agents compared with vessels from normotensive rats. This hyper-responsiveness has been observed in two distinct forms: an increase in reactivity (Hinke, 1965; Beilin, Wade Honour and Cole, 1970; Folkow, Hallback, Lundgren and Weiss, 1970; Hallback, Lundgren and Weiss, 1971; Beilin and Ziakas, 1972; Haeusler and Finch, 1972; Finch and Haeusler, 1974; Finch, 1975; Bhattacharya, Dadkar and Dohadwalla, 1977) and an increase in sensitivity (Holloway and Bohr, 1973; Lais and Brody, 1975; Rascher, Dietz, Schomig, Weber and Gross, 1980; Whall, Meyers and Halpern, 1980). These two terms have been defined by Sivertsson (1970). An increase in sensitivity implies a parallel shift to the left of the dose response curve with a decrease in threshold and no change in slope. An increase in reactivity implies an increase in the slope of the dose response curve. This may be associated with no change in threshold as is the case with an increased wall/lumen ratio, but in other situations a decrease in threshold may accompany an increase in slope of the dose response curve. These definitions are commonly used as a basis for deciding whether changes associated with DOCA/salt hypertension are related to neurogenic changes or structural changes in the vessels studied.

In the above studies it has been assumed that changes in responsiveness occur in all the smooth muscle cells. However, the results in the preceding chapters have shown that the factors which control sensitivity to NA depend on the surface of entry of the amine. A primary aim of the studies described in this chapter was to determine whether the increase in response of the artery to NA extended to all smooth muscle cells in the artery wall, or whether it was selective for cells which

are more closely exposed to released transmitter (outer cells) or to circulating amine (inner cells).

By comparing the sensitivities to NA of the perfused vessels from DOCA/salt hypertensive rats, and normotensive rats, it was hoped, not only to determine the extent of the increased responsiveness of the smooth muscle cells, but also to establish whether neuronal uptake activity may be modified in DOCA/salt hypertension. It was shown in the preceding chapters that neuronal uptake was the process largely responsible for the removal of extraluminally applied NA from the receptor sites.

As indicated in the results section, pronounced changes in sensitivity were observed. Analysis of the changes led to the inclusion of other constrictor stimuli in the study, namely, K^+ and electrical stimulation, together with measurement of the release of NA from the nerve terminals in the hypertensive and normotensive vessels.

The period of DOCA/salt treatment used in these studies was restricted to three weeks. This was in an attempt to minimize structural changes in the vessel wall associated with hypertension (Hansen and Bohr, 1975; Rascher et al., 1980). A phenomena often reported in perfused vessels and vascular beds from spontaneously hypertensive and DOCA/salt animals is an increased resistance to perfusion, presumably resulting from an increased wall/lumen ratio (Hinke, 1965; Folkow et al., 1970; Beilin and Ziakas, 1972; Finch and Haeusler, 1974; Lais and Brody, 1975; Berecek and Bohr, 1977), although other workers have reported no such differences (Beilin et al., 1970; Haeusler and Haefely, 1970; Haeusler and Finch, 1972). Consequently, the effect of DOCA/salt hypertension on resting vascular resistance in the perfused rat tail artery was investigated. (Hinke (1965) reported that the media of tail arteries from 6-13 week DOCA/salt rats was

thickened, so this study examined arterial dimensions from 3 week DOCA/salt rats.

It should be noted that changes in activity and function of sympathetic nerves, so called neurogenic changes, have been reported in DOCA/salt hypertension. de Champlain, Mueller and Axelrod (1969) reported an increased turnover of cardiac NA; de Champlain, Farley, Cousineau and Ameringen (1976) reported an increased level of circulating NA in plasma from anaesthetized hypertensive rats; and de Champlain, Krakoff and Axelrod (1968) suggested that there is a decreased retention and storage of endogenous NA in sympathetic nerve terminals of DOCA/salt rats, a finding consistent with an enhanced release of NA from sympathetic nerve terminals. As well as studying the efflux of ^{3}H-NA from DOCA/salt arteries, the present study also examined the catecholamine levels in the arteries. It was hoped that, by analyzing the possible influence of neuronal uptake on the sensitivity of DOCA/salt arteries to NA, the study would provide a further indication of the role which changes in sympathetic nerve activity may play in the DOCA/salt hypertension.

METHODS

Perfusion

Single and double cannulated arteries were perfused as described in Chapter 2.

The slopes of the dose response curves from control and DOCA/salt arteries to NA were measured by a method similar to that described by Langer and Trendelenburg (1969). Concentrations of NA required to produce increases in perfusion pressure of 150 mm Hg ($[NA]_{150}$) and 50 mm Hg ($[NA]_{50}$) were determined and the slopes calculated as:

$$\frac{150-50}{\log[NA]_{150}-\log[NA]_{50}}$$

Generation of DOCA/Salt Hypertension

Young male rats, weighing about 120 gm, were anaesthetized with pentabarbitone and their left kidneys removed. Ten days later, DOCA/salt treatment was commenced and continued for 3 weeks. The rats were given DOCA (20 $mg.Kg^{-1}$) subcutaneously twice a week and an unrestricted access to 0.9% saline drinking water. A second group of rats, to serve as age matched controls, were sham operated and then given a normal food and water diet. Results from three groups of DOCA/salt and age matched control rats are presented in this chapter, and are designated series 1, 2 and 3.

Determination of Blood Pressure

Either once or twice a week, starting just prior to DOCA/salt treatment, the rats' blood pressures were determined, indirectly, using a tail cuff and a Doppler flow meter (Parks Electronics) as described by Rowberg et al. (1969). Heart weights were recorded at the time of sacrifice.

Measurement of Arterial Dimensions

Segments of tail artery from series 1 and 2 rats were removed at sacrifice and put into formal saline. These tissues were processed as described in Chapter 2 and stained with haematoxylin and eosin. Sections of arteries were photographed through a Leitz microscope and printed on photographic paper using a standard enlarger setting. The internal and external circumferences of the photographed arteries were measured using a Curvimeter map reading device. The internal (r_i) and external (r_o) radii were calculated from the formula $C = 2\pi r$. The thickness of the media (TM), where $TM = r_o - r_i$, the ratio TM/r_i, and the area of the media, $Aw = \pi(r_o^2 - r_i^2)$, were also calculated.

Raised Perfusion Pressures

The effects of an increased resting perfusion pressure of 5 mm Hg were calculated from data from control arteries from series 1. This procedure was executed by considering an actual rise in perfusion pressure of 5 mm Hg to be the new resting perfusion pressure and then reading off, from the original dose response curves, doses that would produce increases in perfusion pressure of 150, 100, 50, 20, 10 and 5 mm Hg (i.e., those doses that actually produced responses of 155, 105, etc., mm Hg in the arteries). Dose ratios of the original and new values were then calculated.

Efflux Studies

Tail arteries from series 2 and 3 rats were incubated in ^{3}H-NA solution and the efflux and retention of ^{3}H was measured. The experimental procedures are illustrated in Fig. 6.1. A standard length of caudal artery (7 cm) was removed from the tail, weighed, and pre-incubated in 5 ml of Krebs solution for 30 min. All solutions were bubbled with 95% O_2 + 5% CO_2 and warmed to 37^{o}C. Arteries were incubated for 30 min in ^{3}H-NA (0.5 μmol.l^{-1}), put through four 10 min washes in 10 ml of Krebs solution, a 5 min wash in 1 ml of Krebs, incubated in 1 ml of Krebs containing an additional 54 mmol.l^{-1} KCl for 5 min, and finally washed twice more for 5 min periods in 1 ml of Krebs. After washing, the arteries were put into 0.1 M HCl overnight to extract catecholamines. Krebs solution contained EDTA (10.8 μmol.l^{-1}) and ascorbic acid (290 μmol.l^{-1}). Samples were added to 10 ml of scintillation fluid (toluene triton, POP and POPOP) and counted in a Packard Model 3310 liquid scintillation spectrometer.

Series 2 arteries were incubated in ^{3}H-NA labelled on the 7,8,-positions of the side chain, and series 3 arteries were incubated in 2,5,6,-ring labelled NA.

