Postjunctional vascular alpha-$_2$ adrenoceptors: modulation and interactions.

A thesis presented for the degree of
Doctor of Philosophy

by

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November 1989

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Acknowledgements

I would like to thank Professor J. C. McGrath for the giving me the opportunity to undertake this research, and for his help and support during its completion. I would also like to thank Professor Shiela Jennett and the Institute of Physiology for allowing me to work in the department. A particular thank you must be made to Vince Wilson, my "co-supervisor" (What happened Tony ?) for the scientific discussions and for introducing me to "Hawkins" and all things good in Birmingham (with the possible exception of the Villa).

Thanks must also go to everyone else in the physiology department, particularly the boys in the Physiology all stars for allowing me to display my skills ! Thanks to Craig (for allowing me to reformat his discs), to both Karens (for their presence), to Eddie (a poor victim of fashion), to Joyce (for the scones), to Anita (for the TLC) and to Alison (for pushing a smile my way) and to all other members of the APU past and present. An experience is only as good as the people you experience it with !

I would also like to thank my family, particularly my mother, for the support and encouragement every young boy requires.

Declaration

The experimental work and other research contained within this thesis was undertaken wholly by myself, with the exception of figures 6, 9, 31, 32, 35 and 36 which was carried out as part of a collaborative project with V. G. Wilson. Some of the results have been published during the period of this study, details of which are given below.

Publications

DALY, C. J., DUNN, W. R., McGRATH, J. C. & WILSON, V. G. (1988). An attempt at selective protection from phenoxybenzamine of postjunctional α-adrenoceptor subtypes mediating contractions to (-)-noradrenaline in the rabbit isolated saphenous vein. *Br. J. Pharmacol.*, **95**, 501-511.

DALY, C. J., DUNN, W. R., McGRATH, C. J., & WILSON, V. G. (1988) The effects of angiotensin II on postjunctional α_1- and α_2-adrenoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.*, **95**, 699P.

DUNN, W. R., McGRATH, J. C. & WILSON, V. G. (1989). Expression of postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery - a permissive role for angiotensin II ? *Br. J. Pharmacol.*, **96**, 259-261.

DALY, C. J., DUNN, W. R., McGRATH, J. C., & WILSON, V. G. (1989). A comparison of the action of Bay K 8644 and angiotensin II at calcium channels that mediate contractile responses to postjunctional alpha$_2$-adrenoceptors. *Annals N.Y. Acad. Sci.*, **560**, 440-443.

DALY, C. J., DUNN, W. R., McGRATH, J. C., MILLER, D. J. & WILSON, V. G. (1989). An examination of the sources of calcium for contractions mediated via postjunctional α_1- and α_2-adrenoceptors in several isolated blood vessels from the rabbit. *Br. J. Pharmacol.*, in press.

McGRATH, J. C., DALY, C. J., DUNN, W. R., MacDONALD, A., MacMILLAN, J. B., TEMPLETON, A. G. B. & WILSON, V. G. (1989). Variation in participation of post-junctional α_1- and α_2-adrenoceptors and P_{2x}-purinoceptors in vascular sympathetic co-transmission: modulation by drugs and hormones. *Proceed. of the XXXIst International Congress Physiol. Sci.*. **P4736** 399.

DUNN, W. R., McGRATH, J. C. & WILSON, V. G. (1989). Isolation of postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery: influence of angiotensin II. *Br. J. Pharmacol.* **98,** 728P.

McGRATH, J. C., DALY, C. J., DUNN, W. R., MacLENNAN, S. J., MacMILLAN, J. B., TEMPLETON, A. G. B. & WILSON, V. G. (1989). Physiological modulation of α-adreno- and 5-HT-receptor expression in blood vessels. *J. Vasc. Biol.* **1,** 187.

SUMMARY

SUMMARY

The work presented herein represents an examination of the α-adrenoceptors mediating contractions in isolated vascular preparations from the rabbit and the factors involved in modulating these responses.

1) The α-adrenoceptor population in the rabbit isolated lateral saphenous vein, based upon agonist and antagonist potency profiles, could not be ascribed to be either a homogeneous population of either postjunctional α_1- or α_2-adrenoceptors, but had characteristics of both.

2) A homogeneous population of postjunctional α_2-adrenoceptors could be isolated in the lateral saphenous vein using receptor protection experiments with the combination of rauwolscine and phenoxybenzamine.

3) Only limited success was achieved in attempting to isolate a homogeneous population of postjunctional α_1-adrenoceptors in the lateral saphenous vein using receptor protection experiments with the combination of YM 12617 and phenoxybenzamine. The residual response to NA remaining after this procedure had characteristics of a mixed population of both postjunctional α_1- and α_2-adrenoceptors.

4) A comparison of the effects of angiotensin II and Bay K 8644 revealed marked differences in their ability to modulate responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors. A II produced a selective enhancement of responses mediated via postjunctional α_2-adrenoceptors, while the action of Bay K 8644 was not dependent upon receptor subtype.

5) In the lateral saphenous vein after isolation of postjunctional α_2-adrenoceptors, both Bay K 8644 enhanced responses to NA. The mechanism of this potentiation also appears to differ for these agents. Bay K 8644 enhanced responses mediated via voltage-dependent Ca^{2+} channels, while A II inhibited the influx of Ca^{2+} mediated via these channels.

6) The effects of A II on responses mediated via postjunctional α_2-adrenoceptors, was mimicked by its physiological precursor angiotensin I, suggesting that local vascular production of A II may be important for the facilitatory action of this peptide.

7) Nifedipine, like Bay K 8644, had a non-differential effect on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors in a number of isolated vascular preparations.

8) Under normal experimental conditions, based upon agonist and antagonist potencies, the rabbit isolated distal saphenous artery contains a homogeneous population of postjunctional α_1-adrenoceptors.

9) In the presence of A II, there was a marked increase in the responsiveness of the distal saphenous artery to UK-14304, which was prazosin-resistant, rauwolscine-sensitive, and so mediated via postjunctional α_2-adrenoceptors.

10) After attempted isolation of postjunctional α_2-adrenoceptors in the distal saphenous artery using receptor protection experiments, with the combination of rauwolscine and phenoxybenzamine, no responses were observed.

11) A II uncovered responses to α-adrenoceptor agonists after the combination of rauwolscine and phenoxybenzamine. The agonist and antagonist potencies after this protocol were consistent with a homogeneous population of postjunctional α_2-adrenoceptors.

12) Some of the results in the present study indicate an interaction between postjunctional α_1- and α_2-adrenoceptors in vascular smooth muscle. The implications for such an interaction is discussed in detail. Furthermore, evidence is presented demonstrating an interaction between postjunctional α_2-adrenoceptors and a number of vasoactive agents.

13) Sympathetic neurotransmission in the rabbit isolated distal saphenous artery is the resultant of an interaction between three receptor systems, postjunctional α_1- and α_2-adrenoceptors and purinoceptors. α_1-adrenoceptors are of principal importance, although a role for the two other receptor systems can be demonstrated under the appropriate experimental conditions.

INTRODUCTION

INTRODUCTION

Section 1 : Alpha-adrenoceptors on vascular smooth muscle

Historical subdivision of adrenoceptors

Some of the earliest experimental evidence suggesting that adrenoceptors could be differentiated into two distinct classes was provided by Dale (1906). He demonstrated in the pithed cat, that pressor responses to adrenaline could be blocked by ergot alkaloids, unmasking a depressor response to the agonist. The prevailing view at this time was that adrenaline was the most likely mediator of sympathetic neurotransmission (Elliot, 1905). In a more comprehensive study, Barger & Dale (1910) demonstrated that noradrenaline mimicked the effects of sympathetic nerve stimulation more closely than adrenaline, but this observation was overlooked for many years. However in this study, these authors, comparing the potency ratios for a large series of catechols, again emphasised that motor and inhibitory sympathomimetic activity varied to some extent independently. Therefore even at the begining of the century, when the concept of receptors was very much in its infancy, the use of relatively selective antagonists and comparing agonist potency ratios, provided retrospective evidence for receptor subdivision.

It appears however, that workers at this time were rather sidetracked by believing that the polarity of the response was crucial. One explanation forwarded to explain the fact that adrenaline could excite some smooth muscles but inhibit others was that of Cannon & Rosenbleuth (1933), who called the sympathetic transmitter sympathin, to emphasise that its actions were not identical to adrenaline. These authors suggested that sympathin, released upon nerve stimulation, could combine with one of two hypothetical substances. These substances made the

sympathin excitatory or inhibitory so that two sympathins could be produced: sympathin E (excitatory) or sympathin I (inhibitory). This hypothesis persisted for some time, before different adrenoceptor subtypes were defined on a pharmacological basis in 1948.

It was in this year that Ahlquist noticed that there were two distinct orders of potency for a series of catecholamines, adrenaline (AD), noradrenaline (NA), α-methylnoradrenaline (α-MeNA), α–methyladrenaline (α-MeAD) and isoprenaline (ISO), when tested on a wide variety of preparations. For excitatory events, but excepting stimulation of the heart, the order of potency was:

(-) AD > (±) AD > (±) NA > α-MeNA > α-MeAD > (±) ISO

while for inhibitory events, but excluding inhibition of the gut, the order of potency was:

(±) ISO > (-) AD > α-MeAD > (±) AD > α-MeNA > (±) NA

He concluded that the differences in potency orders could only be explained by assuming differences in the receptors. Therefore he classified the receptors that mediated mainly excitatory effects, but that included inhibition in the gut, "α" and the other which was mainly inhibitory, but included stimulation of the heart, "β". This dual receptor theory was generally ignored at the time until the selective β-blocking drug dichloroisoproteronol was introduced (Powell & Slater, 1958). This compound, followed by pronethalol (Black & Stephenson, 1962) and propranolol (Black *et al.*, 1964) then allowed the rigorous examination and confirmation of Ahlquist's classification.

Subdivision of α-adrenoceptors

The pharmacological subdivision of α-adrenoceptors awaited the realisation that NA was able to inhibit its own release from sympathetic nerves via pre-junctional α-adrenoceptors in a negative feedback system (see: Starke, 1977,

Westfall, 1977). The first evidence to indicate this mechanism, was from the experiments of Brown and Gillespie (1956, 1957), who observed that phenoxybenzamine was able to increase the amount of NA appearing in venous blood from the cat spleen following nerve stimulation. This observation was complicated by the fact that the end organ response was reduced by the α-adrenoceptor antagonist. Several explanations were forwarded to explain the paradox that phenoxybenzamine increased NA bioavailability, but reduced tissue response, generally concentrating on factors relating to the metabolism and/or uptake of the transmitter (Brown & Gillespie, 1957, Gillespie, 1966).

A prejunctional mechanism of action however became a more likely explanation from studies by Langer *et al.* (1971) and Starke (1972). These authors demonstrated, in guinea-pig atria and perfused rabbit heart respectively, that α-adrenoceptor antagonists increased transmitter overflow following nerve stimulation, without altering NA metabolism or uptake. The paradoxical postjunctional action on α-adrenoceptors was not a complication since the end organ response in these preparations is mediated via β-adrenoceptors. Further evidence favouring prejunctional α-adrenoceptor modulation of transmitter release was the demonstration that α-adrenoceptor antagonists increased stimulation induced overflow of NA and dopamine β-hydroxylase, a marker for exocytotic release of NA, in the dog spleen, while cocaine only increased NA levels (De Potter *et al.*, 1971). It is now a wideheld belief that NA can inhibit its own release by interacting with α-adrenoceptors located on nerve endings.

Differences between pre- and postjunctional α-adrenoceptors were soon implicated by examining agonist and antagonist potencies. Phenoxybenzamine was shown to be more potent at blocking contractions induced by nerve stimulation, than it was at increasing transmitter release in cat spleen (Dubovich & Langer,

1974), while clonidine was shown to be equipotent with NA at inhibiting transmitter release, but less potent than NA in eliciting contractions in the rabbit pulmonary artery (Starke *et al.*, 1974). These pharmacological differences, and the different locations of α-adrenoceptors, led Langer (1974) to propose the subdivision into "α_1"-postjunctional sites and "α_2"-prejunctional sites. This subclassification of α-adrenoceptors has subsequently been consolidated with numerous reports of differences between agonist and antagonist selectivity profiles (see: Starke, 1977, Westfall, 1977, Vizi, 1979).

Postjunctional α-adrenoceptors on vascular smooth muscle

In Vivo

Prior to the subdivision of α-adrenoceptors on the basis of anatomical location, it was believed that postjunctional α-adrenoceptors were a homogeneous population ie. solely α_1-adrenoceptors. The potency order for agonists at these receptors was as follows :- adrenaline > noradrenaline > phenylephrine >> isoprenaline, while the pA_2 value for phentolamine was generally close to 8.0 (Furchgott, 1972). With the discovery that subtypes of α-adrenoceptors existed, and with the availability of newer drugs with greater selectivity between subtypes, in particular the selective α_1-adrenoceptor antagonist prazosin, Starke and Langer (1979) suggested that each α-adrenoceptor could be defined as follows. At α_1-adrenoceptors, phenylephrine was a more potent agonist than clonidine, while prazosin was a more potent antagonist than yohimbine. In contrast at α_2-adrenoceptors clonidine was a more potent agonist than phenylephrine, while yohimbine was a more potent antagonist than prazosin. With these "rules of thumb", particularly the relative antagonist potencies, any given response mediated via α-adrenoceptors could then be defined as being α_1- or α_2-adrenoceptor

mediated.

The first report in favour of receptor heterogeneity of vascular postjunctional α-adrenoceptors, based on the potency of an antagonist, came from studies with prazosin in whole animals. In the pithed rat and perfused organs of the anaesthetised cat, it was observed that the pressor effects of phenylephrine were more susceptible to blockade by prazosin than were the equivalent responses to NA (Drew & Whiting, 1979). These authors did not attempt to define the prazosin resistant response to NA. However other experimental evidence supported the possibility that this component may have been α_2-adrenoceptor mediated. For example, pressor responses in pithed rats to the relatively selective α_2-adrenoceptor agonists, guanabenz and xylazine, were shown to be relatively resistant to prazosin, but susceptible to yohimbine, while the converse was true for phenylephrine (Docherty *et al.*, 1979, Docherty & McGrath, 1980). NA stimulation of both postjunctional α_1- and α_2-adrenoceptors was subsequently demonstrated in pithed rabbits, with the sequential administration of the antagonists prazosin and rauwolscine, the combination of which produced a greater effect than either antagonist alone (McGrath *et al.*, 1982). The presence of postjunctional vascular α_2-adrenoceptors, mediating pressor responses, has subsequently been demonstrated in a wide number of species, including man (see: McGrath, 1982).

Postjunctional α_2-adrenoceptors In Vitro

The demonstration of postjunctional α_2-adrenoceptors *in vivo* precipitated an extensive search for equivalent responses *in vitro*, in order to allow rigorous pharmacodynamic analysis of these receptors. In contrast to the relative ease of demonstrating postjunctional α_2-adrenoceptors in whole animals however, incontrovertible evidence showing responses mediated via these receptors in isolated vascular preparations, with the criteria outlined below, has proven much

more difficult. McGrath (1982) proposed that α_2-adrenoceptor agonism could be assumed if responses were susceptible to rauwolscine (or some other suitable antagonist) but resistant to prazosin (or equivalent) and other aminergic receptor blockers. Responses to α-adrenoceptor agonists in the vast majority of isolated vascular preparations, particularly arterial vessels, have been shown to be sensitive to prazosin (McGrath, 1982). A number of factors may be responsible for the elusive nature of postjunctional α_2-adrenoceptors *in vitro*. These are discussed below.

The first report of the existence of postjunctional α_2-adrenoceptors in isolated preparations were those in the canine saphenous and femoral veins (De Mey & Vanhoutte, 1981). In comparison to the corresponding arterial preparations, the "selective" α_1-adrenoceptor agonists methoxamine and phenylephrine were much less potent than NA in the veins. In addition, responses to NA in the veins were antagonised competitively by yohimbine, but only weakly and non-competitively by prazosin. The presence of α_2-adrenoceptors in the canine saphenous vein was corroborated by the demonstration that, after exposure to phenoxybenzamine, the contractile response to clonidine could be blocked by yohimbine but not by prazosin (Constantine *et al.*, 1982).

These results led others to examine the α-adrenoceptor characteristics in a number of venous preparations from different species. In an extensive study of 15 venous preparations, Shoji *et al.*, (1983) concluded that the canine saphenous, cephalic, femoral and external jugular veins contained mixed populations of α-adrenoceptors. The existence of both postjunctional α_1- and α_2-adrenoceptors, in the same preparation, mediating the same functional response, has complicated the unequivocal demonstration of postjunctional α_2-adrenoceptors. Their presence however, has subsequently been reported in the saphenous veins of the rat

(Cheung, 1985), human (Docherty & Hyland, 1985) and of the rabbit (Alabaster *et al.*, 1985, Schumman & Lues, 1983). It should be noted that even in some of these preparations, eg. the rabbit saphenous vein, despite the fact that the postjunctional α-adrenoceptor population had many characteristics of an α_2-adrenoceptor, in terms of agonist potencies and sensitivity to rauwolscine, prazosin was also a very potent, though non-competitive antagonist (Schumman & Lues, 1983, Daly *et al.*, 1988a). In the latter study, the authors suggested that postjunctional α_2-adrenoceptor mediated responses were susceptible to prazosin due to an interaction between the α-adrenoceptor subtypes. Such an interaction would obviously interfere with the demonstration of α_2-adrenoceptors *in vitro*. The existence of venous preparations, which have a homogeneous population of postjunctional α_2-adrenoceptors is not precluded however, as evidenced from the recently characterised adrenoceptor population in the rabbit ear vein (Daly *et al.*, 1988b).