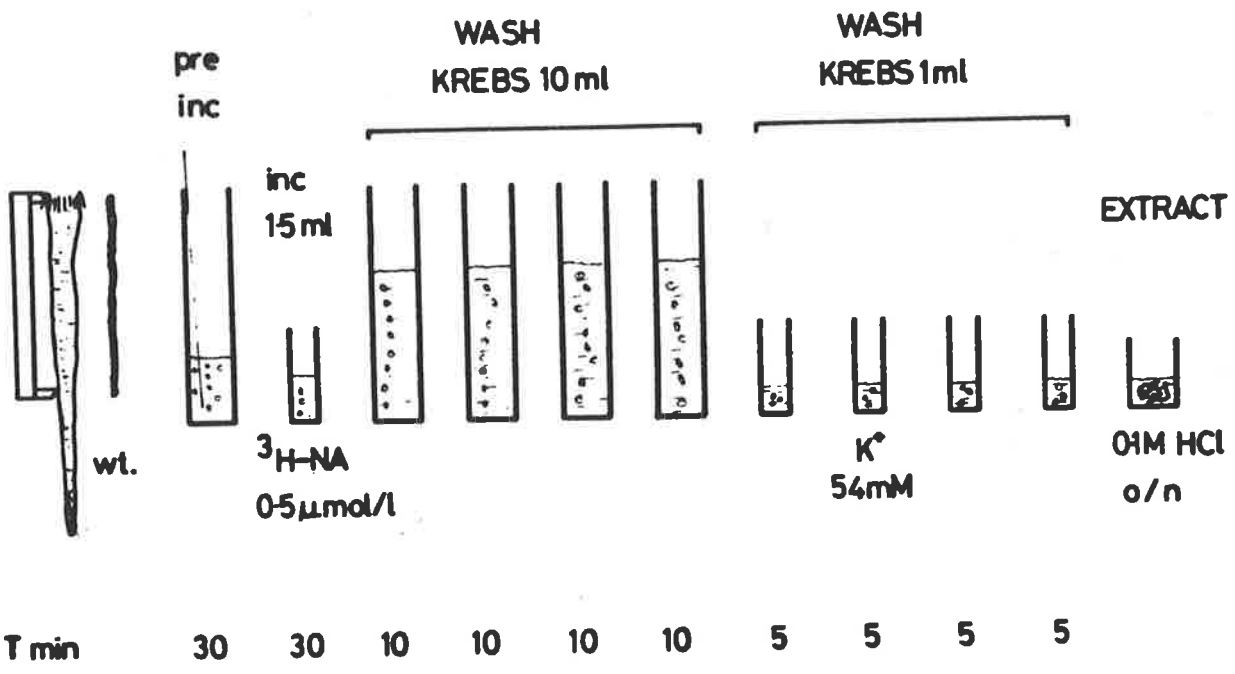

Figure 6.1

A diagrammatic representation of the procedures used to study the efflux of tritium from standard lengths of rat tail artery incubated in tritiated NA solution.

Catecholamine Contents

Amounts of NA, A and dopamine (DA) present in tail arteries from control and hypertensive rats were measured using the radio enzymatic method of da Prada and Zürcher (1976).

Metabolism Studies

Parallel studies on catecholamine metabolite changes in DOCA/salt arteries were carried out during the course of these studies and will be referred to.

RESULTS

General Data

Table 6.1 summarizes a variety of data collected from control and DOCA/salt rats. Mean blood pressures of DOCA/salt rats were higher than of controls as were heart weights and perfused artery resistances. Body weights of DOCA/salt rats were lower than those of controls. In series 1 and 3 there was no difference in weights of a standard length (7 cm) of artery, and in series 2 artery weights from DOCA/salt rats were lower than those from controls. There were no differences in arterial contents of A, NA and DA between DOCA/salt and control rats, except in series 2 where DA was lower in arteries from DOCA/salt rats.

Arterial Dimensions

The only difference seen in arterial dimensions of arteries from DOCA/salt and control rats was that the area of the media of DOCA/salt arteries was smaller than that from controls (Table 6.2). This difference was only seen in artery segments immediately adjacent to the proximal perfused segment and not in segments of artery removed 7 cm further down

Table 6.1

Means ± standard errors of parameters stated above for control and DOCA/salt (3-week treatment) rats from three series of experiments. Numbers in brackets refer to the number of observations from which each mean was derived.

	Series 1		Series 2		Series 3	
	Control	DOCA/Salt	Control	DOCA/Salt	Control	DOCA/Salt
Blood Pressure mm Hg	136±2.8 (7)	196±5.7 *** (7)	117±2.2 (17)	168± 3.7 *** (21)	142± 3.7 (9)	206± 6.4 *** (9)
Heart Weight $g.Kg^{-1}$	3.16±0.05 (7)	5.02±0.1 *** (7)	3.44±0.14 (16)	4.53± 0.13 *** (21)	3.02± 0.09 (9)	4.51± 0.18 *** (9)
Body Weight g	292±7.2 (7)	222±7.1 *** (7)	277±8 (11)	227±10 ** (12)	296±10.2 (9)	254±11.2 * (9)
Artery Resistance mm Hg	7.4 ±0.9 (7)	15±2.1 ** (8)	7±0.4 (6)	12.5 ± 0.7 *** (8)	-	-
Artery Weight $mg.7cm^{-1}$	12.8 ±0.63 (7)	11.2 ±0.66 (6)	12.6 ±0.4 (11)	10.9 ± 0.4 ** (12)	11.1 ± 0.5 (9)	11.7 ± 0.44 (9)
Catecholamine Content $nmol.g^{-1}$						
NA	13.1 ±1.9 (7)	12.3 ±2.8 (6)	33.2 ±4 (6)	38.2 ± 4.4 (6)	22.9 ± 2 (11)	22.8 ± 1.8 (10)
A	0.1 ±0.05 (7)	0.04±0.02 (7)	0.30±0.05 (6)	0.39± 0.13 (6)	0.24± 0.05 (11)	0.25± 0.05 (10)
DA	2.4 ±0.3 (7)	2.2 ±0.6 (7)	0.48±0.07 (6)	0.30± 0.05 * (6)	0.38± 0.08 (11)	0.36± 0.05 (10)

* $p<0.05$. ** $p<0.01$. *** $p<0.001$.

Results of unpaired t-tests between control and DOCA/salt groups within each treatment group.

Note: Catecholamine contents for series 3 were taken from a subsequent series of experiments and were not data from the same arteries that the other series 3 data came from.

Table 6.2

A collection of arterial dimensions derived from measurements of the circumferences of arteries from control and DOCA/salt treated rats. Ri, internal radius; TM, media thickness; Aw, area of the media. The top panel includes values obtained from pieces of artery immediately adjacent to the perfused segment, and the bottom panel includes values from pieces of artery taken 7 cm distal to the perfused segment.

Arterial Dimensions

	DOCA/Salt	Control
Ri (μ)	89.6±5.3	102±6.2
TM (μ)	73.1±3.0	79.6±5.2
TM/Ri	0.86±0.06	0.86±0.11
Aw (μ^2)	58135±3438 n=17	70147±4529 * n=14

Adjacent to perfused segment.

	DOCA/Salt	Control
Ri	90.4±9.5	77±5.6
TM	75.6±4.9	78.6±5.7
TM/Ri	0.96±0.14	1.07±0.10
Aw	60278±4914 n=11	58766±6991 n=10

7 cm Distal to perfused segment.

* $p<0.05$ (unpaired t-test between control and treatment groups)

the tail.

Perfused Arteries

Arteries from both control and DOCA/salt rats were more sensitive (approximately 10 fold) to NA applied IL than to NA applied EL. However, the arteries from the DOCA/salt rats were more sensitive to NA than those from control rats (Fig. 6.2). The difference tended to be greater at higher levels of response, particularly in the case of IL NA (Table 6.3). The increased sensitivity of the arteries from DOCA/salt rats was significant at the lowest response levels (5 mm Hg), indicating that the threshold of sensitivity was lower in these arteries.

In both groups, cocaine caused a marked increase in the sensitivity to EL NA and a much smaller increase in the sensitivity to IL NA (Table 6.4). The nett effect was that the differences between IL and EL NA sensitivity became insignificant. Neither the magnitudes of cocaine's actions on IL and EL NA, nor the ratio of the two sensitivities in the presence of cocaine were significantly different between the DOCA/salt and control groups. Hence, in the presence of cocaine, the sensitivities of the arteries from DOCA/salt rats remained greater than those of arteries from control rats. It will be noted, however, from Table 6.3, that the difference between sensitivities to EL NA was now more than two times greater at higher response levels than at lower response levels, as was previously seen with IL NA in control arteries. There was no indication that the relative sensitivities to NA, or the action of cocaine, was influenced by the level of perfusion pressure increase at which the sensitivities were determined. In both the presence and absence of cocaine, the slopes of only the IL dose response curves to NA for DOCA/salt arteries were significantly steeper than those from controls.

DOCA/salt arteries were more sensitive to K^+ than were arteries from

control rats by 1.2-1.4 fold. This sensitivity difference was apparent at both low and high response levels (Fig. 6.3, Table 6.5). When phentolamine was present to block the constrictor effects of any endogenously released NA, the dose response curves from both groups of arteries were shifted to the right and the slopes reduced, but the sensitivity difference between the DOCA/salt and control arteries still remained the same.

Fig. 6.4 shows constrictor responses of perfused arteries to graded frequencies of electrical pulses stimulating their sympathetic nerves. Although there is no difference between DOCA/salt and control arteries at low threshold and low response levels, there is a pronounced and significant difference at higher frequencies (3.8 fold) which approached the differences observed with exogenous NA, and much greater than those seen with K^+ (Table 6.5).

Sensitivity ratios of arteries with an increased resting perfusion pressure of 5 mm Hg induced by NA, compared with a normal resting perfusion pressure, are also shown in Table 6.5. The sensitivity increase due to a raised resting perfusion pressure is small, ranging from 1.96 at low response levels to 1.04 at higher levels of response.

Efflux Studies

In experiments using NA labelled in the 7,8,-position of the side chain, DOCA/salt arteries took up more ^3H than control arteries and showed a greater release of ^3H (Fig. 6.5). These differences, however, were not observed when 2,5,6,-ring labelled ^3H-NA was used. K^+ increased the release of ^3H in both control and DOCA/salt vessels with both types of ^3H-NA ($p<0.05$, unpaired t-test). However, the incremental release of ^3H, i.e., that ^3H released over and above the expected resting release, was not significantly different for either treatment group.

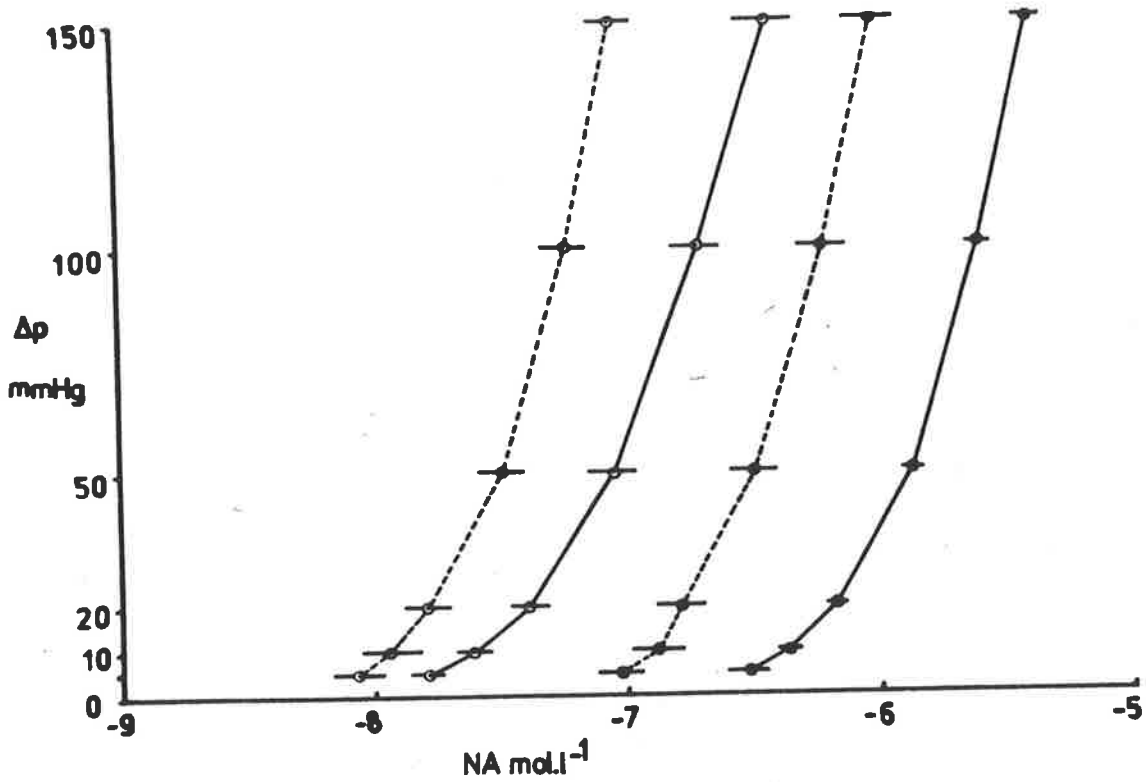

Figure 6.2

Concentration response curves for extraluminal NA (closed circles) and intraluminal NA (open circles) on arteries from control rats (solid lines) and arteries from DOCA/saline rats (broken lines). Each point represents the geometric mean and standard error derived from 4-8 observations (see Table 6.3) for numbers of arteries). For both EL and IL plots the points for arteries from DOCA/salt rats were shifted significantly to the left compared with those for arteries from control rats (unpaired t-test, $p<0.05$). There is a significant increase in slope for the DOCA/salt IL curve compared with the IL control curve (unpaired t-test, $p<0.05$, 13 d.f.).

Table 6.3

Ratios of geometric means of doses of NA - $\frac{\text{control}}{\text{DOCA/saline}}$ - that produced increases in perfusion pressure, ΔP. Smaller numbers refer to numbers of arteries from which the means were derived (control on the left and DOCA/salt on the right). Results of unpaired t-tests between control and DOCA/saline groups are indicated. Cocaine was present at a concentration of 2.9×10^{-5} $mol.l^{-1}$.

ΔP mm Hg	EL	IL	Cocaine EL	Cocaine IL
150	4.1 *** 7 8	4.0 ** 7 8	7.3 * 4 7	7.5 * 4 7
100	4.1 *** 7 8	3.3 ** 7 8	6.6 * 4 7	6.4 * 4 7
50	4.3 *** 7 8	2.8 ** 7 8	5.6 ** 4 7	6.4 * 4 7
20	4.1 *** 7 8	2.6 * 7 8	3.7 * 4 7	4.1 a 4 7
10	3.3 *** 7 5	2.2 * 7 6	4.0 ** 5 5	4.4 * 5 6
5	3.2 *** 5 4	1.9 * 7 5	2.6 * 6 2	4.5 a 4 4

*** $p<0.001$

** $p<0.01$

* $p<0.05$

Table 6.4

Dose ratios showing potentiation of NA by cocaine (2.9×10^{-5} $mol.l^{-1}$). The dose ratio is the ratio of doses, shown in the left hand column, that produce increases in perfusion pressure of 100 mm Hg. Values shown are geometric means and standard errors derived from the number of observations shown in brackets. No significant difference between corresponding ratios from control and DOCA/saline rats was found (unpaired t-test).

	Control	DOCA/Saline
EL/IL	12.8 10.6-15.6 (7)	10.3 8.5-12.4 (8)
EL/EL Coc	20.9 13.9-31.4 (4)	32.1 24.2-42.5 (7)
IL/IL Coc	1.9 1.6- 2.3 (4)	2.9 2.4- 3.6 (7)
EL Coc/IL Coc	0.9 0.8- 1.0 (4)	0.9 0.8- 1.0 (7)

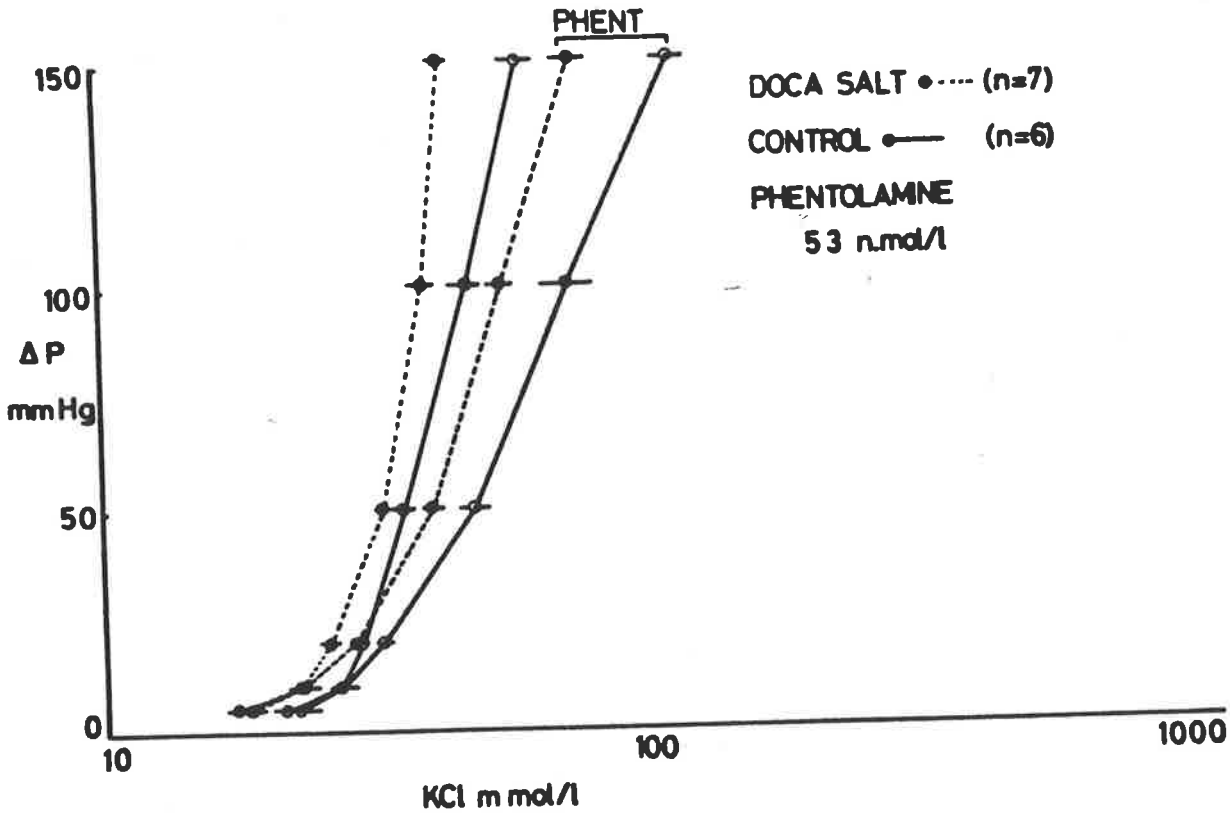

Figure 6.3

Concentration response curves to KCl for single cannulated arteries from control (solid lines) and DOCA/salt (broken lines) treated rats, in the presence and absence of phentolamine (5.3×10^{-8} $mol.l^{-1}$). Each point represents the geometric mean and standard error of n observations. There is a significant difference between the slopes of the DOCA/salt and control curves in both the presence and absence of phentolamine. ($p<0.05$, unpaired t-tests, 10 d.f.)