It has been even more difficult to demonstrate postjunctional α_2-adrenoceptors in isolated arterial preparations, the likely source of α_2-adrenoceptor-mediated pressor responses in whole animals. However, some success has been achieved in studies utilising human arterial vessels. Indeed, one of the main stimuli toward recent research into heterogeneity of postjunctional α-adrenoceptors were the observations of Jauering *et al.* (1978), who demonstrated that responses of human digital arteries were antagonised very weakly by prazosin. Recent comparisons of human arteries and veins (femoral and digital) indicate the presence of both α_1- and α_2-adrenoceptors mediating contractions in both tissues but with a dominance of α_1-adrenoceptors in the arterial preparations and α_2-adrenoceptors in the veins (Glusa & Markwardt, 1983, Stevens & Moulds, 1985). The greater success in identifying postjunctional α_2-adrenoceptors in human isolated blood vessels may be due to the peripheral location of the vessels studied in

contrast to the large conduit arteries used from rats and rabbits. This is supported by the recent finding of an inverse correlation between human arterial vessel diameter and the magnitude of the α_2-adrenoceptor mediated response observed (Neilsen *et al.*, 1989). In addition, when small arterioles were examined from the rat cremaster region, α_2-adrenoceptor mediated responses could be demonstrated (Faber, 1988).

One additional explanation for the difficulty in demonstrating postjunctional α_2-adrenoceptors *in vitro* may be the lack of humoral agents normally present in the whole animal. In 1983, Schumman & Lues demonstrated that responses to BHT-920 were potently antagonised by prazosin in the rabbit saphenous vein. However in the presence of angiotensin II (A II) responses to BHT-920 became resistant to prazosin and therefore more classically α_2-adrenoceptor mediated. This led to the conclusion that α_2-adrenoceptors depended on the presence of A II for their complete expression. The requirement for the presence of tissue stimulants for the expression of α_2-adrenoceptors has also been reported in the canine saphenous artery with Bay K 8644 (Sulpizio & Hieble, 1987), the canine portal vein with a number of pharmacological and physiological stimulants (Furuta, 1988) and in the perfused rat tail after raising tone with vasopressin (Templeton *et al.*, 1989).

Difficulties in demonstrating postjunctional α_2-adrenoceptors on isolated vascular smooth muscle are therefore complicated by a number of factors, including the co-existence of both α_1- and α_2-adrenoceptors in the same preparation, the size of the vessel used in isolated studies and the absence of factors normally found in the whole animal. The difficulties have also been compounded by observations in recent years suggesting further subdivision of both α_1- and α_2-adrenoceptors in both vascular and non-vascular tissues. As yet no consensus is apparent for proposed heterogeneity of either receptor subtype. The relative merits of each proposed subdivision are considered in a critical review by McGrath *et al.* (1989).

Function and Location of α-adrenoceptors in the Vasculature

As described above, α_1- and α_2-adrenoceptors are present on both arterial and venous smooth muscle. Therefore activation of either receptor subtype can influence blood pressure by increasing peripheral resistance and/or by reducing venous capacitance, thereby increasing cardiac output. The relative importance of these mechanisms in mediating pressor responses to α-adrenoceptor agonists have been studied in recent years.

In pithed rats, stimulation of either receptor subtype has been shown to result in an increase in total peripheral resistance (Gerold & Haeusler, 1983, Richer *et al.*, 1987, Hiley & Thomas, 1987, MacLean & Hiley, 1988). For equivalent sized pressor responses, this effect was greater for α_1-adrenoceptor agonists, suggesting a more important role for α_1-adrenoceptors in small arterioles. Using radioactive microspheres it has been demonstrated that α_1-adrenoceptor stimulation induced vasoconstriction in the splenic, mesenteric, caudal, and most markedly in the kidney vascular beds resulting in a redistribution of cardiac output to the heart, lungs and liver (Waldron & Hicks, 1985, Hiley & Thomas, 1987). By comparison, α_2-adrenoceptor activation produced an increase in mesenteric and caudal vascular resistances.

In addition to increasing peripheral resistance, both α_1- and α_2-adrenoceptors can mediate an increase in cardiac output (Gerold & Haeusler, 1983, Kalkman, *et al.*, 1984, Hiley & Thomas, 1987, MacLean & Hiley, 1987). For α_2-adrenoceptors, this effect is due largely to an increase in venous return, while α_1-adrenoceptor-mediated increases in cardiac output, may additionally involve an

increase in cardiac rate (McGrath *et al.*, 1982, Hiley & Thomas, 1987) or contractility (Scholz, 1980). A lack of effect for α_1-adrenoceptors on venous smooth muscle is suggested from observations in conscious rats, where only α_2-adrenoceptor agonists increased mean circulatory filling pressure, an indicator of total body venous tone (Pang & Tabrizchi, 1986). However, such generalisations are difficult to sustain between species, for example in the dog, α_1-adrenoceptors appear to be the more important in controlling venous capacitance (Appleton *et al.*, 1986).

In addition to their presence on vascular smooth muscle, α-adrenoceptors can influence cardiovascular haemodynamics at other sites in the vasculature, although in general the physiological relevance of these mechanisms is not clear. For example, in the heart α_1-adrenoceptors mediate a positive chronotropic (McGrath *et al.*, 1982) and inotropic action (see: Scholz, 1980). These actions may become important in abnormal conditions, such as ischaemia (see: McGrath, 1987). In addition to the presence of prejunctional α_2-adrenoceptors modulating transmitter release from sympathetic nerve terminals, they have also been shown to modulate transmitter release from parasympathetic nerve terminals (see Gillespie, 1980). A similar negative feedback mechanism for α_1-adrenoceptors has also been proposed (Story *et al.*, 1985). The stimulation of α_2-adrenoceptors has also been demonstrated to mediate the release of EDRF from endothelial cells of canine coronary and femoral vascular smooth muscle (Cocks & Angus, 1983, Angus *et al.*, 1986), a feature which may be important for flow regulation in individual vascular beds.

Mechanism of action of α-adrenoceptors in vascular smooth muscle

Ca^{2+} sources for contraction

In parallel with the subdivision of postjunctional α-adrenoceptors was an examination of the Ca^{2+} sources utilised upon stimulation of each subtype. De Mey & Vanhoutte (1981) initially suggested that α_1-adrenoceptor mediated responses were more sensitive to the action of calcium channel blockers (CCB's) than were those to α_2-adrenoceptor stimulation. The susceptibility of α_1-adrenoceptors to CCB's was consistent with observations made earlier in the rabbit aorta (a preparation containing α_1-adrenoceptors), which were shown to consist of two phases, a transient response via release of intracellular Ca^{2+}, sustained by a secondary component, dependent upon the influx of extracellular Ca^{2+} (Bohr, 1963, Deth & VanBreemen, 1977). However, Van Meel *et al.,* (1981) provided contrary evidence in pithed rats and reached the conclusion that α_1-adrenoceptor mediated responses were independent of the entry of extracellular Ca^{2+}, while α_2-adrenoceptors were highly dependent on such a component to maintain contraction. This conclusion has been hotly contested in the literature, with suggestions that differential attenuation is a consequence of receptor reserve and agonist efficacy rather than on receptor subtype (see: Nichols & Ruffolo, 1988) or on the time course of response to a given agonist (O'Brien *et al.,* 1985). This prolonged disagreement demonstrates one of the limitations of analysing pharmacological phenomena quantitatively from whole animal studies, principally because the use of bolus injections of agonists *in vivo* do not reflect "equilibrium" responses. This further highlights the necessity to find suitable *in vitro* preparations containing homogeneous populations of either subtype, to permit rigorous pharmacodynamic analysis of responses. Greater clarification for the effects of CCB's on α_1- and α_2-adrenoceptor mediated responses in pithed rats were obtained by using infusions of agonists, in order to give a more equilibrium response. Under these conditions

responses mediated via either α-adrenoceptor subtype were attenuated (McGrath & O'Brien, 1987). Regardless of the source, the net effect of α_1- and α_2-adrenoceptor stimulation is to increase the free cytosolic levels of Ca^{2+} within the cell, with the subsequent activation of contractile proteins resulting in the contraction of vascular smooth muscle. A number of transduction processes linking receptor occupation with end organ response have been proposed for both subtypes. These are briefly discussed below.

Membrane Potential

α-adrenoceptor activation can result in changes in membrane potential. Depolarisation can occur simultaneously with contractions produced by either α_1-adrenoceptors (eg. Suzuki 1983) or α_2-adrenoceptors (Cheung, 1985). However, there is not always a direct correlation between the two events (see: McGrath *et al.*, 1989), such that the primary action of most α-adrenoceptors appears, not to be a direct change in membrane potential, but rather a modulation of biochemical or electrophysiological properties pre-existing in the cell.

Second Messengers

Most of the available evidence suggests that α_1-adrenoceptors in vascular smooth muscle stimulate the breakdown of phosphatidylinositol-4,5-bisphosphate (PIP_2). This results in the generation of inositol 1,4,5-triphosphate (IP_3), which in turn releases Ca^{2+} from non-mitochondrial intracellular stores (Michell & Kirk, 1981). Two other putative second messengers are also produced, namely, 1,2-diacylglycerol (DAG) and 1,3,4,5-tetrakisphosphate (IP_4), which may be involved in permitting the entry of extracellular Ca^{2+} into the cell (Putney, 1987, Campbell *et al.*, 1985, Spedding, 1987).

Few studies have examined the biochemical consequences of α_2-adrenoceptor stimulation in vascular smooth muscle because of the lack of suitable preparations. There is evidence however, in other tissues, that α_2-adrenoceptor occupation results in an inhibition of cyclic AMP production by adenylate cyclase (Exton, 1985, Fain & Garcia-Sainz, 1980). In agreement with such a mechanism Fredholm, *et al.*, (1985) demonstrated that clonidine dose-dependently reduced forskolin induced stimulation of adenylate cyclase activity, in cat isolated middle cerebral artery. However, α_2-adrenoceptor stimulation is not limited to changes in cyclic AMP levels, since in NG108-15 cells α_2-adrenoceptor agonists have been shown to accelerate Na^+/H^+ exchange (Isom, *et al.*, 1987).

Sympathetic Neurovascular Transmission

As mentioned previously Barger & Dale (1910) were the first authors to demonstrate that noradrenaline mimicked responses to sympathetic nerve stimulation more closely than did adrenaline. It was not until 1946 however, that noradrenaline was shown to be the main sympathetic transmitter, when Von Euler demonstrated that noradrenaline concentrations in sympathetically innervated tissue were very high in comparison to adrenaline. Furthermore, noradrenaline levels were reduced after sympathectomy, indicating a close association with nerves rather than muscle. Noradrenaline was subsequently accepted to be the mediator of sympathetic nerve stimulation. More recently however this concept has had to be modified, since many examples have been found where vascular responses to nerve stimulation remain partly resistant to α-adrenoceptor antagonists.

This has led to two alternative proposals to α-adrenoceptor mediated sympathetic transmission. Firstly, Hirst & Neild (1980) have suggested that noradrenaline is the neurotransmitter in arteriolar smooth muscle, but that it acts

upon a distinct class of adrenoceptors, namely "gamma"-adrenoceptors. Another explanation which has gained more credence, is the "co-transmission" hypothesis, which puts forward the idea that another transmitter is released along with noradrenaline. The most likely candidate for "co-transmitter" involved in sympathetic vascular transmission is ATP (see: Burnstock & Sneddon, 1985). A number of experimental observations suggest this; one example being that in pithed rats, the pressor response to sympathetic nerve stimulation can be blocked by the sequential administration of α-adrenoceptor antagonists and the purinergic receptor antagonist α-,β-methylene ATP (Flavahan *et al.*, 1985).

The fact that α_1-adrenoceptor antagonists were found to be more effective at blocking responses to sympathetic nerve stimulation than they were at blocking equivalent sized responses to exogenous NA, led to the suggestion that postjunctional α_1-adrenoceptors were neurogenic receptors, while α_2-adrenoceptors were located extrajunctionally, responding to circulating catecholamines (McGrath, 1982). It is now apparent however, that this generalisation is not always the case. Postjunctional α_2-adrenoceptors have been implicated in mediating the end organ response to sympathetic nerve stimulation in canine, human and rat saphenous veins (Flavahan *et al.*, 1984, Docherty & Hyland, 1985, Cheung, 1985). Figure 1 illustrates the two major transmitters and the receptors through which they act at the sympathetic neurovascular junction.

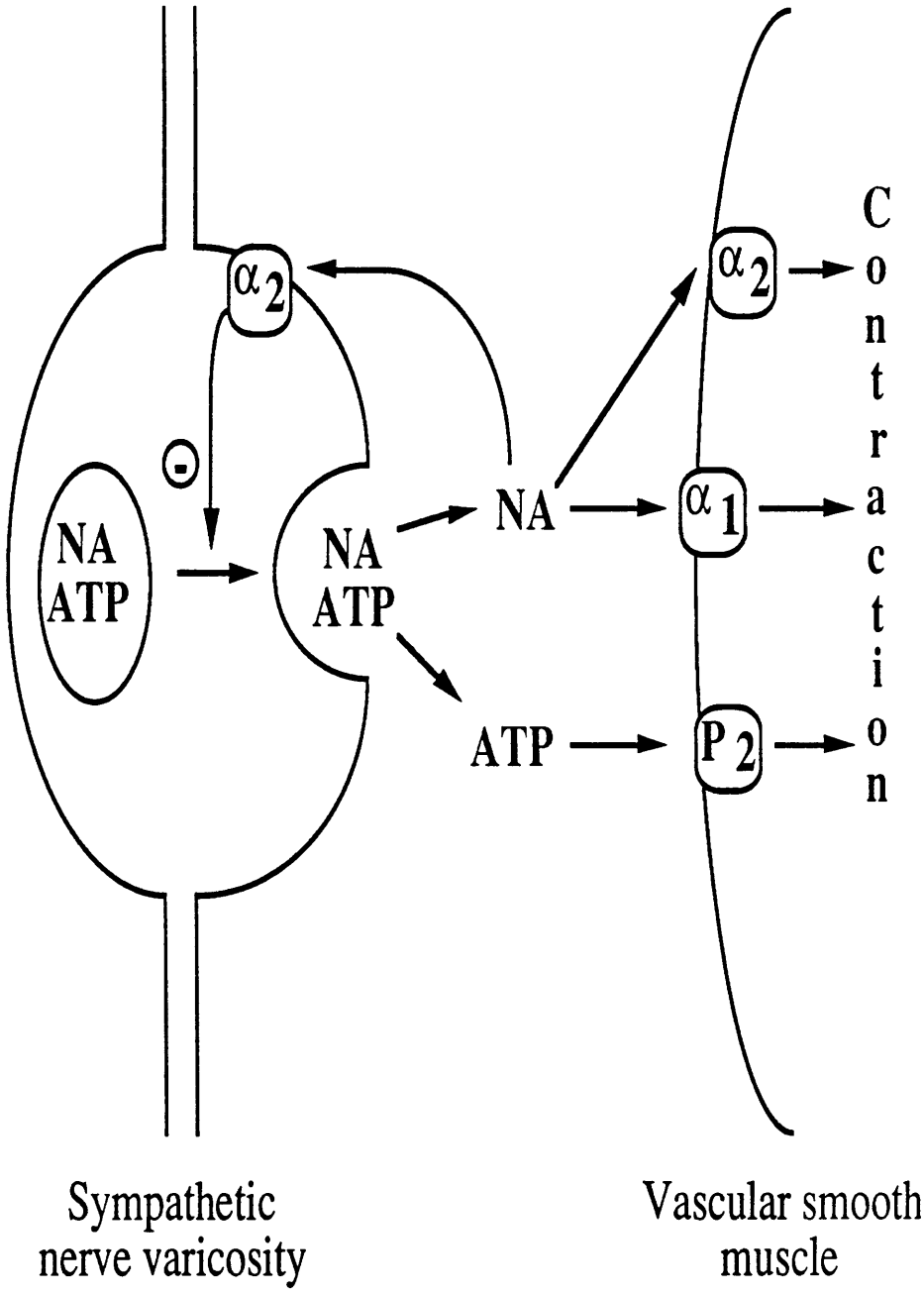


Figure No. 1

This diagram represents the current, accepted view of sympathetic neurovascular transmission and demonstrates the location of the α-adrenoceptors and purinoceptors involved.

Section 2 : The Renin-Angiotensin System

Historical overview

The first direct demonstration that the kidney could influence blood pressure was made in 1898 when Tigerstedt & Bergman observed that injection of saline extracts from renal homogenates into anaesthetised rabbits produced pressor responses. These authors termed the responsible pressor agent renin. However it was not until 1934, when Goldblatt *et al.* demonstrated that a persistent hypertension could be induced in dogs, by producing renal ischemia, that greater interest in a pressor substance of renal origin was rekindled.

The focus of attention was subsequently directed back to renin and its presence in kidney extracts was reconfirmed in 1938 (Kohlstaedt *et al.*, Pickering & Printzmetal). It was observed however, that renin itself was not the active pressor substance. Partially purified renal renin extract, which increased blood pressure when injected into whole animals, had little effect on the isolated dog tail or perfused rabbit ear artery preparations, unless plasma was added to the perfusate (Kohlstaedt *et al.*, 1940, Helmer & Page, 1939). These authors suggested that renin was an enzyme-like substance activated by a renin activator found in blood. In 1940 Page & Helmer named the responsible pressor substance formed from the above interaction "angiotonin". Independently, Braun-Menendez *et al.* (1940) found that a pressor substance could be obtained from venous blood from ischaemic kidneys and that this substance had similar pharmacological properties to the product of the interaction between renin and blood globulins. They termed this substance "hypertensin" and postulated that renin was an enzyme whose substrate was a blood protein. Page *et al.*, (1943) subsequently agreed that renin activator was in fact a substrate, and should be designated "renin substrate" accordingly.

Renin substrate has subsequently been shown to be an alpha-globulin of approximately 57,000 MW (Skeggs *et al.,* 1963) largely synthesized in the liver (Nasjletti & Masson, 1971). The dual nomenclature for the pressor substance, angiotonin or hypertensin, continued for almost two decades until a compromised name of angiotensin was adopted (Braun-Menendez & Page 1958).

After its discovery, attempts were made to purify and elucidate the structure of angiotensin. In the course of such studies Skeggs *et al.* (1954) were able to separate two hypertensins, designated I and II respectively and demonstrated that hypertensin I was rapidly converted to hypertensin II by an enzyme in plasma. These compounds were shown to be peptides and the amino acid composition of each was determined (Skeggs *et al.,* 1955, Lentz *et al.,* 1956). Horse hypertensin II was shown to be an octapeptide with the amino acid sequence:

Asp-Arg-Val-Tyr-Ileu-His-Pro-Phe-His-Leu

being produced by the action of a converting enzyme upon a non-pressor decapeptide (hypertensin I) by cleavage of a histidylleucine dipeptide (Skeggs *et al.*, 1956). The sequence of ox hypertensin I was also determined in the same year by Elliot & Peart (1956) and found to be almost identical to that of horse hypertensin I:

Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu

varying only at the fifth amino acid residue. Human angiotensin has subsequently been shown to be identical to that of horse angiotensin (Arakawa *et al.,* 1967). Angiotensin was produced synthetically in 1958 (Schwarz *et al.,* 1957) and this led to the discovery that this compound had many physiological roles relating to the maintenance of blood pressure.

Circulating and Local Angiotensin II production

As described above angiotensin II (A II) is produced from a biochemical cascade process involving two substrates, angiotensinogen and angiotensin I (A I), and two enzymes, namely, renin and a plasma angiotensin converting enzyme. The traditional concept of the renin-angiotensin system has been of a circulation-borne endocrine system, whose components are secreted by different organs, with the biological end product of this cascade, A II, being conveyed by arterial blood to act on specific target organs (for review see: Peach, 1977). More recently evidence in favour of an additional, local tissue A II generating system has accumulated (see: Dzau, 1987, 1988, Campbell, 1987, Unger *et al.*, 1989).

Renin

Under normal circumstances the kidney appears to be the most important source of renin. In 1976, Thurston demonstrated that plasma renin fell to undetectable levels after bilateral nephrectomy, and despite the fact that renin has also been shown to be present in and to be synthesized by vascular smooth muscle cells (Gould *et al.*, 1964, Re *et al.*, 1982), vascular renin levels are also reduced after bilateral nephrectomy (Thurston *et al.*, 1979), albeit at a slower rate. In addition, Loudon *et al.*, (1983) have shown that arterial smooth muscle renin levels are related to the exogenous administration, ie. the plasma levels, of renin. These results suggest that the majority of tissue renin is also of renal origin. Locally produced renin may be important in some vascular beds; for example, in the saline perfused rat hindlimb, tetradecapeptide renin substrate, in the absence of exogenous renin, produced pressor responses which could be blocked by renin-inhibitory peptide (Oliver & Sciacca, 1984). However, it is known that a large number of enzymes found in peripheral tissues have renin-like activity and could be responsible for the ultimate production of A II (for references see: Peach, 1977).

Alternatively, the importance of the local production of renin may be pathophysiological. Assad & Antonaccio (1982) have demonstrated that vascular renin levels in spontaneously hypertensive rats remain elevated even after bilateral nephrectomy, when plasma levels of renin are undetectable.

Angiotensinogen

The liver has been suggested to be the major source of the substrate for renin, angiotensinogen (Nasjletti & Mason, 1971). This site of production however is not exclusive, since it has also been shown to be synthesized in other tissues within the blood brain barrier (Ganten *et al.*, 1972), and in addition, angiotensinogen messenger RNA has been detected in a number of extrahepatic tissues including vascular smooth muscle (Campbell & Habener, 1986).

Angiotensin Converting Enzyme

It was initially suggested that A I was converted to A II by angiotensin-converting enzyme (ACE) solely in the blood, from observations demonstrating the lack of effect of A I in, for example, saline perfused kidneys (Skeggs *et al.*, 1956) or rabbit aortic strips (Helmer, 1957), where A II was active. Carlini *et al.*, (1958) however, were able to demonstrate activity for A I in a number of preparations. Then in 1968, Ng & Vane demonstrated that conversion of A I into A II in plasma, was very slow in comparison to the rate of generation of A II when passing through the pulmonary circulation. This led to the wideheld belief that the lung was the most important site for ACE and the subsequent generation of circulating A II. ACE itself is a fairly non-specific peptidyl dipeptide hydrolase which cleaves peptide bonds of a variety of amino acids (Yang *et al.*, 1971) and is identical to kininase II which inactivates bradykinin.

It is now apparent that production of A II locally in vascular smooth muscle is also very important. ACE has been demonstrated to be present in a wide variety of tissues, and is very prominent on vascular endothelial cells (Wilson *et al.*, 1987), with some evidence suggesting that it may be present on vascular smooth muscle (Velletri & Bean, 1982).There are now numerous reports to suggest that A II can be produced locally, by conversion of A I, in isolated vascular smooth muscle (eg. Malik & Najletti, 1976, Oliver & Sciacca, 1984, Juul *et al.*, 1987), an effect which can be blocked by ACE inhibitors. Indeed the use of ACE inhibitors has provided perhaps the most compelling evidence for an important physiological role for local vascular A II production. These agents have been found to be very effective antihypertensive agents in various models of experimental hypertension (Rubin *et al.*, 1981), even when circulating A II levels are normal or low. In some of these models, eg. the spontaneously hypertensive rat (SHR), the magnitude and duration of blood pressure reduction correlates better with the inhibition of tissue ACE, than with inhibition of serum enzyme activity (Cohen & Kurtz, 1982, Unger *et al.*, 1984). This is perhaps demonstrated most strikingly in the latter study whereby, several hours after a single dose of an ACE inhibitor was administered, when serum ACE activity as well as the pressor response to exogenous A I had returned to normal, blood pressure remained reduced. The duration of the antihypertensive response was paralleled by the suppression of aortic and kidney ACE activity.

Direct evidence for the existence of the vascular renin-angiotensin system, acting independently from the circulating system, is provided by the observations that basal A II production occurs in cultured bovine aortic endothelial cells (Kifor & Dzau, 1987), although the true physiological importance of this remains to be determined. However it is very much apparent that in addition to the traditional

concept of the circulating renin-angiotensin system, there also exists a functionally important local A II generating system in vascular smooth muscle cells, which is independent to some degree from the circulating system. The components of the renin-angiotensin system are outlined in figure 2.

Metabolism of Angiotensin II

It has been known since the discovery of A II, that enzymes found in the blood and tissues function to metabolise and inactivate the pressor substance (Braun-Menendez *et al.*, 1940). These enzymes have been termed angiotensinases and are generally non-specific in their actions. They include aminopeptidases (angiotensinase 'A') (Khairallah *et al.*, 1963) which hydrolyse A II at the N-terminal bond, endopeptidases (angiotensinase 'B') (Regoli *et al.*, 1963) which hydrolyse the middle portion of the peptide and carboxypeptidases (angiotensinase 'C') (Johnson & Ryan, 1968) which hydrolyse the carboxy terminal of A II.

Release of Renin

In most instances the rate limiting step in the renin-angiotensin biochemical cascade is the amount of renin produced. Since plasma renin and most vascular renin, under normal conditions, is of renal origin (Thurston *et al.*, 1979, Loudon *et al.*, 1983), the amount of renin released from the kidney will determine the levels of production of the biologically active angiotensins. To this extent, a number of mechanisms exist which control renal renin secretion.

These mechanisms which control renin secretion include:- i). An intrarenal baroreceptor, which is dependent on the balance between renal perfusion pressure and ureteral pressure (Kaloyanides *et al.*, 1973) and appears to utilise cyclo-

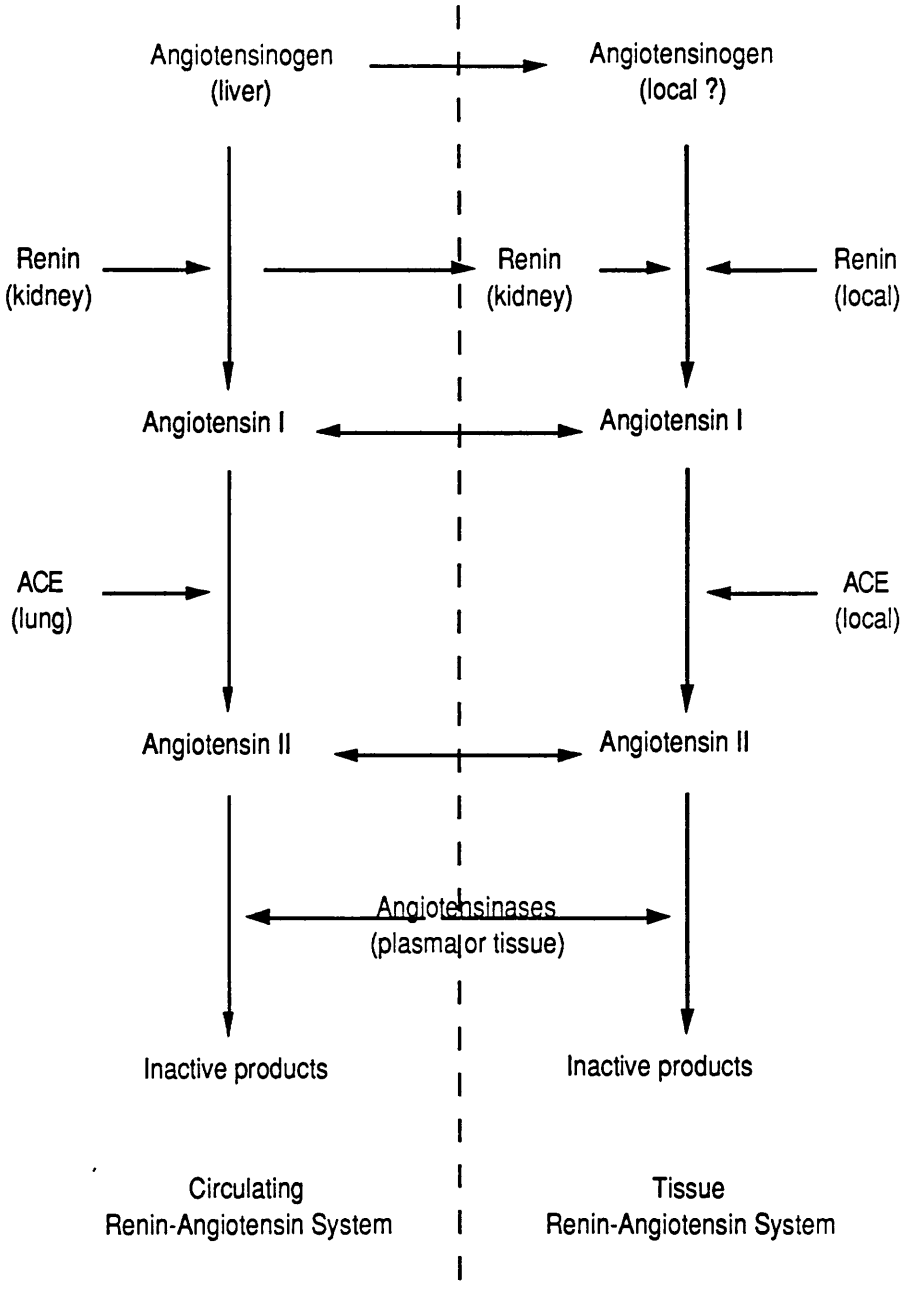


Figure No. 2

The renin-angiotensin cascade system incorporating the classical circulating endocrine system and the local tissue angiotensin generating system.

oxygenase products (Blackshear *et al.*, 1979). ii). Decreased Na^+ levels in the distal tubule of the kidney reduce renin release from the closely associated juxtaglomerular cells (Churchill *et al.*, 1978). iii). Sympathetic nerve stimulation to the juxtaglomerular cells evokes renin release into the circulation, an effect mediated via β-adrenoceptors (Weber *et al.*, 1983) which can also be activated by circulating catecholamines (Pettinger *et al.*, 1972). and v). A II can itself inhibit renin release in a 'short loop' mechanism (Keeton *et al.*, 1976).

Physiological and Pharmacological Effects of Angiotensin II

Actions of Angiotensin II on Vascular Smooth Muscle

A II is a potent vasoconstrictor agent in all species studied. On a molar basis it is about 40 times more potent than noradrenaline (DeBono *et. al.*, 1963). The regional haemodynamics of A II suggest that its vasoconstrictor activity, mediated via arteriolar constriction, is greatest in the splanchnic beds and the kidney, by an action at both pre- and postglomerular arterioles (Wilson, 1986), moderate in skin and least in skeletal muscle (Douglas, 1980). A II has been reported to cause constriction of coronary blood vessels (Frank *et al.*, 1970), although paradoxical coronary vasodilatation may occur due to the stimulation of prostaglandin synthesis by the peptide (Needleman *et al.*, 1975). A II also contracts large conduit arteries, a function which may be important in the pathophysiology of hypertension (see: Dzau & Safar, 1988). It was initially suggested to show selectivity for the arterial rather than the venous vasculature (Folkow *et al.*, 1961), although some venous preparations, such as rat portal and mesenteric veins, clearly respond to A II (Somlyo & Somlyo 1966). More recently, Pang & Tabrizchi (1986) demonstrated that A II infusion in conscious rats increased mean circulatory filling pressure, an indicator of venous tone, while, Kaufman & Vollmer (1985) and Maclean & Hiley (1988) have shown, using ACE inhibitors in pithed rats, that A II contributed to the

maintenance of blood pressure by decreasing venous capacitance, thereby maintaining cardiac output. Furthermore, Stokland *et al.*, (1982) concluded that half the rise in systemic pressure produced by A II infusion, was caused by venoconstriction with the resultant increase in preload and cardiac output.

In addition to the acute contractile effects of A II on vascular smooth muscle, this peptide raises blood pressure gradually when administered in low or subpressor rates of infusion. This "slow pressor" effect was first demonstrated in the rabbit (Dickinson & Lawrence, 1963) and appears to be particularly marked in the rat (Brown *et al.*, 1981). The mechanism of action for the "slow" pressor effect of A II is unknown, although it has been proposed that inducing vascular hypertrophy may be important (Lever, 1986).

Relaxatory responses to A II can be demonstrated, under certain conditions, in blood vessels such as rabbit renal vein (Webb, 1982), or the rabbit mesenteric vasculature (Pure & Needleman, 1979). These A II-induced relaxations can be blocked with cyclo-oxygenase inhibitors, and may be due to the production of prostaglandin E_2 or I_2 (Pure & Needleman, 1979). The physiological significance of A II-induced relaxation remains unclear.

The contractile effects of A II on vascular smooth muscle are mediated by specific receptors. As early as 1976 it was suggested that angiotensin receptors were heterogeneous (Moore *et al.*, 1976). Subdivision of these receptors has subsequently been suggested in vascular smooth muscle (Tabrizchi & Pang, 1987), in the kidney cortex (Douglas, 1987). However, the lack of suitable competitive antagonists of A II has meant that no general consensus on subtypes of angiotensin receptors is apparent.

Mechanism of action of Angiotensin II in vascular smooth muscle

Deth and van Breemen (1977) observed in the rabbit aorta that responses to A II were largely dependent on the release of Ca^{2+} from an intracellular store. In general responses to A II are poorly attenuated by omitting extracellular Ca^{2+} from the bathing medium or by Ca^{2+}-entry blockers (see: Peach, 1977, Hof *et al.*, 1982). It is now apparent that A II, in common with many other vasoactive agents, stimulates the breakdown of phosphatidylinositol-4,5-bisphosphate (PIP_2) and the generation of inositol triphosphate (IP_3), which subsequently mobilises Ca^{2+} from intracellular stores (Alexander *et al.*, 1985). A II stimulated breakdown of polyphosphoinositides concomitantly releases another putative 2nd messenger, 1-,2-diacylglycerol (DAG) (Griendling, *et al.*, 1986), which may be involved in regulating the entry of extracellular Ca^{2+} into the cell (Campbell *et al.*, 1985). A II also stimulates Na^+/H^+ exchange, with subsequent intracellular alkalinization, in vascular smooth muscle cells (Greindling *et al.*, 1988, Bingham, 1986). The physiological response of any vascular tissue represents the integration of these intracellular signals.

A feature of responses to A II in vascular smooth muscle, particularly with higher concentrations, is the inability to maintain the contraction either *in vivo* or *in vitro* (Bock & Gross, 1961, St. Louis *et al.*, 1977). The desensitisation to A II has been attributed to a number of factors including, the production of prostaglandins (Aiken, 1974), or the formation of a stable drug-receptor complex (St. Louis *et al.*, 1977). In favour of the latter, Danthuluri & Deth (1986) have recently demonstrated the inability of A II to maintain production of DAG, an agent which may regulate the entry of extracellular Ca^{2+} into the cell (Campbell *et al.*, 1985). This suggests that the inability of A II to maintain the response is due to a loss of effectiveness of the hormone-receptor complex in signal transduction.