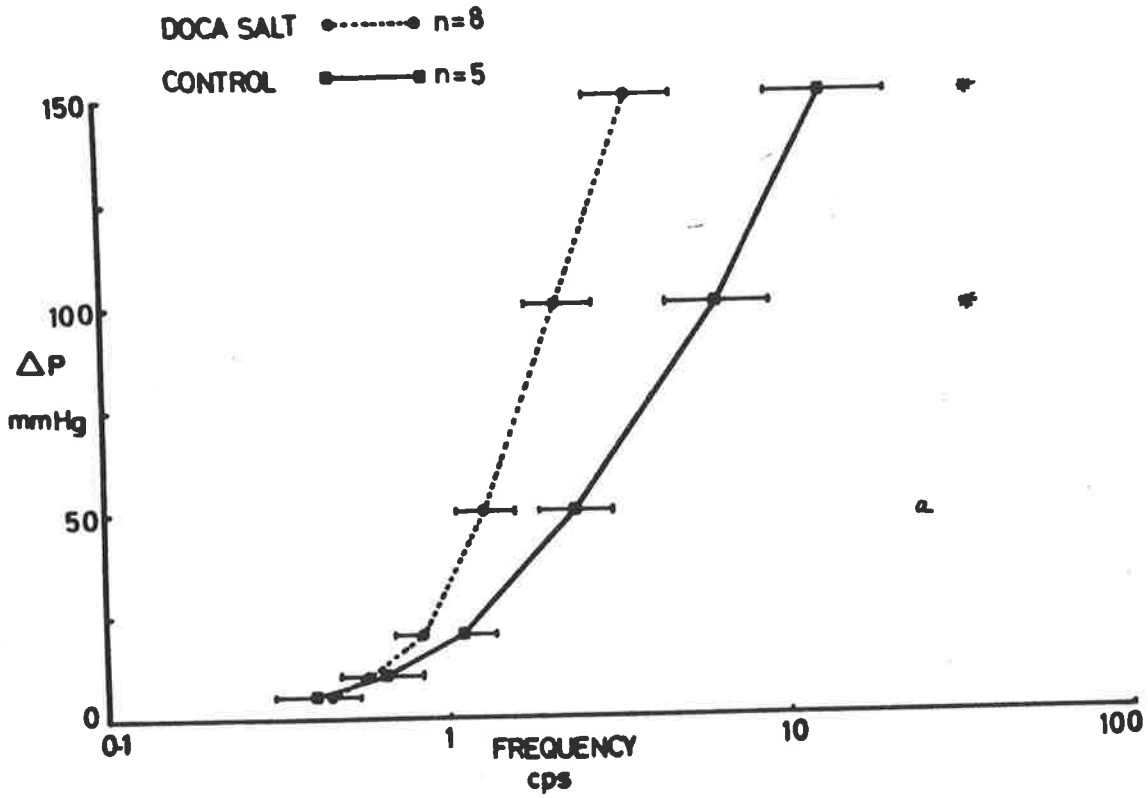

Figure 6.4

Frequency response curves to electrical stimulation for single cannulated arteries from control (solid lines) and DOCA/salt (broken lines) treated rats. Each point represents the geometric mean and standard error of n observations.

* $p<0.05$
(unpaired t-tests between control and treatment groups)
a $p<0.1$

Result of unpaired t-test on slopes of curves; $0.1>p>0.05$ (11 d.f.).

Table 6.5

Table showing the ratios of mean concentrations of NA and KCl, and mean frequencies of electrical stimulation, that produce similar responses (increases in perfusion pressure) in perfused rat tail arteries from control (N) and DOCA/salt (H) treated rats. Phentolamine, when present, was in a concentration of 5.3×10^{-8} $mol.l^{-1}$.

Sensitivity Ratios N/H

						Raised P 5 mm	
mm Hg	NA EL	NA IL	KCl	KCl/Phent	Stim	NA EL	NA IL
150	4.1 *	4.0 *	1.4 *	1.5 *	3.8 *	1.04	0.97
100	4.1 *	3.3 *	1.2 *	1.3 *	2.8 *	1.07	1.04
50	4.3 *	2.8 *	1.1 a	1.2 a	1.8 a	1.15	1.1
20	4.1 *	2.6 *	1.2 *	1.1 *	1.3	1.29	1.23
10	3.3 *	2.2 *	1.2 *	1.2 a	1.1	1.66	1.46
5	3.2 *	1.9 *	1.2 *	1.2 *	0.9	1.84	1.96

* $p<0.05$
(unpaired t-tests between control and treatment groups)
a $p<0.1$

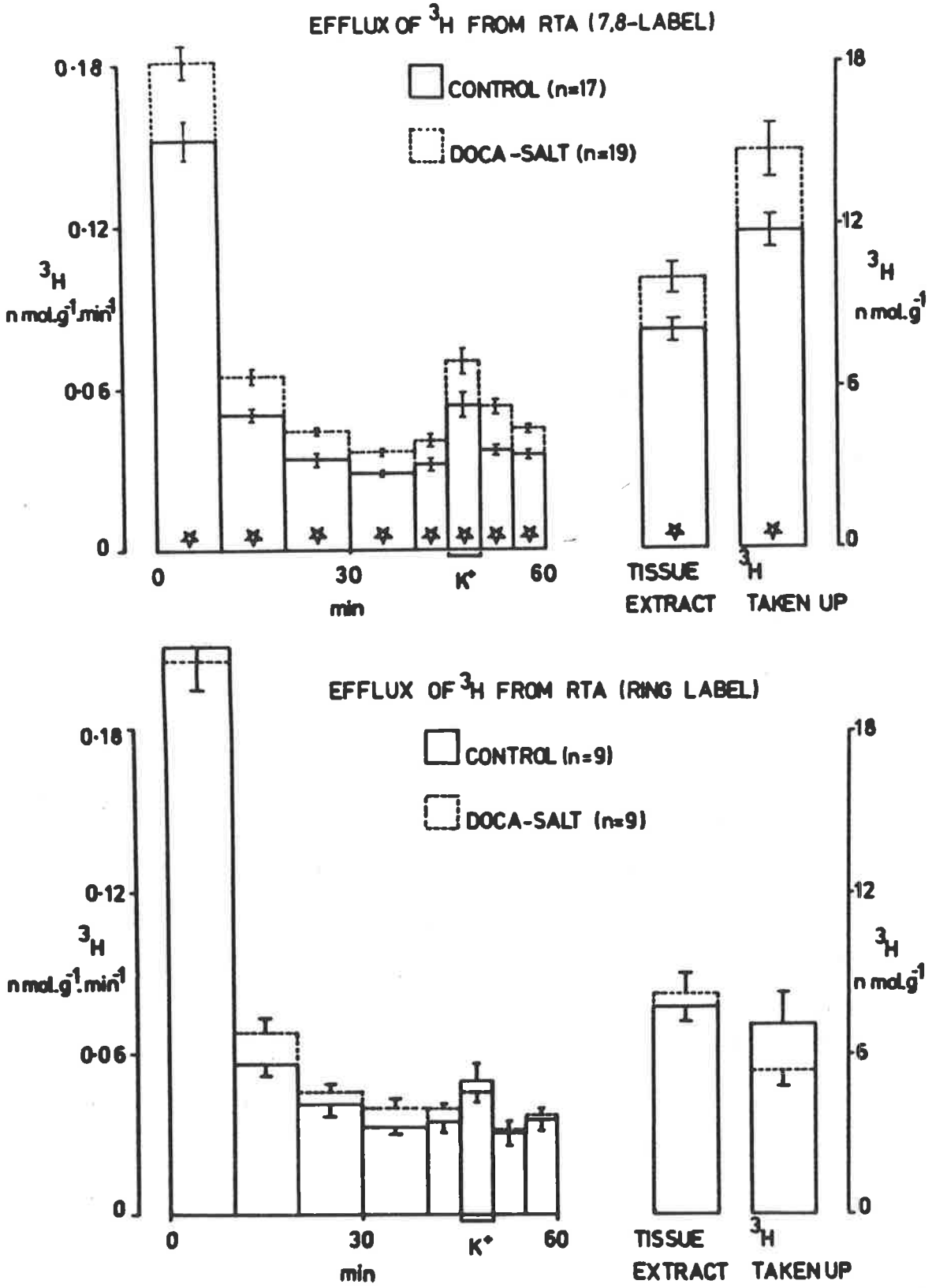

Figure 6.5

Bar diagrams summarizing the results of studies on the efflux of ^{3}H from rat tail arteries incubated in 7,8,-labelled and 2,5,6,-ring labelled ^{3}H-NA solutions and the effect of K^+. Each value represents the mean ± sem of n observations.