Aldosterone secretion

Another action of the renin-angiotensin system, pertinent to blood pressure control, is its influence on aldosterone secretion. Aldosterone is a mineralocorticoid secreted from cells in the zona glomerulosa in the adrenal cortex, whose function is to enhance sodium reabsorption in the distal tubules and collecting duct of the kidney. The overall effect of enhanced aldosterone secretion is to increase circulating blood volume, with a subsequent tendency for increased arterial pressure (Horton, 1973). It is now evident that the renin-angiotensin system is a potent stimulant of aldosterone secretion (Brown *et al.*, 1979), although there is some doubt as to whether A II or the des-Asp metabolite (A III) is physiologically the more important (Semple & Morton, 1976).

Central actions of Angiotensin II

In addition to the actions of A II in the periphery, this peptide also produces a number of effects, pertaining to blood pressure control, which are mediated via the central nervous system. It was initially difficult to comprehend how a circulating peptide had access to the brain to produce such central effects. However, it is now believed that circulating A II mediates its central actions via the circumventricular organs, small areas of the brain that lack a blood brain barrier (see Reid, 1984). In addition an endogenous brain renin-angiotensin system has been demonstrated (Ganten, 1972).

A central action of A II on blood pressure was first demonstrated by Bickerton & Buckley (1961). These authors used cross-circulation techniques and observed that injections of A II into the central circulation of an animal's head, isolated from its own trunk, produced a hypertensive effect in the vasculature of its

own trunk, which was mediated by an increase in sympathetic efferent activity. This central pressor action of A II, by increasing sympathetic outflow, has been confirmed subsequently with infusions of A II via vertebral arteries (Dickinson & Yu, 1967, Yu & Dickinson, 1971) and with direct central injections of A II (Scholkens *et al.*, 1982). In addition to enhancing sympathetic discharge, it has been suggested that part of the central pressor action of A II may be due to a reduction of vagal tone with a subsequent increase in cardiac output (Reid *et al.*, 1982), or by stimulating vasopressin release, an effect which may be important in the rat (Severs, *et al.*, 1973). A II has also been shown to have a dipsogenic effect, which can be induced by either peripheral or central administration of A II (Fitzsimons, 1972, 1975) and in addition it can stimulate ACTH secretion from the pituitary gland (Sobel, 1983). Overall the central actions of A II can potentially increase blood pressure directly by increasing sympathetic output or indirectly by increased fluid volumes. A more extensive account of the actions of angiotensin(s) in the central nervous system can be found in review papers by Reid (1984) and Phillips (1987).

SECTION 3: Interactions between the Renin-Angiotensin System and the Peripheral Sympathetic Nervous System

It is perhaps not surprising that the sympathetic nervous system and the renin angiotensin system, both of which play an integral part in the control of cardiovascular haemodynamics, do not work independently from one another. As mentioned previously, activation of the renin angiotensin system can increase sympathetic outflow from the central nervous system. In addition both systems have been suggested to interact with each other at a number of sites in the periphery.

Prejunctional Action of Angiotensin II on Sympathetic Nerve Terminals

An interaction between A II and the peripheral sympathetic nervous system was first implicated by the observations that pressor responses to A II were reduced following sympathectomy or pretreatment with reserpine (Zimmerman, 1962, Baum, 1963). These results suggested that part of the pressor response to A II was mediated via the sympathetic nervous system. Confirmation of such an action was forthcoming in studies which detailed enhanced responses, in the presence of A II, to procedures which caused the release of NA from nerves (McCubbin & Page, 1963), or to direct sympathetic nerve stimulation in the guinea-pig vas deferens and cat spleen (Bennelini *et al.*, 1964). This facilitatory action for A II has subsequently been described in a number of vascular preparations from a range of species (eg. Panisset & Bourdois, 1968, Johnson *et al.*, 1974, Nicholas, 1970, Majewski *et al.*, 1984) and can be mimicked with tetradecapeptide renin substrate and A I, suggesting a possible role for A II produced at a local tissue level (Malik &

Najletti, 1976, Boke & Malik, 1983).

This enhancement of responses produced by A II was attributed principally to a prejunctional action, since equivalent sized responses to exogenous NA were either not affected (rarely), or potentiated to a smaller degree than nerve stimulation responses. Such a prejunctional mechanism was directly demonstrated when A II was observed to cause an increase in ^{3}[H]-NA overflow in perfused canine hindlimb, rabbit portal vein and rabbit coeliac artery, in response to sympathetic nerve stimulation (Hughes & Roth, 1971, Zimmerman *et al.*, 1967). A number of suggestions were forwarded to explain this effect, including increased transmitter synthesis (Roth, 1972), blockade of neuronal uptake of NA (Panisset & Bourdois, 1968, Khairallah 1972) or a facilitatory action on NA release during nerve stimulation. Of these mechanisms, the latter appears to be most important. This is demonstrated by the fact that A II potentiation of NA release, persisted in the presence of cocaine (Hughes & Roth, 1971, Eikenberg, 1982) and perhaps more convincingly, A II increased the release of both NA and dopamine β-hydroxylase during sympathetic nerve stimulation (Ackerly *et al.*, 1976), while cocaine has been shown to increase only the former (De Potter *et al.*, 1971). In more recent years, Trachte and co-workers have re-examined the prejunctional facilitatory action of A II on sympathetic nerve stimulation, in light of the proposed existence of 'co-transmitters'. These authors have demonstrated that A II and in addition its des-Asp metabolite, A III, enhanced responses to sympathetic nerve stimulation in rabbit vas deferens. This effect was due to facilitation of the adrenergic component of the response, while in contrast, the non adrenergic (purinergic) component of the response was reduced. These observations are particularly interesting because they provide evidence suggesting that co-transmitters are not released concomitantly, and that their relative importance in mediating the end organ response, could vary depending upon the prevailing circumstance (Saye *et al.*, 1986, Trachte *et al.*,

1987, Trachte, 1988).

Postjunctional Interaction between Angiotensin II and Adrenoceptors

Part of the facilitatory action of A II on responses to sympathetic nerve stimulation, may be due to an increase in the responsiveness of vascular smooth muscle to NA. This proposed action has been more controversial than the well documented prejunctionally mediated increase in transmitter release, because it is not evident in all vascular preparations. For example responses to injection or infusion of NA in the canine renal and hindpaw vascular beds, rabbit portal vein and coeliac artery were unaffected by A II (Bell, 1972, Hughes & Roth, 1971, Zimmerman & Whitmore, 1967). In contrast, A II has been shown to enhance responses to NA in other preparations such as the perfused rat caudal artery (Nicholas, 1970), rabbit femoral artery (Purdy & Weber, 1988), rat, canine and feline mesenteric blood vessels (Malik & Najletti, 1976, Panisset & Bourdois, 1968, Chiba & Tsukada, 1986), human digital arteries (Moulds & Worland, 1980) and in rabbit aorta (Day & Moore, 1976). Despite these contrasting observations in isolated preparations, the use of ACE inhibitors in whole animals has clearly demonstrated a role for endogenous A II in influencing pressor responses to α-adrenoceptor stimulation.

A number of ACE inhibitors, and the A II receptor antagonist saralasin, have been demonstrated to reduce responses to both sympathetic nerve stimulation and to exogenous NA, or α-adrenoceptor agonists in pithed animals, presumably by preventing endogenous A II formation (Hatton & Clough, 1982, Clough *et al.*, 1982, Antonaccio & Kerwin, 1981, De Jonge *et al.*, 1983). This has not always been easy to demonstrate however, since re-infusion of A II does not consistently reverse the effects of ACE inhibition (Bull & Drew, 1984, Kaufman & Vollmer, 1985, Grant & McGrath, 1988a). This inability of A II infusion to reverse the

effects of endogenous A II removal, may reflect an important role for inhibition of local tissue A II formation independent of the circulating renin-angiotensin system (Bull & Drew, 1984). Alternatively, it may reflect a complex interaction between A II and cyclo-oxygenase products (Grant & McGrath 1988a).

The mechanism whereby A II enhances vascular responsiveness to NA or α-adrenoceptor agonists is not clear. It has been suggested that ACE inhibition attenuates pressor responses to NA, by the reduction in blood pressure evoked by these agents *per se*, since restoring basal blood pressure with vasopressin also restored responses to NA (De Jonge *et al.*, 1982). This suggests a non-angiotensin dependent mechanism with the degree of vascular tone being crucial. In contrast however, captopril attenuated pressor responses to NA without reducing basal blood pressure (Hatton & Clough, 1982) and in addition, teprotide had no effect on pressor responses to 5-HT and A II, but markedly decreased both blood pressure and responses to α-adrenoceptor agonists in pithed rats (Grant & McGrath, 1988b). These latter observations provide strong evidence for a specific interaction between A II and α-adrenoceptor mediated responses. Two recent reports have implicated small resistance arterioles as the site for this interaction (Kaufman & Vollmer, 1986, MacLean & Hiley, 1988). In these studies, ACE inhibition antagonised α-adrenoceptor mediated increases in total peripheral resistance without influencing corresponding changes in cardiac output..

It has been suggested that ACE inhibitors selectively attenuate responses to α_2-adrenoceptor stimulation (De Jonge *et al.*, 1981, De Jonge *et al.*, 1982, Timmermans *et al.*, 1982, Docherty, 1988). These authors demonstrated that responses to NA in the presence of prazosin, and to α_2-adrenoceptor agonists, were reduced by captopril, while corresponding α_1-adrenoceptor mediated responses were unaffected. Such a selective interaction between A II and

postjunctional α_2-adrenoceptors is supported in the rabbit lateral saphenous vein, by the observation that responses to BHT-920 were resistant to prazosin only in the presence of A II (Schumman & Lues, 1983). However attractive such a proposal may seem, a number of observations suggest that this generalisation cannot be sustained. For example, it has been shown that A II increased contractile responses to BHT-920 in rabbit isolated thoracic aorta, but that these responses remained susceptible to prazosin (Lues & Schumman, 1984). There is also evidence suggesting that the inhibitory effect of ACE inhibitors depends not upon receptor subtype, but rather on the time course of the response produced by α-adrenoceptor agonists (O'Brien *et al.*, 1985). Indeed it has recently been reported that ACE inhibitors are more selective against the secondary component of responses to α-adrenoceptor agonists, irrespective of their selectivity for α-adrenoceptor subtype (Grant & McGrath, 1988b, MacLean & Hiley, 1988). Purdy & Weber (1988) have also demonstrated A II induced enhancement of responses to NA, mediated via α_1-adrenoceptors, in rabbit isolated femoral artery. This facilitation produced by A II, was greater if the α_1-adrenoceptor reserve was reduced with benextramine. Therefore no generalisation is apparent with regard to the interaction of A II and a particular α-adrenoceptor subtype. It is interesting to note, that the second phase of the pressor response to α-adrenoceptor agonists, and procedures which reduce receptor reserve, are associated with an increasing dependency of responses on extracellular Ca^{2+}. Since A II facilitates responses best under these circumstances, this suggests a possible causal link.

The Release of Catecholamines from the Adrenal Medulla

As early as 1940, A II, in a crude peptide preparation, was shown to cause the release of adrenal medullary catecholamines (Braun-Menendez *et al.*, 1940). This has subsequently been confirmed in a number of studies (for review see:

Peach, 1974), although recently Vollmer *et al.*, (1988) have demonstrated that ACE inhibitors do not reduce the output of catecholamines upon electrical stimulation of the adrenal gland, even when the renin-angiotensin system is highly activated, in the pithed rat. The physiological relevance of this action of A II is therefore questionable.

β-Adrenoceptor Mediated Production of Angiotensin II

In 1984 Kawasaki *et al.*, demonstrated that β-adrenoceptor mediated increases in responses to sympathetic nerve stimulation in the rat perfused mesenteric bed, could be blocked by captopril or saralasin. These results therefore suggested that β-adrenoceptor stimulation resulted in the production of A II, and that this agent subsequently increased stimulation-induced responses. This mechanism was confirmed in 1986 by Nakamura *et al.*, who observed an increase in immunoreactive A II in response to the β-adrenoceptor agonist isoprenaline and has also been shown to occur in the subendothelial layer of rat vena cava (Gothert & Kollecker, 1986). These results provide further evidence for an important role of local tissue production of A II, and the possibility that circulating catecholamines, particularly adrenaline, could potentiate their own contractile effects on vascular smooth muscle, by stimulating the production of A II with the subsequent pre- and postjunctional facilitatory action that this peptide produces.

It is therefore apparent that, in addition to producing direct vasoconstriction themselves, the sympathetic nervous and renin-angiotensin systems interact in a complex manner. This interaction involves A II increasing sympathetic function at the level of the central nervous system, peripheral sympathetic nerves and at α-adrenoceptors on vascular smooth muscle.

Aims of the study

One of the principal effects of angiotensin II (A II) in the cardiovascular system is to facilitate responses produced via the sympathetic branch of the autonomic nervous system. At the outset of the project, much of the available evidence indicated that A II produced such an effect by a dual action. There was a well documented enhancement of transmitter output via a prejunctional mechanism (Zimmerman, 1979) and secondly a postjunctional facilitation of responses also seemed to be involved, particularly on responses mediated via postjunctional α_2-adrenoceptors.

Such a selective interaction between A II and postjunctional α_2-adrenoceptors on vascular smooth muscle, was suggested from two seminal studies. Firstly, De Jonge *et al.*, (1984) demonstrated that angiotensin-converting enzyme (ACE) inhibitors selectively attenuated pressor responses to selective α_2-adrenoceptor agonists. Secondly in the rabbit isolated lateral saphenous vein contractions produced by B-HT 920 were sensitive to low concentrations of both prazosin and rauwolscine but, in the presence of A II, the response possessed characteristics consistent with an action at α_2-adrenoceptors. The selective α_1-adrenoceptor agonist phenylephrine was unaffected by A II. These latter findings suggested that A II may play an obligatory role in the functional expression of postjunctional α_2-adrenoceptors *in vitro*.

A number of other observations however, suggested that the above interpretation was too simplistic. Grant & McGrath (1988a,b) reported that ACE inhibitors modulated responses to α-adrenoceptor agonists independently of the subtype of α-adrenoceptor activated, but dependent upon the nature of the response to a bolus injection of a particular agonist. Similarly A II was observed to confer

α_1-adrenoceptor agonism on B-HT- 920 in the rabbit isolated thoracic aorta, a compound which in the absence of A II had acted as a weak α_1-adrenoceptor antagonist

All of these observations suggested that there were interesting interactions between A II and α-adrenoceptor agonists, which needed to be clarified if the physiological interaction between the renin-angiotensin and sympathetic nervous systems were to be understood. Initially I concentrated on seeking a greater haemodynamic characterisation of the interaction between ACE inhibitors and α-adrenoceptors in pithed rats, by measuring the additional parameter of cardiac ouput. It became apparent however, that this model was inappropriate to study these phenomena for a number of reasons. For example, bolus injections of α-adrenoceptor agonists produce biphasic pressor responses in the pithed rat, which could be differentially modulated. Responses to the bolus injection of an agonist do not represent an 'equilibrium response'. Furthermore, ACE inhibitors reduced basal blood pressure, an effect which complicated the interpretation of their interaction with postjunctional α-adrenoceptor-mediated responses. It was therefore more desirable to examine the effects of A II on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors in isolated vascular preparations. Of particular importance to us, was to use the endogenous ligand, NA, the definitive α-adrenoceptor agonist (Furchgott, 1972).

This was rather difficult however, since, at the outset of this project, one of the most paradoxical observations in adrenergic pharmacology, was the difficulty in clearly demonstrating isolated vascular preparations which contained postjunctional α_2-adrenoceptors, despite the relative ease of demonstrating α_2-adrenoceptor-mediated responses in whole animals (McGrath, 1982). The only convincing examples were to be found in the canine or human saphenous veins, the cat cerebral

artery or the human isolated digital artery, preparations which were very difficult to obtain on a regular basis. The starting point of this project therefore, was to find a suitable vascular preparation which contained a homogeneous population of postjunctional α_2-adrenoceptors from the rabbit. Having achieved this we would then have a good basis to examine the effects of A II (and other modulatory influences) on responses to NA mediated via these receptors. Since there was some evidence in the rabbit isolated lateral saphenous vein that postjunctional α_2-adrenoceptors were present (Alabaster *et al.*, 1985, Schumman & Lues, 1983), this vessel was chosen for study.

METHODS & MATERIALS

METHODS & MATERIALS

1) Isolated vascular smooth muscle preparations

A wide variety of vascular preparations were employed in a number of different experiments. Tissues were obtained from white albino rabbits of either sex, weighing between 2.0-3.0 kg, which were killed by stunning followed by exsanguination. Segments of the lateral saphenous vein, left renal vein, ear vein, distal saphenous artery, central ear artery, renal artery, femoral artery or superior mesenteric artery were cleaned of fat and connective tissue *in situ* and placed in ice-cold modified Krebs-Henselite solution (Krebs). When necessary, preparations were cleaned further with the aid of a dissecting microscope. 3-5 mm length segments were taken from each preparation and suspended between two 0.2 mm thick wire supports. The upper support was connected by cotton to a Grass FT03 isometric transducer while the lower support was connected to a glass tissue holder. The venous or arterial segments were mounted in 30 ml isolated organ baths, bathed in Krebs maintained at 37^{o}C, and gassed with 95% O_2 plus 5% CO_2. They were then placed under an initial tension as follows: lateral saphenous vein 2 g.wt., left renal vein 0.5 g. wt., ear vein 0.3 g. wt., distal saphenous artery 1.5 g. wt., central ear artery 2.0 g. wt., renal artery 2 g. wt., femoral artery 1.5 g. wt. or superior mesenteric artery 2 g. wt. Isometric contractions were measured by a Grass FT03 transducer connected to a Linseis 6052 pen recorder. A diagram of the experimental apparatus is shown in figure 3.

In all experiments, tissues were left to equilibrate for a 60 min period, during which time a steady resting tension was achieved. Each preparation was then exposed to 3μM NA and allowed to contract for 10 min. Exposure to a "sighting"

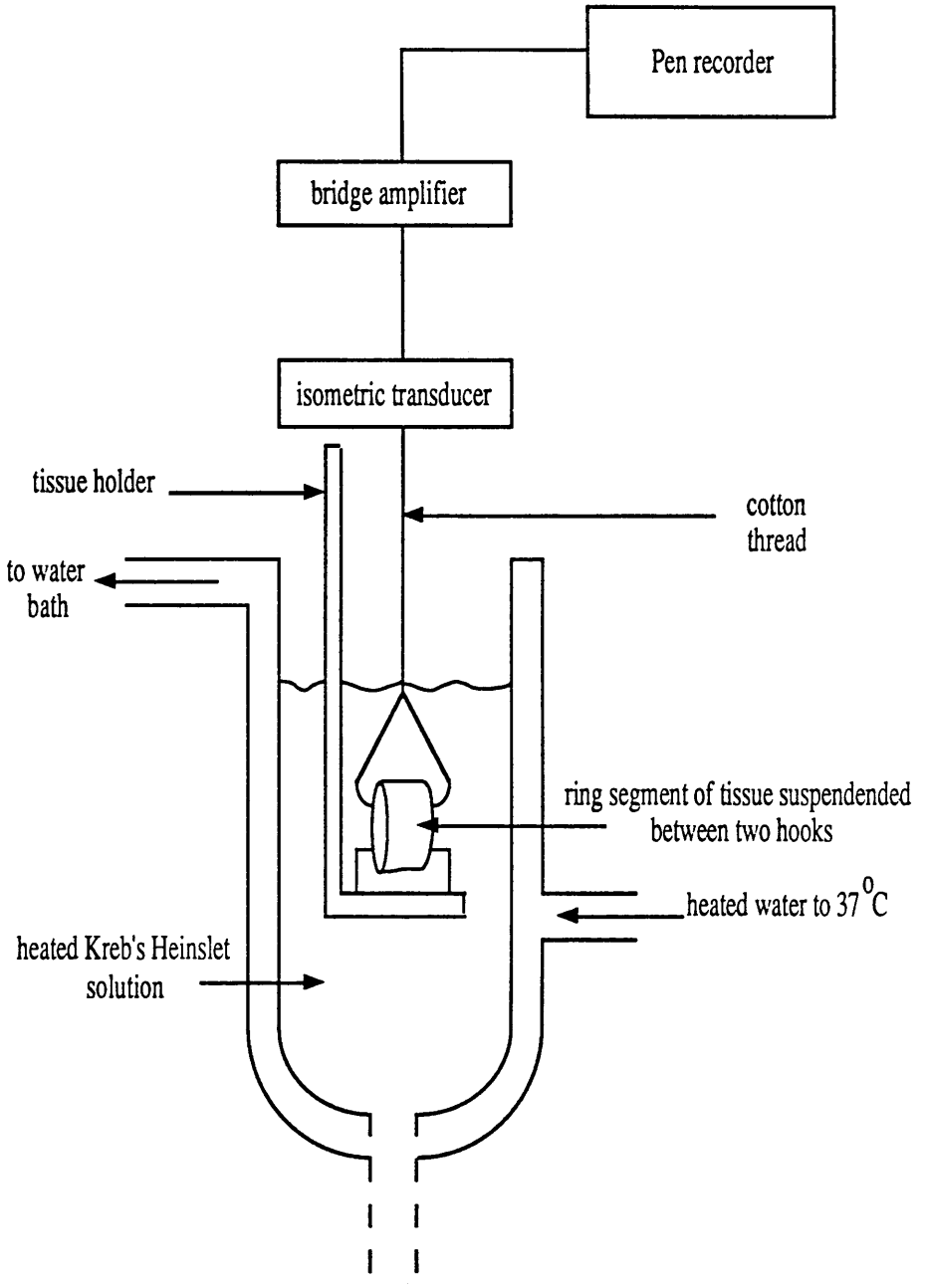


Figure No. 3

Diagrammatic representation of the experimental apparatus used in studying contractions of isolated vascular smooth muscle preparations.

concentration of an agonist minimised changes in the sensitivity of the preparations to further addition of agonists and is similar to the method used by Ruffolo *et al.*, (1979). Following complete washout, an additional one hour equilibration period was allowed before commencement of any other experimental procedure. Basal tension, following the sighting response, remained stable in all preparations for the rest of the experiment and was as follows: lateral saphenous vein 0.3 - 0.4 g.wt., left renal vein 0.15 - 0.2 g. wt., ear vein 0.05 - 0.1 g. wt., distal saphenous artery 0.7 - 0.9 g. wt., central ear artery 0.9 - 1.2 g. wt., renal artery 0.5 - 0.7 g. wt., femoral artery 0.7 - 0.9 g. wt. or superior mesenteric artery 0.7 - 0.9 g. wt.

2) Effect of agonists and antagonists

In order to examine the effects of various agonists and antagonists in rabbit blood vessels, the following standard protocol was adopted:

Cumulative concentration response curves (CCRC) to agonists were constructed by increasing the concentration of agonist in the bath by approximately 3-fold increments. When responses to agonists were not well maintained, addition of the next concentration was made as close to the peak as possible. An initial control CCRC, to any given agonist, was obtained in each preparation. Following attainment of the maximal control contraction, preparations were washed until complete relaxation was effected. The preparations were then left for a further period of 45-60 min before re-exposure to the agonist.

In experiments using reversible antagonists, these drugs were added to the organ bath at least 45 min prior to the onset of a second CCRC. In general only two CCRC's were obtained from each segment of a preparation, with the exception of receptor isolation experiments (see below) and some experiments in the left renal vein using phenoxybenzamine. In the latter, after obtaining an initial control CCRC

to NA, the receptor reserve was reduced by exposing the tissue to phenoxybenzamine (1 nM) for a period of 15 min. The tissues were subsequently washed at least four times and 30 min later a CCRC to NA was constructed. After complete washout, a 60 min period was allowed before a further CCRC was obtained. When examining the effects of angiotensin II, angiotensin I and Bay K 8644 on agonist responses, these drugs were added approximately 15 min prior to the onset of a CCRC to an agonist. In some experiments saralasin was added 10 min prior to A II.

3) Receptor isolation experiments

In receptor isolation experiments a control CCRC to NA was obtained in each preparation. Following washout, to effect complete relaxation, one of the following protocols was adopted:

a) Isolation of postjunctional α_2-adrenoceptors

Segments of lateral saphenous vein were exposed to 1μM rauwolscine 5 min prior to the addition of 0.3μM phenoxybenzamine. This combination of antagonists was allowed to remain in contact with the tissue for a further period of 30 min (total time: 35 min for rauwolscine; 30 min for phenoxybenzamine). The preparations were then washed a minimum of seven times over the period of one hour. During the first two washes 1μM rauwolscine was present in an attempt to maximise protection of postjunctional α_2-adrenoceptors. 20 min following the final washout, a CCRC to NA was obtained in each preparation. This CCRC to NA then served as an internal control in examining the effects of antagonists and of facilitatory agents such as A II on a third CCRC constructed 60 min later. The experimental and theoretical protocol for α_2-adrenoceptor isolation is represented

schematically in figure 4.

A very similar protocol was followed in an attempt to isolate postjunctional α_2-adrenoceptors in the distal saphenous artery. It was found necessary, however, to leave the combination of rauwolscine 1μM and phenoxybenzamine 0.3μM in contact with the tissue for a 60 min period (total times: rauwolscine 65 min; phenoxybenzamine 60 min) in order to effect complete removal of postjunctional α_1-adrenoceptors. In addition only one CCRC to an agonist was obtained in the distal saphenous artery after the isolation procedure.

b) Isolation of postjunctional α_1-adrenoceptors

Essentially, experiments attempting to isolate postjunctional α_1-adrenoceptors in the lateral saphenous vein followed the same protocol as those carried out in order to isolate postjunctional α_2-adrenoceptors. The exception was that YM 12617 was used to mask α_1-adrenoceptors (as opposed to rauwolscine masking α_2-adrenoceptors) thereby preventing phenoxybenzamine from irreversibly binding to α_1-adrenoceptors. Therefore, after obtaining an initial CCRC to NA, preparations were exposed to 0.1μM YM 12617 5 min before the addition of 0.3μM phenoxybenzamine and both antagonists were then washed out after a period of 30 min (total time: YM 12617 35 min; phenoxybenzamine 30 min). The preparations were then washed a minimum of 7 times over the period of one hour. YM 12617 was present during the first two washes in an attempt to maximise protection of α_1-adrenoceptors. 20 min following the final washout, a CCRC to NA was obtained in each preparation. The effects of antagonists and of facilitatory agents such as A II on responses to NA were examined on a further CCRC constructed 60 min later. The protocol for attempted isolation of postjunctional α_1-adrenoceptors is outlined in Figure 5.

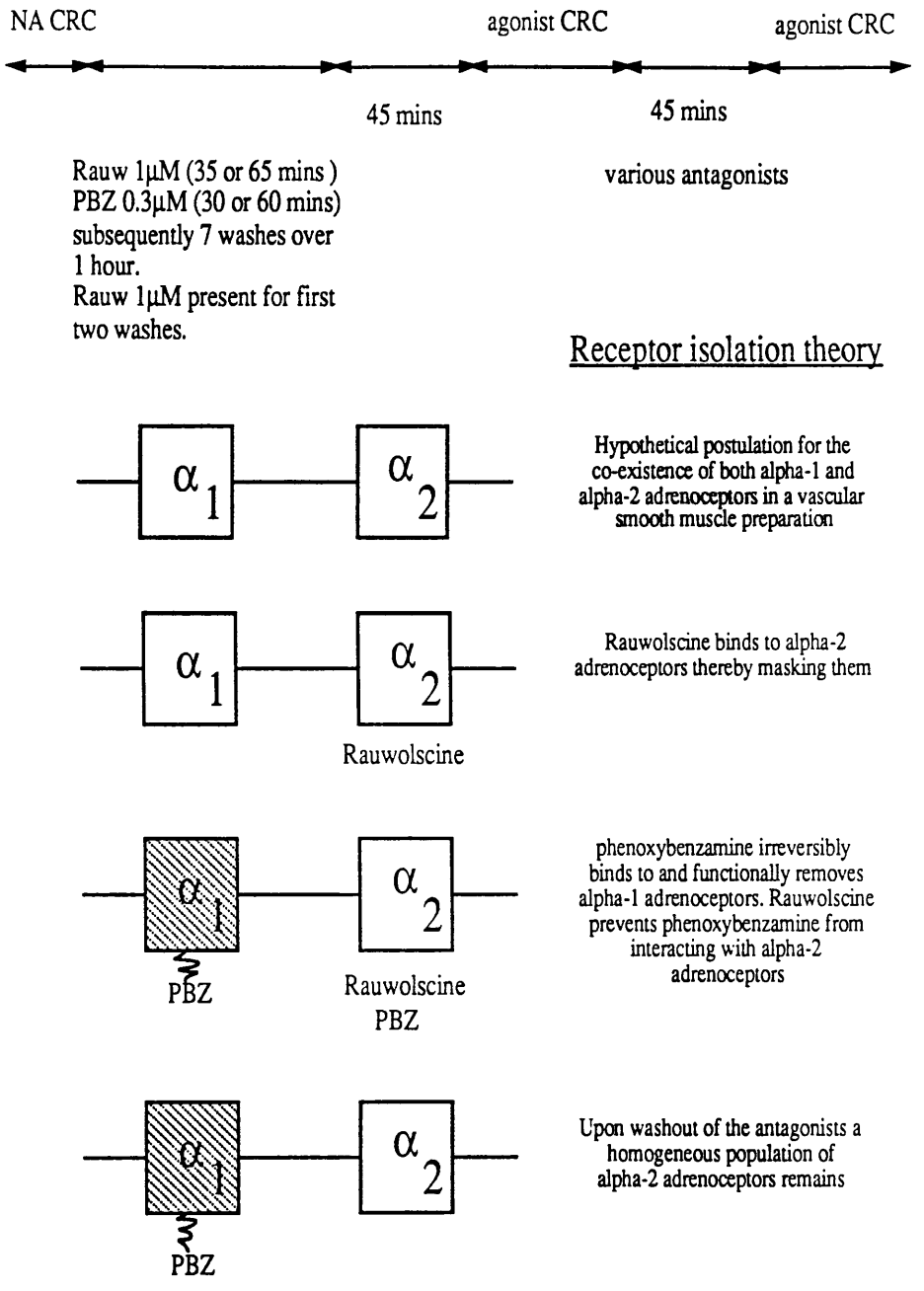


Figure No. 4

Experimental and theoretical protocol adopted in attempting to isolate postjunctional α_2-adrenoceptors.

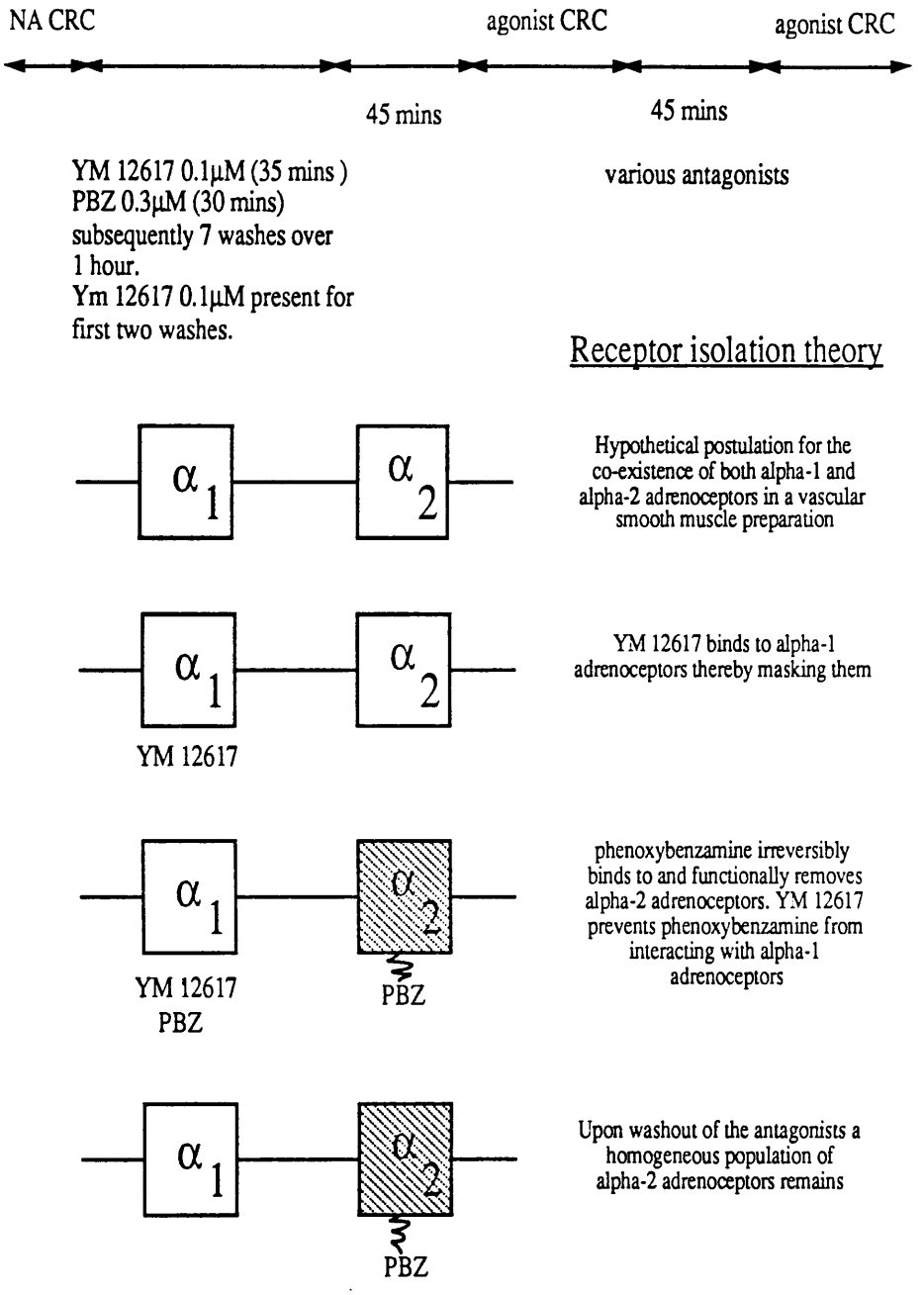


Figure No. 5

Experimental and theoretical protocol adopted in attempting to isolate postjunctional α_1-adrenoceptors.

4) Ca^{2+} readdition experiments

Some experiments, in the lateral saphenous vein, were carried out to analyse the effects of A II and Bay K 8644 on the influx of extracellular Ca^{2+} into the cell, stimulated by either depolarisation or by postjunctional α_2-adrenoceptor activation. Segments of lateral saphenous vein were set up as previously described and an initial CCRC to NA was obtained.

To examine responses upon the addition of extracellular Ca^{2+} in depolarised preparations, tissues were subsequently washed and bathed in nominally Ca^{2+}-free Krebs solution containing 65mM KCl (exchanged with NaCl on an equimolar basis). Subsequently a Ca^{2+} CCRC was obtained by adding Ca^{2+} to the organ baths in approximately 2-fold increments. Following attainment of the control maximum response, tissues were washed in nominally Ca^{2+}-free Krebs until complete relaxation was effected and allowed to bathe in this solution. A second Ca^{2+} CCRC was obtained 45-60 min later in the presence of A II or Bay K 8644.