☆ $p<0.05$ (unpaired t-test between control and DOCA/salt groups)

DISCUSSION

Exogenous Noradrenaline and Sensitivity

Arteries from DOCA/salt rats were more responsive to NA than arteries from control rats. This raises a number of questions. Is this increased responsiveness due to an increased responsiveness of the effector cells (smooth muscle), or is it mediated by changes in disposition of NA, i.e., by changes in the neuronal or extraneuronal uptake systems? From the evidence presented in this chapter it would seem that it is probably due to a change in the effector cell because the increased responsiveness occurs in cocaine treated arteries and, therefore, changes in neuronal uptake could not be responsible for the observed increase in response. A decrease in extraneuronal uptake of NA cannot account for the increase in sensitivity since the increase is of the order of 3-4 fold, whereas, as shown in Chapter 5, inhibition of extraneuronal uptake, in normotensive vessels, caused a much smaller (1.4 fold) increase in sensitivity.

Is the increase in responsiveness confined to the inner or the outer cells? The results suggest that the changes occurred in both populations of cells, as the sensitivity increases to both IL and EL NA are comparable.

Is the increase in responsiveness specific for NA? The results suggest that it is relatively specific for NA, since although there was a small increase in responsiveness of the DOCA/salt arteries to K^+ (1.1 -1.5 fold), this was much less than that seen with NA (3-4 fold).

Is the increase in responsiveness a change in sensitivity or a change in reactivity? The fact that there is a decrease in threshold to NA in DOCA/salt arteries suggests that there is a true sensitivity change. As the slope of the dose response curve to EL NA is unchanged

by DOCA/salt treatment, it is concluded that there is a true sensitivity increase to EL NA. There was, however, an increase in slope in the dose response curve to IL NA. This indicates that for IL NA there may be changes in both sensitivity and reactivity.

A related question is whether there are morphological changes in the vessel. The only change observed was a tendency of the media to be thinner in the hypertensive vessels. This may be related in some way to the lower body weight of the hypertensive rats. Nevertheless, the increase in resting perfusion pressure remains to be explained.

The relationship between the above findings and relevant results of other investigators (summarized in Table 6.6) will now be considered.

Although the comparative studies after only 3-week DOCA/salt treatment are not reported, the present findings are compatible with the earlier report of Hinke (1965) that, at 6-12 weeks treatment, there was an increased responsiveness to NA (a change in threshold was not specified) that was relatively specific insofar as it did not occur with angiotensin II. They are also compatible with the evidence of Beilin et al. (1970), who reported that, in the whole perfused tail, at a minimum of 4-weeks DOCA/salt treatment, there was an increased responsiveness to NA, specific insofar as there was no change in the reactivity of the tail to vasopressin, with a decreased threshold but no change in resting perfusion pressure or in the wall/lumen ratio. The only evidence of morphological changes is in Hinke's study, who observed in the hypertensive arteries, at 6-8 weeks, a greater lumen diameter but no change in wall/lumen ratio (Table 6.7). However, increases in the latter ratio were observed at 12 weeks. The only evidence of morphological changes observed in this study was the thinner wall thickness mentioned earlier; however, there was no suggestion of an increase in wall/lumen ratio. This may reflect the use of the

Table 6.6

Preparation	Author	Treatment Period (weeks)	Response to NA	Threshold to NA	Reactivity	NA Max Response	Resting BP	Responsiveness to Other Agonists	Morphology	Comments
Rat tail artery	Venning – Current study	3 weeks	↑↑	↓	?	–	↑	K^+ ↑ slight Stim ↑ Slope ↑	Nil	
Rat tail artery	Hinke 1965	6–8 8–13	) ↑)	– –		– –	Resting flow ↓	↑ vasopressin ↑ rat serum Not to AII	Yes Media ↑ TM/Ri	Elasticity during contraction. More elastic at rest.
Perfused rat tail	Beilin, Wade, Honour, Cole 1970	Minimum 4	↑	↓	–	– –	No change at $\frac{1}{2}$ ml. min^{-1}	Vasopressin no change	TM/Ri no change	
Perfused rat tail	Beilin, Ziakus 1972	5 weeks after 7 week DOCA/salt	↑	–	–	Response to large dose NA ↑	↑ at $\frac{1}{2}$,1,2 $ml.min^{-1}$	5-HT ↑	–	BP still ↑
Mesenteric rat	Finch, Haeusler 1974	6					–	Depolarised response to Ca^{++} unchanged	–	
	Finch, Haeusler 1972	6–8	–	–	–	–	Unchanged	↑ 5-HT Max ↑ Threshold ↓	–	NA and K^+ increases same as in genetic hypertension. 5-HT increase > genetic rats.
	Hauesler, Haefley 1970		↑	Unchanged		↑	Unchanged	K^+ ↑ (same as NA)	–	
Hind quarters rat	Finch, Haeusler 1974	6	↑	↓	↑	↑ Slope ↑	–	–		
	Rascher et al. 1980	1	↑							
Femoral rat	Holloway, Bohr 1973	4	↓ A and K^+			?	–	Ad ↑ K ↑ Thresholds ↓	–	Increased lability of smooth muscle cell.

Table 6.7

This table shows arterial dimension parameters and blood pressure values from this study in comparison with similar values from a study by Hinke (1965). Ri, internal radius; TM, thickness of the media. Each value is the mean ± sem of n observations.

	3 Week		6-13 Week	6-8 Week	8-13 Week
	Control	DOCA/Salt	Control	DOCA/Salt	DOCA/Salt
Ri (μ)	102±6.2	90±5.3	37±2.7	52±2.1	37±2.7
TM (μ)	80±5.2	73±3.0	87±3.5	110±5.1	109±2.7
TM/Ri	0.86±0.11	0.86±0.06	2.2±0.16	2.12±0.07	3.0±0.12
BP mm Hg	117±2.2 n=14	168±3.7 * n=17	121±1.6 n=10	157±8.5 * n=6	170±6.2 * n=10
			From Hinke (1965)		

* $p<0.05$ (unpaired t-test between control and treated groups).

shorter DOCA/salt treatment period in the present study.

In other studies, perfused hind-limb and mesenteric vessels, and also mesenteric and femoral strips have been used. In perfused mesenteric vessels, Haeusler and Haefely (1970) and Haeusler and Finch (1972) showed that after 6-8 weeks, there was a non-specific increase in response indicated by an increased responsiveness to NA, 5-HT and K^+. There was a decreased threshold to 5-HT, but no change in the threshold to NA but an increase in the maximum response to NA. It was concluded that a stretching of the artery wall contributed to the increased responsiveness. In the case of the perfused hind-limb, Finch and Haeusler (1974) found an increased response with a decreased threshold, increased slope and maximal response, and also concluded that these reflected an increased reactivity due to an increased wall/lumen ratio (although the latter was not actually mentioned). More recently, Rascher et al. (1980) reported that, even at seven days, before blood pressure had increased, there is an increased responsiveness of the perfused hind-limb to NA. In femoral strips, at four weeks, Holloway and Bohr (1973) showed that the threshold to both K^+ and NA was decreased, and concluded that, in this preparation, smooth muscle cells were more "labile" in response to excitatory agents.

A simple interpretation of these studies would be that, in DOCA/salt hypertension, there is a true decrease in threshold indicating an increased excitability of the smooth muscle cell, and that this can be manifest at a stage where the hypertrophic changes are probably too small to influence the response by increasing vascular reactivity. However, it does seem that by about six weeks morphological changes are sufficiently advanced for the influence of reactivity to predominate.

Furthermore, the present studies, when considered in relation to those of Hinke (1965) and Beilin et al. (1970), suggest that changes

in the rat tail artery may be more specific for NA, compared with those in the mesenteric and femoral arteries.

Neurogenic Factors

The present evidence that the activity of the neuronal uptake system is unaltered in DOCA/salt hypertension is supported by the metabolism studies of Morris and de la Lande (unpublished), who showed that there was no difference between the rates of formation of a metabolite of neuronal origin (DOPEG) in control and DOCA/salt arteries. The findings are also in accord with those of de Champlain et al. (1969) and Giachetti, Rubenstein and Clark (1979), who both reported that neuronal uptake was unchanged in DOCA/salt hypertension in rats. Further evidence against neurogenic changes is provided by the failure to detect changes in endogenous catecholamine contents. However, this may be time related, as Crabb, Head, Hempstead and Berkowitz (1980) reported decreased levels of NA in some vessels (not the tail artery) of the 12-week DOCA/salt hypertensive rat.

It was thought that the efflux studies might indicate whether neurogenic changes occurred in DOCA/salt hypertension. In each of the four comparisons, the resting effluxes of ^{3}H from vessels pre-incubated in ^{3}H-NA tended to be greater from the hypertensive than the normotensive vessels. However, possibly because of the greater numbers involved, the difference was only significantly greater in the three comparisons in which the 7,8, ^{3}H-NA label was used. Morris and de la Lande (unpublished) reported a 30% increase in ^{3}H O-methylated deaminated product after an analysis of the metabolites from the efflux experiments. The increase was only significant in one of the two series analyzed. They concluded that there was no evidence for an association between NA inactivation in the artery and the hypertension induced by

DOCA/salt treatment. The minor and inconsistent changes in the formation of the neuronal deaminated metabolites, and the accumulation of ^{3}H-NA, led them to conclude that decreased neuronal inactivation could not account for the increase in sensitivity of the tail artery to NA in 3-week DOCA/salt rats. de Champlain et al. (1969) reported that the leakage of NA into the neuroplasm appeared to be enhanced in the heart of the DOCA/salt treated rat.

Exogenous Noradrenaline and Sensitivity

K^{+} produced a small, but significantly greater, constrictor response in arteries from DOCA/salt rats. The difference could be due to a greater depolarizing effect on the muscle membrane. K^{+} will also depolarize the nerve terminals. However, it is not necessary to invoke a greater depolarizing effect on the nerve terminals, since the same amount of released transmitter should result in an enhanced response of the hypertensive vessel. These two effects were distinguished in pharmacological experiments by investigating the responses of arteries to K^{+} in phentolamine treated vessels, and in biochemical experiments by examining the effects of K^{+} on the release of ^{3}H from arteries previously incubated with ^{3}H-NA. The sensitivity of both DOCA/salt and control arteries to K^{+} was depressed by phentolamine at higher response levels, but not at threshold, suggesting that low levels of response were a result of K^{+} induced depolarization of the smooth muscle, whereas higher levels of response were due to both muscle depolarization and the effects of K^{+} induced release of endogenous NA. However, the difference between the sensitivities to K^{+} in the hypertensive and normotensive vessels in the presence of phentolamine was comparable to that in the absence of phentolamine (Table 6.5). These effects can be explained as follows. Assuming that the effects seen in phentolamine

treated arteries represented only the direct effects of K^+ on the smooth muscle, the hypertensive vessels were hyper-responsive to K^+. This hyper-responsiveness probably represented both increases in sensitivity (decreased threshold) and reactivity (since the dose response curves of the hypertensive arteries had a steeper slope). Phentolamine's depression of K^+ induced responses reflected the contribution of K^+ induced release of NA from the nerve terminals to the response to K^+. The question of whether this release differed in control and DOCA/salt arteries was examined in the biochemical experiments.

The results showed that the release of ^3H due to K^+, i.e., the incremental release of ^3H over and above the expected resting release, was not significantly different between the control and DOCA/salt arteries. It is concluded, therefore, that hypertension due to DOCA/salt is not associated with an increase in the depolarization induced release of NA. However, the result raises an interesting question. Since the hypertensive arteries were more sensitive to exogenous NA, why were they not more sensitive to the endogenous NA released by K^+? The evidence that they were not is indirect, namely, that phentolamine reduced the sensitivity of the hypertensive and normotensive arteries to K^+ to approximately the same extent. The same question is posed by the observations on electrically stimulated vessels. Here, the difference in sensitivities at higher frequencies approached the differences observed with exogenous NA, and was much greater than that seen with K^+. Although hypertensive vessels were more responsive than control vessels at higher frequencies, there was no difference in response at frequencies close to threshold (Fig. 6.4, Table 6.5).

The work of Hirst and Neild (1980) suggests that the properties of adrenoreceptors in arterioles differ according to whether the receptors are close to the nerve terminals or more distant. On this

basis, the alpha adrenergic receptors in the hypertensive vessels may differ from those in normotensive vessels, either with respect to their affinities for NA or their density. However, this difference may only apply to the "non-innervated" receptors more distant from the nerve terminals (Fig. 6.6). On this basis, the NA released by K^+ may be insufficient to diffuse to more distant receptors. The absence of a difference at low frequency stimulation in the hypertensive and normotensive arteries may reflect the failure of the released NA to diffuse further than the nearby receptors. However, at high frequency stimulation, the transmitter release may overflow to more distant receptors and mimic the effect of exogenous NA. Bevan et al. (1976) have suggested that the increase in contraction of rabbit cephalic and short saphenous veins with rise in carotid artery pressure can be accounted for by an increase in the sensitivity of the alpha adrenergic receptor. An investigation of adrenergic receptor types is presented in Chapter 7.

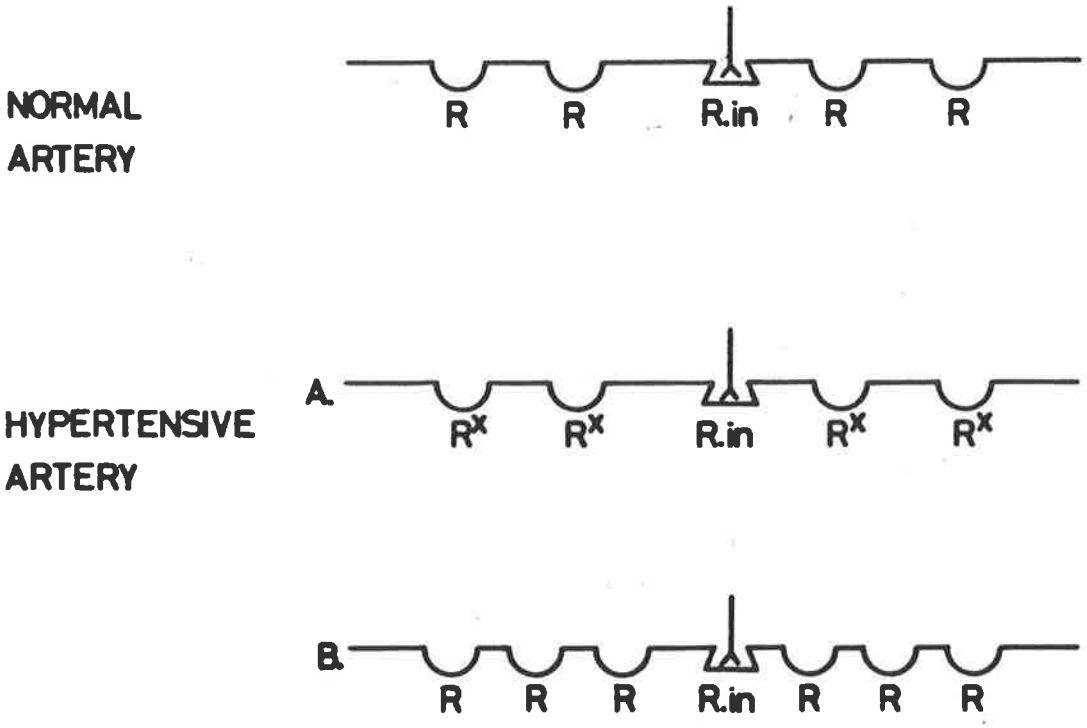

Figure 6.6

Diagrammatic model of some of the changes that may occur in arteries from hypertensive rats. The upper drawing shows "innervated" and "non-innervated" receptors in a normal artery. The lower two drawings depict affinity and density changes in non-innervated receptors in arteries from hypertensive animals.

C H A P T E R 7

INVESTIGATION OF RECEPTOR TYPES

INTRODUCTION

In order to explain the different effects of DOCA/salt on sensitivities of exogenous and endogenous NA, it was suggested in the previous chapter that the post-synaptic alpha receptors mediating responses to endogenous NA may be different from those mediating responses to exogenous NA.

The present study presents a preliminary investigation of this hypothesis. It reports the effects of the highly specific $alpha_1$ receptor antagonist, prazosin, and the relatively specific $alpha_2$ receptor antagonist, yohimbine, on responses of the perfused rat tail artery to exogenous NA and to nerve stimulation.

METHODS

Single cannulated arteries were perfused with Krebs solution at a flow rate of 2 $ml.min^{-1}$ as described in Chapter 2. Cumulative dose response curves were elicited to NA alone and in the presence of increasing concentrations of the antagonists phentolamine, prazosin and yohimbine. Cocaine ($1.5x10^{-5}$ $mol.l^{-1}$) was present in the Krebs solution to inactivate the neuronal uptake mechanism. The effect of the antagonists on the responses of the artery to electrical field stimulation was also determined.

Dose ratios were determined at two response levels (50 and 100 mm Hg) for the effects of a concentration of prazosin ($2.4x10^{-9}$ $mol.l^{-1}$) on the responses of the perfused artery to EL NA and field stimulation. This ratio was the ratio of concentrations of NA or frequencies of electrical stimulation producing the same responses in the presence and absence of antagonist.

From the dose response curves to NA, in the presence and absence of antagonist, K_B, the equilibrium constant of the antagonist was

calculated from the formula, $\log(dr-1) = b.\log[B] + \log \frac{1}{[K_B]}$, where dr is the dose ratio for the antagonist (i.e., the dose of NA in the presence of antagonist producing a response of 150, or 100 mm Hg, divided by the dose of NA producing the same response without antagonist present), and where [B] is the concentration of antagonist (Schild, 1947). K_B was calculated on a programmable Hewlett Packard 25 calculator using a power curve fit programme based on the equation, $\log y = b.\log x + \log a$ ($y = (dr-1)$, $x = [B]$, $a = \frac{1}{[K_B]}$). pA_2 values were then calculated from K_B values ($pA_2 = -\log K_B$ when K_B is expressed as $mol.l^{-1}$).