In order to examine responses to the addition of extracellular Ca^{2+} in response to stimulation of postjunctional α_2-adrenoceptors, the following protocol was adopted:

After obtaining an initial CCRC to NA in the lateral saphenous vein, postjunctional α_2-adrenoceptors were isolated by the method previously described. Tissues were subsequently washed and allowed to bathe in nominally Ca^{2+}-free Krebs. A CCRC to Ca^{2+} was then constructed in the presence of NA 30μM, added 10 min prior to the onset of the Ca^{2+} CCRC. Following attainment of the control maximum response, tissues were washed in nominally Ca^{2+}-free Krebs until

complete relaxation was effected and allowed to bathe in this solution for 45 min. NA 30μM was then added to the organ baths 10 min before a second Ca^{2+} CCRC, in the presence of A II or Bay K 8644, was obtained. In each experiment, one preparation received no ancillary drugs and served as a time control.

5) Electrical field stimulation

Some experiments were carried out in order to determine the neurotransmitter(s) responsible, and the receptors through which the(se) transmitter(s) acted, to producing contractions in the distal saphenous artery, in response to electrical field stimulation.

Segments of distal saphenous artery were suspended between two 0.2 mm thick wire supports as previously described. The upper support was connected by cotton to a Grass FT03 isometric transducer, while the lower support was connected to a modified glass tissue holder. These modified tissue holders encompassed Linton small tissue, platinum plate, electrodes which were connected to a Square One Instruments electrical stimulator.

Tissues were exposed to a "sighting" concentration (3μM) of NA after 45 min. 15 min after complete washout of the NA, tissues were subjected to electrical field stimulation with the following parameters: 16 Hz for 1 second, pulse width 0.03 msec at a supramaximal voltage of 35V. Repetitive stimulation was applied, once every 5 min, until constant responses were achieved. Subsequently, a control frequency-response-curve (FRC), was obtained for each preparation. The FRC consisted of obtaining responses to the following frequencies of stimulation; 4, 8, 16, 32 and 64 Hz at a stimulation duration of 1 second and 4 and 8 Hz at a stimulation duration of 10 seconds.

After obtaining a control FRC, tissues were exposed to either, prazosin (0.1μM), rauwolscine (1μM), or α-,β-,methylene-ATP (3μM). The α-adrenoceptor antagonists were applied 30-45 min prior to the onset of a second FRC, while α-,β-,methylene-ATP was given 25-30 min before. After application of the various antagonists, these compounds remained in contact with that particular preparation for the remainder of the experiment. Further to the second FRC, an alternative antagonist was added cumulatively to the organ bath (eg.α-,β-, methylene-ATP may have been given to the preparation which had already received prazosin, prazosin to that having received rauwolscine etc.) in a random order, to examine the effects of a combination of two of the three antagonists.

In a seperate series of experiments, after obtaining a control FRC, tissues were exposed to two of the three aforementioned antagonists and a second FRC was obtained. The effects of A II (0.05μM) or of the third alternative antagonist were subsequently examined on the remaining residual response when a third FRC was obtained.

6) Calculation of results

Unless otherwise stated, responses to agonists are expressed as a percentage (mean ± s.e.mean) of the maximum response of the first control CCRC to any given agonist. In nerve stimulation experiments, results are expressed as a percentage of the response to 64 Hz obtained in the first FRC. Differences between means were considered statistically significant if $p < 0.05$ for either paired or unpaired observations - Student's *t*-test.

In examining the effects of antagonists, agonist concentration-ratio values were determined from the concentrations producing 50% of the maximum response in the absence and presence of each concentration of antagonist. In all experiments, one preparation was run in parallel with experimental tissues, but received no antagonist, and was used to determine time-dependent changes in agonist sensitivity (Furchgott, 1972). According to Arunlakshana & Schild (1959) if antagonism is competitive, a plot of the logarithm of dose ratio - 1 against the negative logarithm of the molar concentration of the antagonists, yields a straight line whose slope is one and the intercept along the abscissa scale is the pA_2, which is equal to the antagonist dissociation constant (K_B) under equilibrium conditions. Antagonism was considered competitive if the 95% confidence limits for the slope of the Schild plot, drawn by linear regression, overlapped unity. Concentrations of antagonists which did not consistently produce greater than 3-fold rightward displacements of the agonist CCRC were excluded from quantitative Schild analysis. In some experiments, the agonist concentration-ratio values in the presence of antagonist were determined at the 25%, 50% and 75% level of the maximum response to NA as described by Flavahan & Vanhoutte (1986).

7) Solutions & Drugs

The composition of the modified Krebs-Henselite solution was as follows (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11. Na_2EDTA was also included to prevent degradative oxidation of NA and as standard, with the exception of nerve stimulation experiments, propranolol (1μM) and cocaine hydrochloride (10μM) were also included to inhibit β-adrenoceptors and neuronal uptake of NA respectively. The nominally Ca^{2+}-free Krebs was identical to that above without Ca^{2+} being added. The following compounds were used:

prazosin HCl	(Pfizer);
rauwolscine HCl	(Roth);
YM 12617	(Yamanouchi);
CH 38083	(Chinoin);
UK-14304	(Pfizer);
(-)-phenylephrine HCl	(Sigma);
(-)-noradrenaline bitrate	(Sigma);
propranolol HCl	(Sigma);
cocaine HCl	(MacCarthys);
phenoxybenzamine HCl	(S,K&F);
angiotensin II amide	(Ciba);
Bay K 8644	(Bayer);
saralasin ([Sar^1,Ala^8]-angiotensin II)	(Sigma);
captopril	(Squibb);
cilazaprilat	(Roche);
5-Hydroxytryptamine creatinine sulphate	(Sigma);
U46619	(Upjohn);

nifedipine (Bayer).

With the following exceptions all drugs were dissolved in distilled water. NA was prepared in 23μM Na_2EDTA to prevent oxidative degradation. YM 12617, Bay K 8644 and nifedipine, as stock solutions of 1mM, were prepared in 20% absolute alcohol. Phenoxybenzamine (1mM) was also prepared in 20% absolute alcohol in distilled water and a drop of 1N HCl was added to remove turbidity. Further dilutions of YM 12617, Bay K 8644, nifedipine and phenoxybenzamine were made in distilled water.

YM 12617 (5-[2-[[2-(ethoxyphenoxy) ethyl] amino] propyl]-2-methoxy benzene-sulphonamide HCl);

CH 38083 (7,8-(methylenedioxi)-14-α-hydroalloberbane HCl);

UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate);

Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate);

U46619 (5Z,9α,13E,15S)-11,9-(Epoxymethano)prosta-5,13-dien-1-oic acid.

RESULTS

RESULTS

Alpha-adrenoceptors in the rabbit isolated lateral saphenous vein

Most of the evidence in the literature suggests that postjunctional α_2-adrenoceptors can be most easily demonstrated in isolated venous preparations. The rabbit isolated lateral saphenous vein has previously been reported to contain a homogeneous population of postjunctional α_2-adrenoceptors (Alabaster *et al.*, 1985), although other authors have not been able to demonstrate this unequivocably (Purdy *et al.*, 1980, Schumman & Lues, 1983, Daly *et al.*, 1988a). The present study therefore, re-examined the α-adrenoceptor population in this preparation and attempted to isolate different components of the response to the endogenous ligand NA.

Agonist potencies

The α-adrenoceptor agonists NA (non-selective), phenylephrine (selective α_1) and UK-14304 (selective α_2) produced concentration-dependent contractions in the rabbit lateral saphenous vein. The rank order of potencies for these agonists was as follows: UK-14304 $\geq$ NA $>$ phenylephrine. Based upon the maximum contractions, NA and phenylephrine can be classed as full agonists, while UK-14304 is a partial agonist compared to NA (Figure 6). The pD_2 and E_{max} values for each agonist are given in Table 1a.

Effects of α-adrenoceptor antagonists on responses to NA

Unfortunately, consecutive CCRC's to NA were not reproducible, there being a small but significant increase in the maximum response with time (Figure

7). Therefore, one preparation was run in parallel with experimental tissues, but received no ancillary drug, and was used to determine time-dependent changes in agonist responses.

As shown in Figure 8, the α_1-adrenoceptor antagonists, prazosin and YM 12617 produced concentration-dependent, rightward displacements of the NA CCRC. The pA_2 value for prazosin was 7.93, while that for YM 12617 was 8.36. For each antagonist the slope of the Schild plot was significantly different from unity, indicating non-competitive antagonism (Table 1b). Prazosin appeared to have a greater effect on the lower portion of the NA CCRC. The mean log agonist-concentration ratio, in the presence of 0.1μM prazosin, calculated at the 25% level of the maximum response to NA, was greater than that calculated at the 75% level, although this tendency was not significant (Table 2). Conversely, the rightward shift of the NA CCRC, produced by YM 12617, was similar at all levels of the NA CCRC (Table 2).

Increasing concentrations of the selective α_2-adrenoceptor antagonist rauwolscine, produced a non-parallel displacement of the NA CCRC (Figure 9a). A component of the response to NA, equivalent to approximately 20-35% of the maximum, was resistant to rauwolscine. Therefore, in contrast to prazosin, the log agonist-concentration ratio, in the presence of 0.5μM rauwolscine, was significantly greater at the 50 and 75% levels of the maximum response to NA, than it was at the 25% level (Table 2). Based upon a Schild plot of the agonist concentration-ratios at the 50% level of the maximum response, it was observed that the antagonism produced by rauwolscine was also non-competitive, with a pA_2 value of 8.41 (Table 1b). Interestingly, in approximately 30% of the preparations, the addition of rauwolscine to the bathing medium produced a small transient contraction (<15% of the maximum response to NA). This effect was never seen in

the presence of prazosin (0.1μM) or YM 12617 (0.1μM) (n = 5). Based upon pA_2 values, the potency order for the three antagonists in the saphenous vein was rauwolscine = YM 12617 > prazosin (Table 1b).

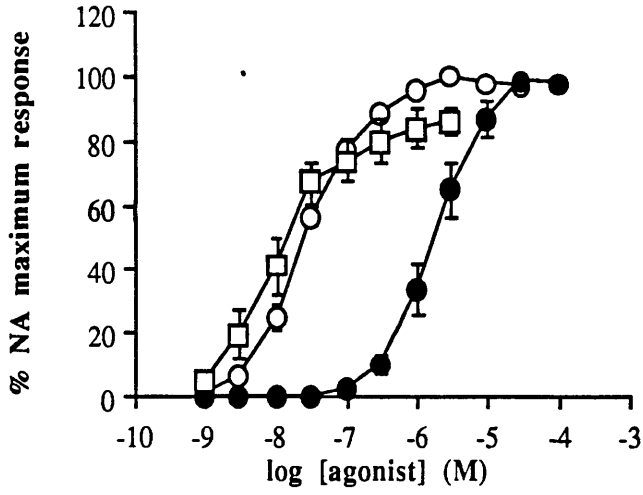
Figure 9b demonstrates the effect of varying concentrations of rauwolscine on responses to NA in the presence of prazosin (0.1μM). Under these conditions the component of the response to NA, which was previously resistant to rauwolscine, was not seen and the log agonist concentration ratio value (50% level of NA maximum response) was increased from 2.16 ± 0.08 in the presence of 2.5μM rauwolscine alone, to 2.53 ± 0.18 in the presence of the combination of 2.5μM rauwolscine and 0.1μM prazosin.

Attempted isolation of postjunctional α_2 -adrenoceptors

The previous results suggested the presence of both α_1- and α_2-adrenoceptors in the lateral saphenous vein. An attempt was therefore made to isolate a homogeneous population of postjunctional α_2-adrenoceptors using the protocol described in figure 4. Phenoxybenzamine (0.3μM) virtually abolished all responses to NA in this preparation (Figure 10a). The rationale employed therefore, was to prevent phenoxybenzamine binding to α_2-adrenoceptors by masking these receptors with 1μM rauwolscine. This concentration of rauwolscine was considered to be without effect at α_1-adrenoceptors, since even in the presence of the higher concentration of 2.5μM, a component of the response to NA which was susceptible to prazosin, remained resistant to rauwolscine (Figure 9).

Following the inclusion of rauwolscine (1μM) before, and during, the incubation period with phenoxybenzamine (0.3μM), a large component of the response to NA was spared. The maximum response to NA was significantly increased after the protection protocol, being 67.3 ± 4.5 compared to 5.8 ± 2.7 of

a)



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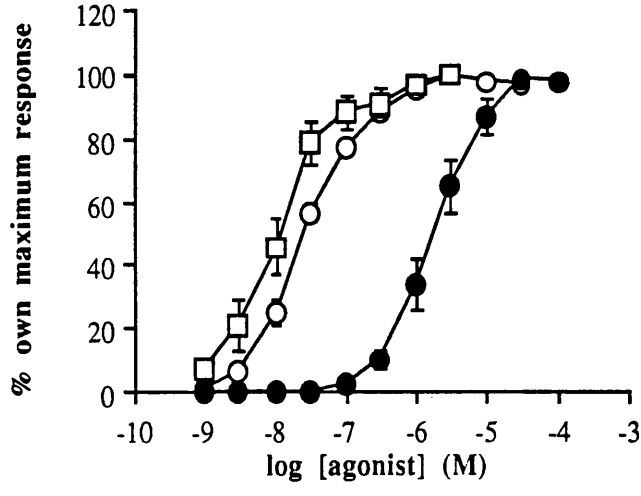
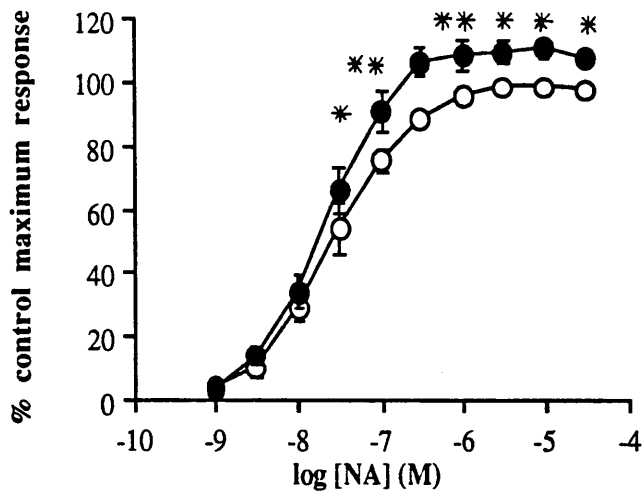


Figure No. 6

Concentration-dependent responses to the α-adrenoceptor agonists NA (○), UK-14304 (□) and phenylephrine (●) in rabbit isolated lateral saphenous vein. Results are expressed as a % of **a)** the maximum response to NA in each preparation or **b)** as a % of the maximum response to each individual agonist.

Each point represents the mean ± s.e.mean (n = 8 -11).

a)



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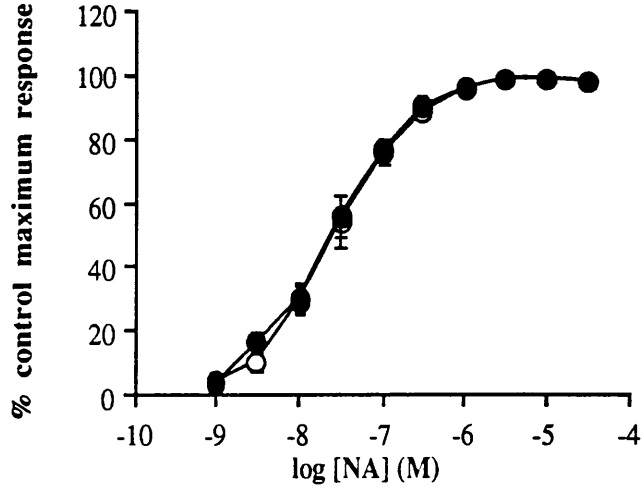
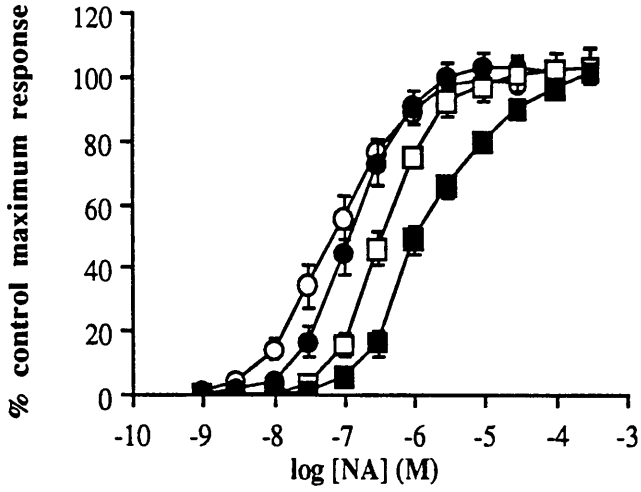


Figure No. 7

Reproducibility of two consecutive concentration-response curves to NA in rabbit isolated lateral saphenous vein expressed as: **a)** a % of the maximum response of the first CCRC to NA or **b)** a % of the maximum response of each individual CCRC. The 1st CCRC to NA is represented by (○) while (●) represents the 2nd CCRC.