RESULTS

Values for pA_2 values for phentolamine, prazosin and yohimbine are shown in Table 7.1. There is close agreement between the values for an individual drug whether they were determined at 150 or 100 mm Hg response levels. Dose ratios for prazosin are shown in Table 7.1. The values determined for electrical stimulation are greater than those for exogenous NA. Dose ratios for yohimbine proved to be difficult to determine and so are not tabulated, because yohimbine changed the slope of the frequency response curves. At low concentrations, yohimbine caused a potentiation of the response to electrical stimulation at response levels over 50 mm Hg, and at higher concentrations, yohimbine depressed all levels of response to electrical stimulation.

DISCUSSION

Prazosin is obviously a potent antagonist for exogenously applied NA. The pA_2 values for prazosin (9.2), estimated at 100 and 150 mm Hg, are comparable with the values reported by Davey (1980) in tissues from the dog (femoral artery, 8.48; femoral vein, 8.5; mesenteric artery,

Table 7.1

Means and standard errors of pA_2 values for the antagonists specified. Cocaine present, 1.5×10^{-5} $mol.l^{-1}$.

Noradrenaline was used as an agonist.

	Response Level mm Hg		
	100	150	
Phentolamine	7.79±0.86	7.65±0.61	n=2
Prazosin	9.25±0.07	9.21±0.06	n=4
Yohimbine	6.67±0.07	6.72±0.07	n=7

Geometric means and standard errors of dose ratios for prazosin (2.4×10^{-9} $mol.l^{-1}$) determined at response levels of 100 and 50 mm Hg.

	Response Level mm Hg	
	100	50
Noradrenaline	4.4 * 3.2- 6.2 n=6	5.0 * 3.5- 7.0 n=6
Electrical Stimulation	9.0 6.7-12.1 n=6	11.3 8.7-14.7 n=6

* $p<0.05$ (paired t-test)

8.60). Yohimbine, a relatively specific alpha$_2$ receptor antagonist, had a lower pA$_2$ value for exogenous NA than did prazosin. The pA$_2$ values for yohimbine (6.7) and phentolamine (7.7-7.8) correspond to their reported pA$_2$ values for post-synaptic alpha receptors (yohimbine, 6.4; phentolamine, 7.7) in the rat anococcygeus muscle with NA as an agonist (Doxey, Smith and Walker, 1977). Both yohimbine and phentolamine are more active at pre-synaptic alpha receptors than at post-synaptic alpha receptors (Doxey et al., 1977). However, yohimbine, at a concentration approximating its K_D for exogenous NA, caused a potentiation of the response to endogenous NA released by electrical stimulation. This suggests that in the rat tail artery, there are alpha$_2$ receptors present, located pre-synaptically. The potentiation of responses by yohimbine was probably related to this drug's ability to antagonise pre-synaptic alpha$_2$ receptors, resulting in an increased release of endogenous NA in response to nerve stimulation (Cambridge, Davey and Massingham, 1977). Although a pA$_2$ value for yohimbine for pre-synaptic alpha$_2$ receptors in response to electrical stimulation was not determined, there seems little reason to doubt that, at levels of response of 50 and 100 mm Hg, this response was mediated by alpha$_1$ receptors, as the dose ratio of prazosin was even greater against responses to electrical stimulation than against responses to exogenous NA. There are two possible explanations for this last observation. Firstly, the amount of NA released by electrical stimulation may not be linearly related to the frequency of stimulation causing the release. Secondly, the density, or affinity for NA, of alpha$_1$ receptors may be greater in the vicinity of the synapse than on areas of smooth muscle more distant from the synapse. The high pA$_2$ value for prazosin suggests that post-synaptic alpha$_2$ receptors, however, are not involved in the initiation of contractions of the smooth muscle of the rat tail artery in accord

with the latter explanation. There is evidence, however, for such post-synaptic alpha$_2$ receptors in other vascular beds (Jauernig, Moulds and Shaw, 1978; Drew and Whiting, 1979; Davey, 1980; Docherty and McGrath, 1980; Langer, Massingham and Shepperson, 1980).

The results of this brief investigation of receptor characteristics in the rat tail artery imply that post-synaptic alpha$_1$ receptors mediate the responses to both endogenous and exogenous NA. In normotensive vessels, post-synaptic alpha$_2$ receptors do not appear to be involved in the mediation of responses to either endogenous or exogenous NA. However, the situation in DOCA/salt hypertensive vessels still remains to be investigated.

C H A P T E R 8

GENERAL DISCUSSION

The majority of the studies reported in this thesis used the double cannulated perfused artery technique to analyse the effect of the surface of entry of agonist into the artery, as used by de la Lande et al. (1966) with the rabbit ear artery, and subsequently by Nicholas (1969) with the rat tail artery. From Kalsner's (1972) evidence on the rabbit ear artery, it seemed that this approach would enable drug effects on the inner and outer cells of the artery to be distinguished.

Using this technique, it was shown, in Chapter 3, that the surface of entry (inner or outer) to the artery influenced the response to NA in the same way as in the rabbit ear artery (de la Lande et al., 1966). Hence, the same model (see Fig. 3.7) was used to interpret the effects on the rat tail artery, i.e., the difference in sensitivity of the artery to EL and IL NA was explained in terms of the location of the sympathetic nerves at the medial-adventitial border. As already discussed in detail in Chapters 3 and 4, this model explains most of the features of the actions of NA, A and methoxamine on the rat tail artery, including the effect of cocaine on the sensitivity to NA and the smaller effect that it exerts on the sensitivity of the artery to A, as well as its lack of potentiating effect on the sensitivity to methoxamine. This is in accord with the findings of de la Lande et al. (1966, 1967, 1970) on the rabbit ear artery.

Cocaine had a greater sensitizing effect on IL A than it did on IL NA (3.5 for A, and 2.6 for NA), and this is not immediately compatible with the evidence of Iversen (1967) that NA has a higher affinity for neuronal uptake than does A. In fact, these results are difficult to interpret without invoking an additional sensitizing effect of cocaine which is not mediated by inhibition of neuronal uptake. As discussed in Chapter 3, a post-junctional sensitizing action may conceivably arise from a weak inhibitory effect of cocaine on

extraneuronal uptake (Iversen, 1967; Kalsner, 1969), or an increase in intracellular mobilization of Ca^{++} (Greenburg and Innes, 1976). It is also conceivable that the endothelial cells of the artery possess a cocaine sensitive amine transport system similar to that of the endothelial cells in the lung (Gillis, 1976). However, if cocaine did possess a post-junctional sensitizing effect, it would be expected that it should be demonstrable with methoxamine. Apart from a small (1.07 fold) effect in augmenting steady state responses, an effect of sufficient magnitude to explain the results with IL NA and A was not observed.

The possibility of a small extraneuronal action of cocaine may also be considered in relation to the effects of methoxamine on arteries from rats pretreated with reserpine. Methoxamine was more potent EL in reserpinized arteries. As mentioned in Chapter 4, it is possible that 24-hour reserpinization caused the development of a post-junctional supersensitivity which was restricted to the outer cells of the artery. The action of cocaine on reserpinized arteries remains puzzling and is an area in which further experimentation is required to elucidate the interaction.

Extraneuronal inactivation appeared to play a minor role in determining the sensitivity of the rat tail artery to NA and A. In Chapter 5, it was established that inhibition of extraneuronal uptake increased the sensitivity to IL A by a factor of only 1.3 fold. The effect on EL A was even less (1.1 fold). Despite the small magnitude of the changes, it was possible to show that sensitization due to inhibition of COMT did not occur after extraneuronal uptake was inhibited with DOCA. This indicated that the extraneuronal inactivation was in accord with the model of Trendelenburg (1977), namely, uptake into a steroid sensitive compartment followed by O-methylation.

Wade and Beilin (1970), on the basis of propranolol's small sensitizing effect on NA and A in the perfused rat tail, had concluded that the adrenoreceptors in the rat tail artery were predominantly of the alpha type. The present study did not include pharmacological investigations of isoprenaline, because, in preliminary experiments, it proved difficult to demonstrate isoprenaline induced relaxation of the vessel, except, to a small extent, at high concentrations (µg concentrations). It is of interest that the low activity of the steroid sensitive O-methylating system for NA and A in the rat tail artery, as deduced from this pharmacological study, does not appear to extend to isoprenaline. de la Lande (1981) has reported that the rates of steroid sensitive O-methylation in the rat tail artery were surprisingly high and comparable with those of the rabbit ear artery. As considered in Chapter 5, the difference between the pharmacological sensitivity of the rat tail artery and the rabbit ear artery to inhibition of O-methylation may reflect either a low affinity of NA and A, compared with isoprenaline for the extraneuronal O-methylation, or may be related to differences in wall thickness, and the much smaller diffusional pathway in the thinner walled rat tail artery (Guimaraes et al., 1975).