Each point represents mean ± s.e.mean (n =24). Statistically significant differences between the 1st and 2nd CCRC's are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

a)



b)

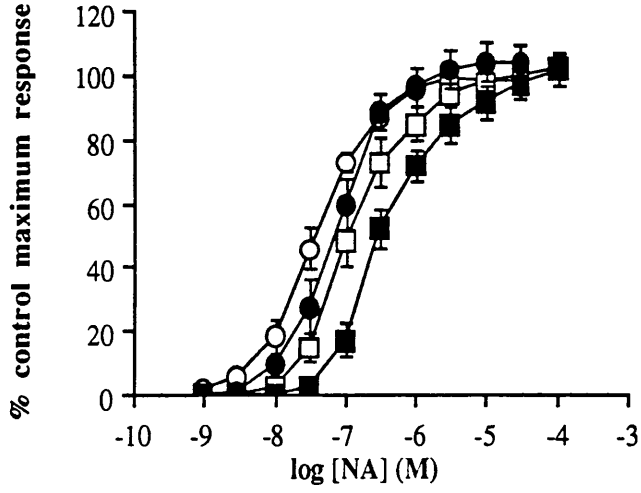
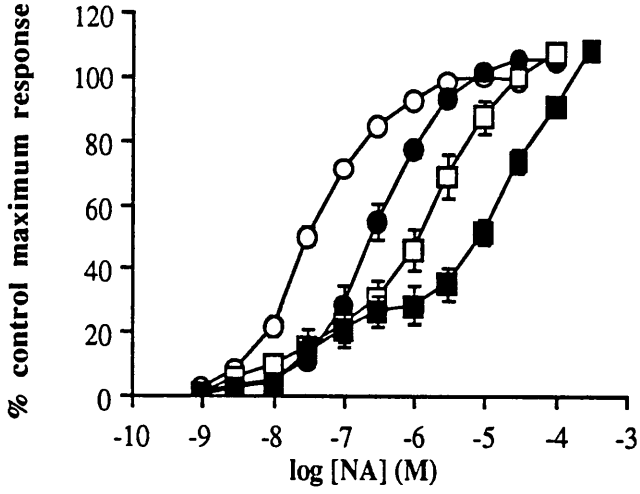


Figure No. 8

Effects of the α_1-adrenoceptor antagonists **a)** prazosin 0.01μM (●), 0.1μM (□), 1μM (■) and of **b)** YM 12617 0.01μM (●), 0.1μM (□), 1μM (■) on responses to NA (○) in rabbit isolated lateral saphenous vein. Each point represents the mean ± s.e.mean (n = 5-12).

a)



b)

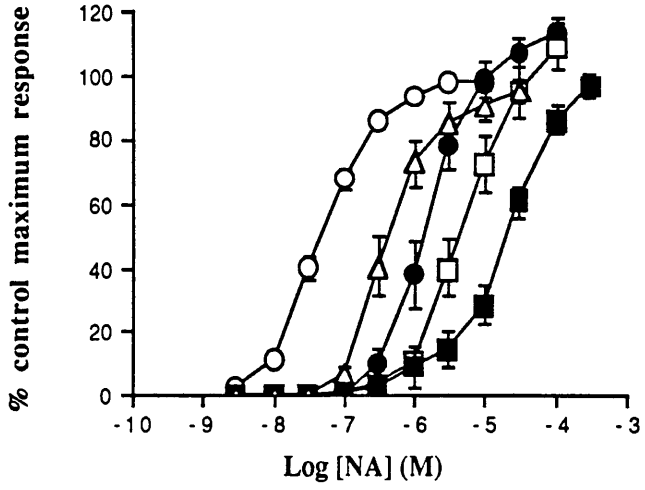


Figure No. 9

Effect of the α_2-adrenoceptor antagonist rauwolscine 0.05μM (●), 0.5μM (□) and 2.5μM (■) on responses to NA (○) in **a)** the absence and **b)** the presence of 0.1μM prazosin in rabbit isolated lateral saphenous vein. The effect of prazosin 0.1μM (△) alone against responses to NA is also shown.

Each point represents mean ± s.e.mean (n = 4-7)

a)	*Agonist*	*pD_2*	*E_{max}*
	NA	7.53 ± 0.08	1
	(n = 8)		
	phenylephrine	5.83 ± 0.06	0.95 ± 0.02
	(n = 7)		
	UK 14304	7.85 ± 0.16	0.86 ± 0.04
	(n = 8)		

b)	*Antagonist*	*pA_2*	*Slope*
	prazosin	7.93	0.62
	(0.1 - 1μM)	(8.37 - 7.57)	(0.51 - 0.72)*
	YM 12617	8.36	0.47
	(0.1 - 1μM)	(8.81 - 7.91)	(0.34 - 0.60)*
	rauwolscine	8.41	0.80
	(0.05 - 2.5μM)	(8.60 - 8.22)	(0.74 - 0.86)*

Table 1

a) List of pD_2 values (with 95% confidence limits) and E_{max} values for α-adrenoceptor agonists; and of b) pA_2 values with the slopes of the Schild plots (with 95% confidence limits) for α-adrenoceptor antagonists against responses to NA in rabbit isolated lateral saphenous vein in the presence of propranolol (1μM) and cocaine (10μM). pA_2 values were determined from a regression analysis of the log agonist concentration ratio from 18-24 individual observations. * denotes slope of Schild plot significantly different from unity.

Antagonist	*25%*	*50%*	*75%*
prazosin (0.1μM) (n = 12)	0.81 ± 0.07	0.69 ± 0.07	0.64 ± 0.10
YM 12617 (0.1μM) (n = 5)	0.69 ± 0.20	0.69 ± 0.12	0.71 ± 0.16
rauwolscine (0.5μM) (n = 5)	1.47 ± 0.14	1.70 ± 0.12	1.85 ± 0.09[a]

Table 2

log agonist concentration-ratio values for NA measured at the 25%, 50% and 75% level of the maximum response to NA, in the presence of prazosin (0.1μM), YM 12617 (0.1μM) or rauwolscine (0.05μM)

[a] denotes a significant difference in the value for rauwolscine (0.5μM) calculated at the 25% and 75% levels of the maximum response to NA. $p<0.05$

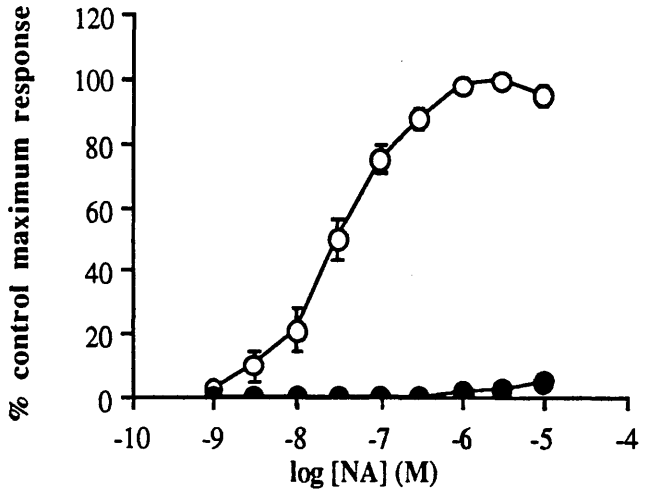
the control maximum response, with phenoxybenzamine alone (Figure 10). Consecutive CCRC's to NA were reproducible with time after the protection protocol (Figure 11).

Effects of α-adrenoceptor antagonists after attempted isolation of postjunctional α_2-adrenoceptors

The residual response to NA, remaining after the combination of rauwolscine and phenoxybenzamine, was relatively resistant to the selective α_1-adrenoceptor antagonists YM 12617 and prazosin (Figure 12a). The log agonist concentration-ratio values for NA in the presence of YM 12617 (0.1μM) or prazosin (0.1μM) were reduced markedly, after the protection procedure, compared to control values (Table 3). Figure 13 shows representative trace recordings of the effects of 0.1μM prazosin on NA-induced contractions under normal experimental conditions and after attempted isolation of postjunctional α_2-adrenoceptors.

In contrast to the α_1-adrenoceptor antagonists, rauwolscine (1μM) produced a marked rightward displacement of the NA CCRC after the protection protocol (Figure 12b. Table 3), with an estimated -logK_b value of 7.98. It was found necessary to use this lower concentration of α_2-adrenoceptor antagonist, since the rightward displacement produced by 2.5μM rauwolscine was sufficiently great that it prevented the calculation of a -logK_b value. Coincident with this protective effect of rauwolscine against phenoxybenzamine, was the removal of the component of the response to NA, which was "rauwolscine resistant" (compare Figure 9a with Figure 12b).

a)



b)

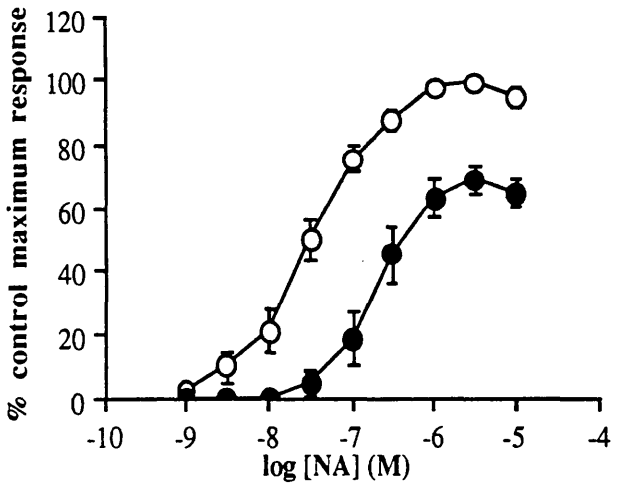
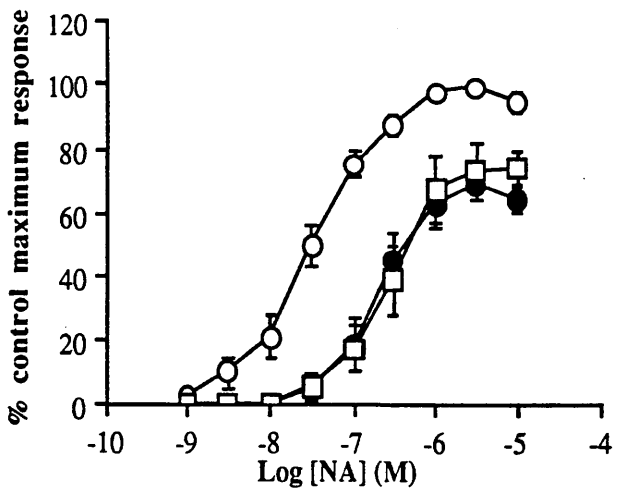


Figure No. 10

Effect of phenoxybenzamine (0.3μM) (●) on responses to NA (○) **a)** in the absence and **b)** in the presence of the α_2-adrenoceptor antagonist rauwolscine (1μM), in the rabbit isolated lateral saphenous vein.

Each point represents mean ± s.e.mean (n = 8). All responses to NA after treatment with the combination of rauwolscine (1μM) and phenoxybenzamine (0.3μM) are significantly different from those in the presence of phenoxybenzamine alone (0.3μM), $p<0.05$ Student's *t*- test.

a)



b)

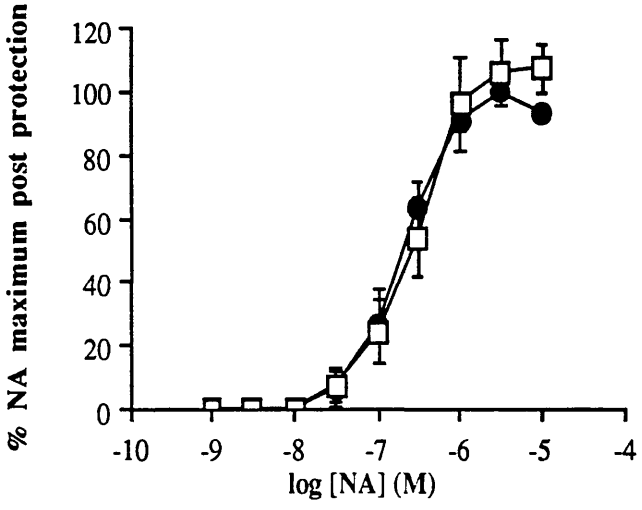
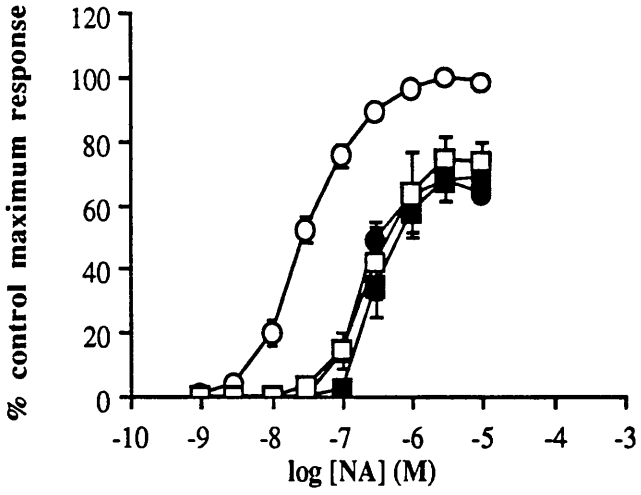


Figure No. 11

Reproducibilty of responses to NA in rabbit isolated lateral saphenous vein after attempted isolation of postjunctional α_2-adrenoceptors. Results are expressed in two ways: **a)** as a % of the initial maximum response to NA (○) or **b)** as a % of the maximum response to NA after the combination of rauwolscine and phenoxybenzamine. (●) represents the first CCRC to NA after the protection protocol, while (□) represents the second.
Each point represents mean ± s.e.mean (n = 8).

a)



b)

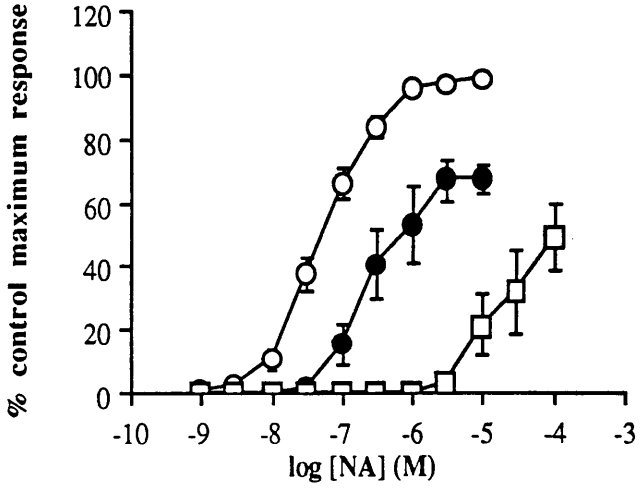


Figure No. 12

Effect of α-adrenoceptor antagonists on responses to NA (●) after attempted isolation of postjunctional α_2-adrenoceptors in rabbit isolated lateral saphenous vein. **a)** Effect of the α_1-adrenoceptor antagonists prazosin (0.1μM) (□) and YM 12617 (0.1μM) (■). **b)** Effect of rauwolscine (1μM) (□). Results are expressed as a % of the maximum response to NA (○) prior to the protection protocol.

Each point represents mean ± s.e.mean (n = 6 - 8).

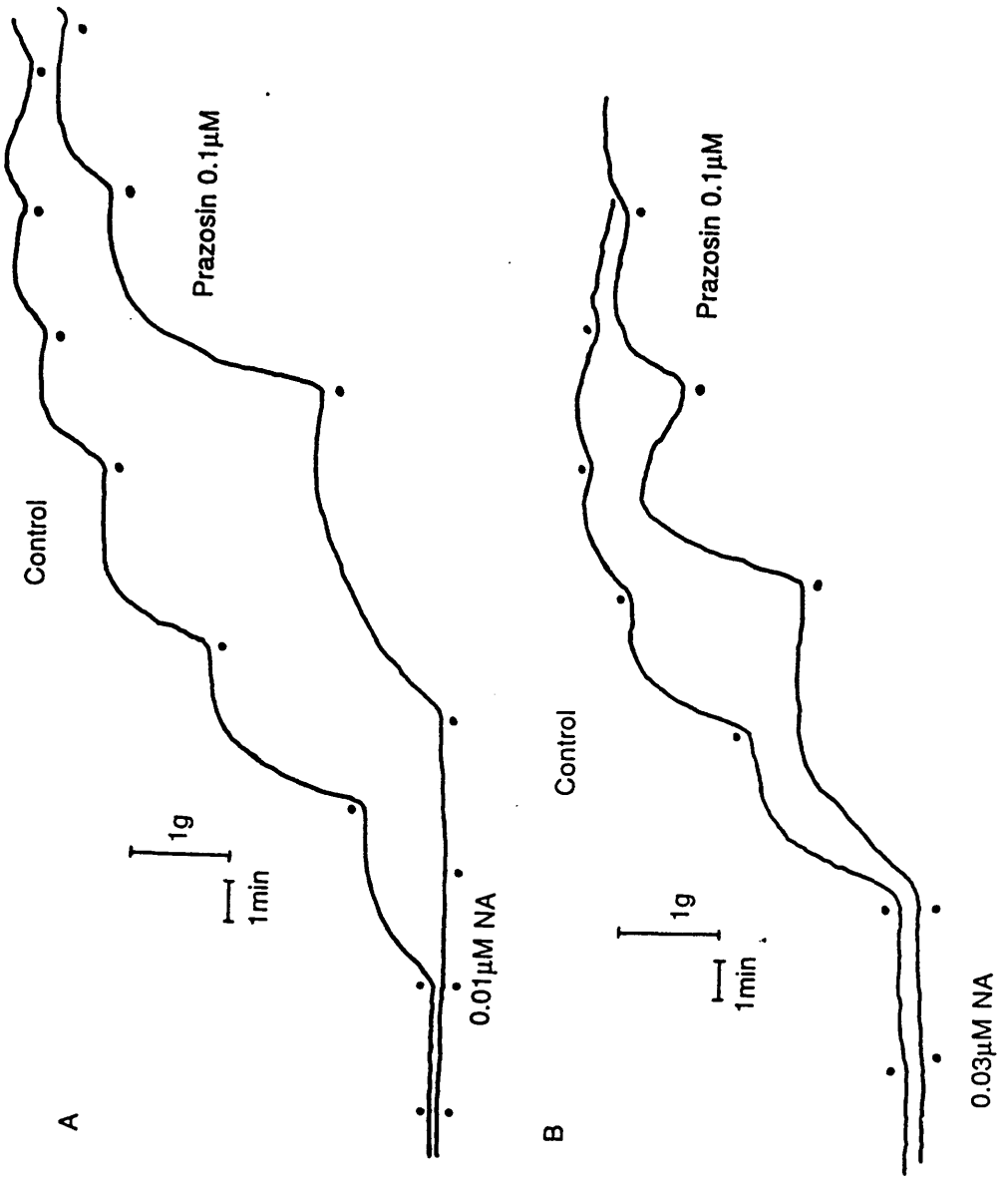


Figure No. 13

Representative trace recording of the effect of prazosin (0.1μM) on NA-induced contractions (cumulative addition; approximately 3 fold increments in concentration) of the rabbit isolated lateral saphenous vein. **a)** under normal experimental conditions and **b)** following attempted isolation of postjunctional α_2-adrenoceptors with the combination of rauwolscine (1μM) and phenoxybenzamine (0.3μM).