In general, these results confirm and extend the conclusions of Wyse (1976), based on the relaxation properties of oil immersed artery strips, that neuronal inactivation of NA predominated in this vessel (as discussed fully in the Introduction of this thesis). Wyse had not reported his finding when this study was carried out. Other evidence favouring the importance of neuronal inactivation in this vessel is that of Morris, Pater and de la Lande (1979), who found that the major metabolites of NA in the vessel were the deaminated metabolites (DOPEG 40%, and DOMA 19%). They showed that these metabolites were mainly

neuronal in origin. In contrast, normetanephrine represented only 6% of the total metabolites; nevertheless, its formation was inhibited by the presence of DOCA. Hence, their metabolite data emphasized the relative unimportance of steroid sensitive extraneuronal inactivation in the metabolism of NA.

One of the gaps in the present study is that the role of neuronal and extraneuronal MAO in the sensitivity of the artery to catecholamines has not been investigated. In one sense, the effects of neuronal MAO are indirectly measured by the effects of cocaine, since the latter, by inhibiting neuronal uptake, will automatically prevent access of substrate to the intraneuronal enzyme. However, there is no certainty that a corticosteroid may have prevented access of the substrate (NA or A) to the extraneuronal MAO. Nevertheless, the absence of information on the effects of MAO inhibition is probably not a serious defect in view of Wyse's (1976) study, in which he reported that the effects of inhibiting MAO were small, compared with those of inhibiting neuronal uptake, in prolonging the relaxation time of oil immersed strips of rat tail artery contracted with endogenous or exogenous NA.

As outlined in the Introduction, there is evidence that when an agonist is applied to one surface of the artery, there is a declining concentration of amine from its surface of entry to the opposite surface. Some evidence for a declining concentration (i.e., a non-uniform distribution across the artery wall) in the rat tail artery was provided by the studies in Chapter 5. Cocaine treated arteries, during a steady state response to IL amine only, constricted further when the amine was applied in the same concentration to the adventitia, or when the external Krebs bathing solution was replaced with oil. The augmentation of steady state response to IL A amounted to a potentiation of 2.7 fold with EL A, and 1.9 fold with oil. The augmentation of the

plateau of the response occurred rapidly with EL amine but was slow with oil (Fig. 5.6). However, when the oil was replaced with amine free Krebs, the response rapidly returned to the previous steady state response level to IL A alone. This strongly suggests that the augmented response was primarily due to amine which had accumulated in the outer region of the vessel wall, so that when the oil was replaced with Krebs solution, the amine was near the adventitial surface, from which it could efflux rapidly. These findings indicate that, in the rat tail artery, as in the rabbit ear artery (de la Lande et al., 1980), a non-uniform gradient of concentration of amine occurs at steady response when the amine is applied to only one surface of the vessel. Hence, they support the assumption that the response to EL and IL agonists, in the rat tail artery, is mediated by the smooth muscle cells closest to the surface of entry of the agonist. The difference in these responses provide a measure of the difference between sensitivities of inner and outer cells in the vessel wall.

The results from Chapter 5 also suggest that extraneuronal inactivation made little contribution to this gradient. This is because the augmenting effects with oil were increased only to a small extent when extraneuronal uptake was inhibited by DOCA. This is different to the situation in the rabbit ear artery, where DOCA has a marked effect, in the sense that it was not possible to achieve a steady state response to oil in cocaine treated vessels in the absence of DOCA (de la Lande et al., 1980).

As reported in Chapter 6, arteries from three week treated DOCA/salt hypertensive rats were more responsive to IL and EL NA than those from normotensive rats. The increased responsiveness appeared to be post-junctional, since it was undiminished in the presence of cocaine. It was concluded that the increased responsiveness to EL NA

was a true increase in sensitivity, since the dose response curve was shifted in a parallel fashion to the left. However, the increased slope to IL NA in the hypertensive vessels suggested that the increased responsiveness to IL NA may have included a reactivity component. This is the only hint seen in the present study that the properties of the smooth muscle cells in the inner and outer regions of the vessel may differ.

It is not known whether in this vessel the maximum responses to EL and IL NA may differ. Here, it should be noted that, while the perfused vessel permits a relatively simple analysis of the effects of surface of entry of agonists on their vascular effects, it is difficult to determine the maximum response, as in the case with artery strip preparations. Furthermore, the resistance to flow provides only an indirect measurement of changes in tension and length in the smooth muscle cells. To overcome these defects, it would be desirable to extend the present study to an analysis of the responsiveness of the artery ring preparations, or strips, coated on one surface with vaseline, as applied to the rabbit aorta by Pascual and Bevan (1979). There is already preliminary evidence that, in the rabbit ear artery, the maximum response to NA applied to the intima is less than to NA applied to the adventitial surface (McCalden and Bevan, 1980).

The relationship between hypertension and sensitivity to NA, observed in these studies, was compared with findings of other investigators in the discussion of Chapter 6. It was concluded that the more clear-cut evidence of sensitivity changes in the vessel in this study may have been due to the shorter DOCA/salt pretreatment time of three weeks; most earlier studies employed 6-12 week treatment periods, in which time irreversible structural changes leading to an increased wall/lumen ratio had occurred (Folkow, 1978).

The remainder of the study was concerned with the apparent differences in responsiveness of the hypertensive and normotensive vessels to exogenous and endogenous NA. The difference was deduced from the effects of K^+ and nerve stimulation. Here, the hypothesis was considered that DOCA/salt hypertension may have been associated with a change in the properties of the alpha receptors of those smooth muscle cells not immediately adjacent to the nerve terminals. This hypothesis was advanced in order to explain the absence of an increased responsiveness of the hypertensive vessels to low frequency nerve stimulation. An attempt was made, in Chapter 7, to assess whether, in normotensive vessels, the alpha receptors responding to nerve stimulation (endogenous NA) differed in their affinity for prazosin from the alpha receptors responding to exogenous NA. The results offered no evidence in support of the hypothesis, in that prazosin proved to be a potent antagonist of both exogenous NA and nerve stimulation at all levels of response. This result implied that the effects of nerve stimulation, like those of exogenous NA, are both mediated by the post-synaptic $alpha_1$ receptors in the vessel. However, it would be worthwhile to establish whether this is also the case in hypertensive vessels.

A P P E N D I X

Krebs Bicarbonate Solution

The composition of the Krebs solution used throughout the study was:

	$mmol.l^{-1}$
NaCl	120.0
KCl	3.9
$CaCl_2$	2.5
$MgCl_2$	1.1
$NaHCO_3$	25.0
KH_2PO_4	1.0
Glucose	5.5
EDTA	0.01

All compounds, except $MgCl_2$, $CaCl_2$ and EDTA (ethylenediamine tetra-acetic acid), were dissolved in the required volume of distilled deionized water. $CaCl_2$ and $MgCl_2$ were added from standardized 10% stock solutions. EDTA was added from a 30 µM stock solution. The Krebs solution was filtered before use and gassed with 95% O_2 and 5% CO_2. The pH of the gassed solution was 7.4.

Liquid Scintillation Spectrometry

Radio-activity was counted using a Packard Model 3310 Liquid Scintillation Spectrometer. The scintillation fluid had the following composition ($g.lit^{-1}$): PPO (2,5-diphenyloxazole) 8.25; POPOP (1,4-di(2[5-phenyl-oxazolyl])benzene) 0.25. The PPO and POPOP were dissolved in one litre of toluene to which was added 500 ml of Triton X-100.

Drugs

The following drugs were used:

l-adrenaline bitartrate	Koch Light Laboratories/Sigma

cocaine hydrochloride	MacFarlane Smith
DOCA (4-pregnen-21-al-3, 20-dione acetate	Koch Light Laboratories
Methoxamine HCl	Burroughs-Wellcome
l-noradrenaline bitartrate	Koch Light Laboratories/Sigma
^{3}H-7,8,-C l-noradrenaline HCl	Radiochemical Centre, Amersham
^{3}H-2,5,6,-C l-noradrenaline HCL	New England Nuclear
pentobarbitone (Nembutal)	Abbott
phentolamine mesylate (Regitine)	Ciba
prazosin HCl	Pfizer
reserpine (Serpasil)	Ciba
U0521 (3',4'-dihydroxy-2-methyl propiophenone)	Upjohn
Yohimbine HCl	Sigma

Preparation of Drugs

Catecholamines and U0521 were prepared in 0.9% saline containing ascorbic acid (0.57 mmol.l^{-1}).

DOCA was prepared as a stock solution of 67 mmol.l^{-1} in ethanol.

All other drugs were prepared in 0.9% saline.

Concentrations of adrenaline, noradrenaline, phentolamine and reserpine refer to the bases. Concentrations of all other drugs refer to the salts.

A B B R E V I A T I O N S A N D S Y M B O L S

NA	noradrenaline
A	adrenaline
IL	intraluminal
EL	extraluminal
mm Hg	millimetres of mercury
DOCA	deoxycorticosterone acetate
UO521	3',4'-dihydroxy-2-methyl-propriophenone (COMT inhibitor)
MAO	mono amine oxidase
COMT	catechol-O-methyl transferase
DOPEG	3,4-dihydroxy phenylethylene glycol
DOMA	dihydroxy mandelic acid
Route of Drug Application	e.g., EL NA means NA applied to the artery extraluminally
Dose Response Curve	≡ concentration response curve

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