Attempted isolation of postjunctional α_1-adrenoceptors

The rationale behind this protocol was to protect the population of α_1-adrenoceptors from phenoxybenzamine with YM 12617 (see Figure 5). This α_1-adrenoceptor antagonist prevented the complete abolition of responses caused by phenoxybenzamine (0.3μM) alone (Figure 14a). In the presence of 1μM YM 12617 a residual response to NA of 57.5 ± 5.8% remained, while 0.1μM prevented the abolition of 32.2 ± 5.6% of the maximum response to NA. Subsequent experiments were carried out using the lower concentration of the antagonist. This concentration was chosen, since 0.1μM YM 12617 has been reported to be inactive at both prejunctional α_2-adrenoceptors, in the rat isolated vas deferens (Honda *et al.*, 1985), and postjunctional α_2-adrenoceptors in the rabbit isolated ear vein (Daly *et al.*, 1988b).

In approximately 25% of preparations, particularly those from young animals (< 2.3kg.), the responses were less than 15% of the original NA maximum, poorly maintained and subject to changes unrelated to the addition of NA to the bathing medium. These preparations were discarded from the subsequent quantitative analysis. The residual response in the remaining preparations treated with 0.1μM YM 12617 and 0.03μM phenoxybenzamine, varied from 20 to 40% of the control maximum response. Unfortunately, successive CCRC's to NA following the protection protocol were not reproducible, there being an approximately 30% increase in the maximum response (Figure 15).

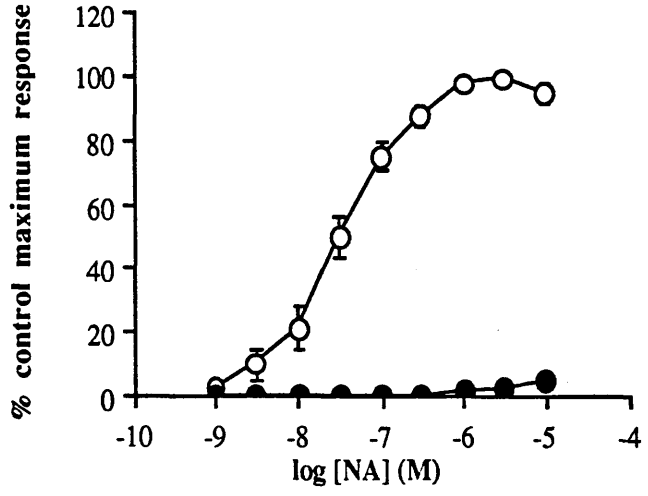
Effects of α-adrenoceptor antagonists after attempted isolation of postjunctional α_1-adrenoceptors

YM 12617 produced a concentration-dependent rightward shift in the

CCRC to NA after attempted isolation of postjunctional α_1-adrenoceptors (Figure 16a). The antagonism produced by YM 12617 appeared not to be competitive. The log agonist-concentration ratio for NA in the presence of 0.01μM YM 12617 was 0.16 ±0.06 (n = 6), while a 10-fold higher concentration of YM 12617 produced only a 5-fold greater rightward displacement, 0.67 ± 0.10 (n = 11) (Table 3).

Surprisingly, following receptor protection with 0.1μM YM 12617, rauwolscine (2.5μM) produced a significant leftward shift of the NA CCRC (Figure 16b). This potentiating action of rauwolscine on the residual response to NA was abolished by YM 12617 (0.1μM), and the combination of the two antagonists produced a greater inhibition of responses to NA, than either antagonist alone (Figure 17a). The log agonist-concentration ratio value, in the presence of both rauwolscine (1μM) and YM 12617 (0.1μM), was increased to 0.93 ± 0.10 (n = 5). In contrast to rauwolscine, the selective α_2-adrenoceptor antagonist CH 38083 (1μM) (Vizi *et al.*, 1986), produced a significant rightward displacement of the NA CCRC following attempted isolation of postjunctional α_1-adrenoceptors. This was associated with the uncovering of a small 'resistant' component of the response to NA (Figure 17b).

a)



b)

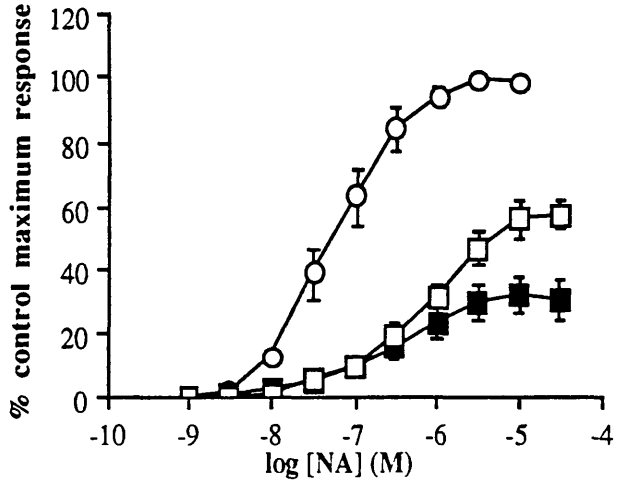
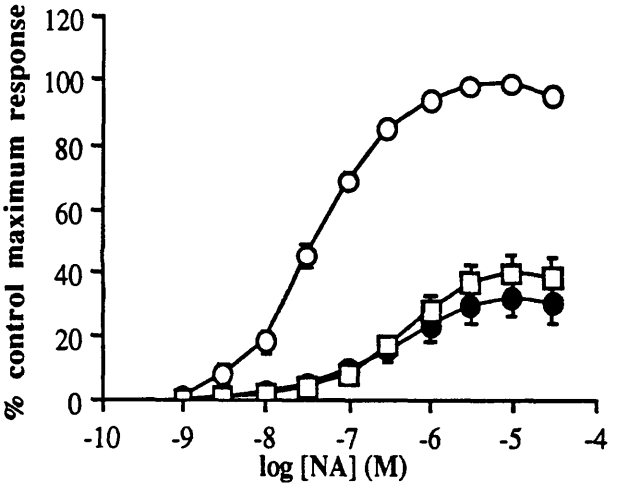


Figure No. 14

Effect of prior treatment with phenoxybenzamine (0.3μM) on responses to NA (○) **a)** in the absence (●) and **b)** in the presence of the α_1-adrenoceptor antagonist YM 12617 (1μM) (□) and (0.1μM) (■), in the rabbit isolated lateral saphenous vein.

Each point represents mean ± s.e.mean (n = 4 - 11). All responses to NA after treatment with the combination of YM 12617 (0.1-1μM) and phenoxybenzamine (0.3μM) are significantly different from those in the presence of phenoxybenzamine alone (0.3μM), $p<0.05$ Student's *t*- test.

a)



b)

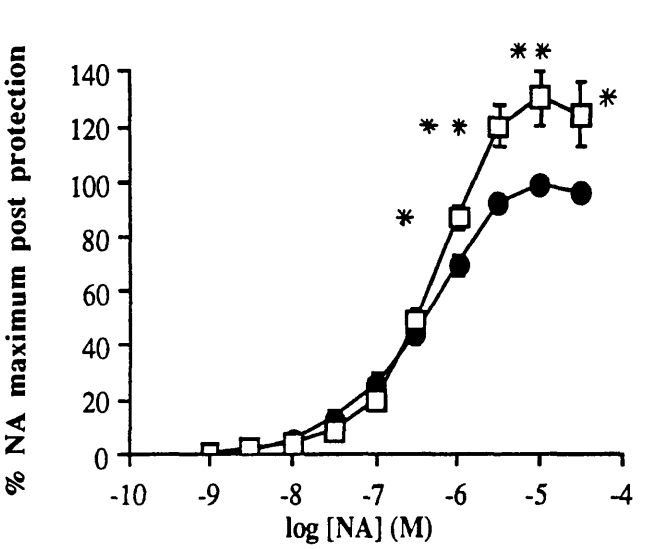
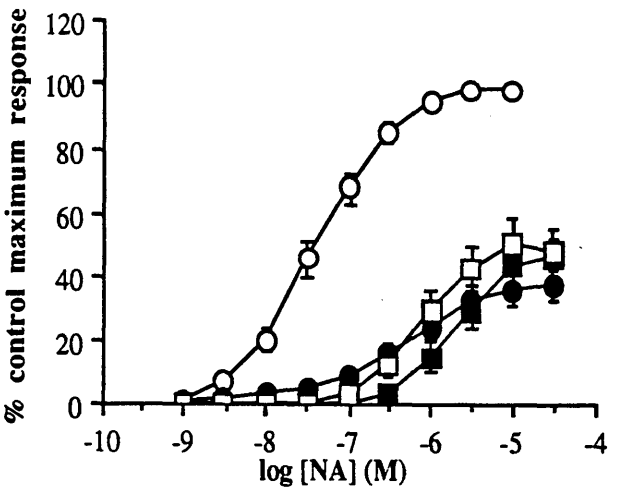


Figure No. 15

Reproducibility of responses to NA in rabbit isolated lateral saphenous vein after attempted isolation of postjunctional α_1-adrenoceptors. Results are expressed in two ways **a)** as a % of the initial maximum response to NA (○) or **b)** as a % of the maximum response to NA after the combination of YM 12617 and phenoxybenzamine. (●) represents the first CCRC to NA after the protection protocol, while (□) represents the second.

Each point represents mean ± s.e.mean (n = 11). Statistically significant differences between the 1st and 2nd CCRC's after the protection protocol are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

a)



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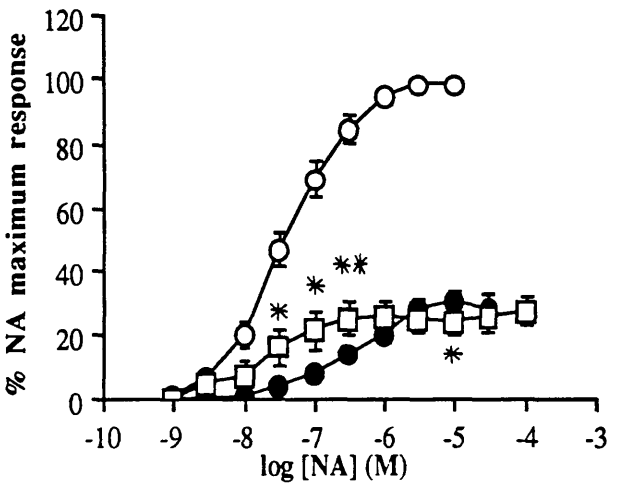
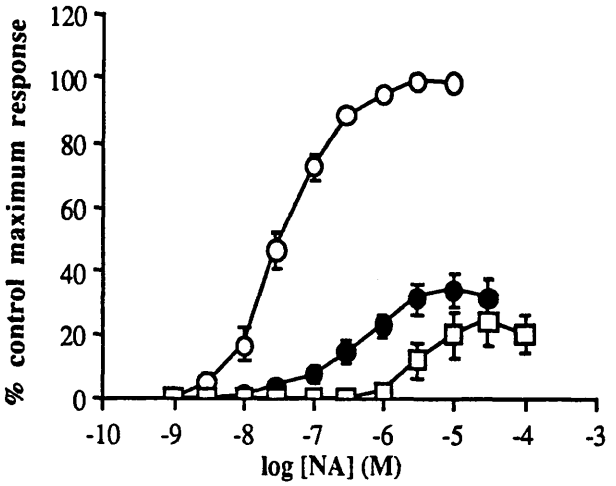


Figure No. 16

Effect of α-adrenoceptor antagonists on responses to NA (●) after attempted isolation of α_1-adrenoceptors in rabbit isolated lateral saphenous vein. **a)** the effect of the α_1-adrenoceptor antagonist YM 12617 (0.01μM) (□) and (0.1μM) (■). **b)** the effect of the α_2-adrenoceptor antagonist rauwolscine (1μM) (□). Results are expressed as a percentage of the maximum response to NA (○) prior to the protection protocol.

Each point represents mean ± s.e.mean (n = 6 - 11). Statistically significant differences between responses in the absence and presence of rauwolscine (1μM) (Figure b) are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

a)



b)

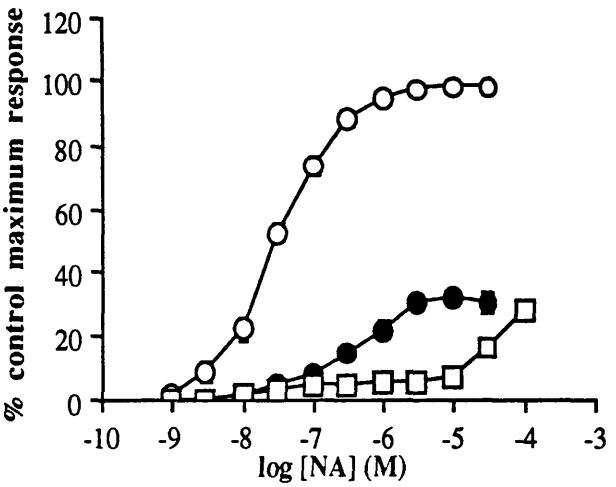


Figure No. 17

a) Effect of the combination of YM 12617 (0.1μM) and rauwolscine (1μM) (□) and **b)** of the α_2-adrenoceptor antagonist CH 38083 (1μM) (□) on responses to NA (●) after attempted isolation of postjunctional α_1-adrenoceptors in rabbit isolated lateral saphenous vein. Results are expressed as a % of the maximum response to NA (○) prior to the protection protocol.

Each point represents mean ± s.e.mean (n = 5).

Antagonist	*control*	*α_1-isolation*	*α_2-isolation*
prazosin	0.69 ± 0.07	n. a.	0.27 ± 0.07*
(0.1μM)	(n = 12)		(n = 6)
YM 12617	0.69 ± 0.12	0.67 ± 0.10	0.26 ± 0.06*
(0.1μM)	(n = 5)	(n = 11)	(n = 5)
rauwolscine	2.16 ± 0.08	n. p.	1.98 ± 0.11[a]
(2.5μM)	(n = 4)		(n = 6)

Table 3

Log agonist concentration-ratio values for NA in the presence of various antagonists, under control conditions and after attempted isolation of postjunctional α_1- and α_2-adrenoceptors respectively. *denotes a significant difference from control values ($p < 0.01$).

n. a. - this experiment was not attempted.

n. p. - this value could not be calculated since rauwolscine enhanced responses to NA under these conditions.

[a] - this log agonist concentration ratio was obtained with 1μM rauwolscine.

Comparison of the effects of A II and Bay K 8644 on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors in vascular smooth muscle

It has been suggested from whole animal studies that pressor responses mediated via α_2-adrenoceptor stimulation, but not via α_1-adrenoceptor activation, can be selectively attenuated by calcium entry blockers (van Meel *et al.*, 1981) and by angiotensin-converting enzyme inhibitors (De Jonge *et al.*, 1981, 1982). There is evidence however, that this differential attenuation may be depend, not on receptor subtype, but on the nature of the response produced by a given agonist (O'Brien *et al.*, 1985, Grant & McGrath, 1988b, MacLean & Hiley, 1988). Because of these conflicting observations, the present study examined the effects of angiotensin II (A II) and the calcium channel facilitator Bay K 8644 on responses to NA, mediated via postjunctional α_1- and α_2-adrenoceptors, *in vitro*. Three isolated venous preparations, with differing α-adrenoceptor populations were used: the lateral saphenous vein, which contains a mixture of both α-adrenoceptors; the lateral saphenous vein after attempted isolation of homogeneous populations of both α_1- and α_2-adrenoceptors; the ear vein, which contains almost exclusively postjunctional α_2-adrenoceptors (Daly *et al.*, 1988b) and the left renal vein, which contains a homogeneous population of α_1-adrenoceptors (Daly *et al.*, 1988c).

Lateral Saphenous Vein

Effects of A II and Bay K 8644 on responses to NA

A II produced a concentration-dependent contraction in the lateral saphenous vein (Figure 18a). Responses to higher concentrations of A II, consisted of an initial rapid response which transiently returned to baseline after 6-8 mins. Lower

concentrations produced contractions which were slower in onset and longer lasting, returning to baseline after 12-15 mins. Baseline tension remained stable after the transient contraction produced by A II, for at least the duration of the experiment.

A II (0.05-0.5μM) had no effect on responses to NA when the full receptor complement was present in the lateral saphenous vein (Figure 19a). In contrast Bay K 8644 (0.03μM), a concentration which had no effect on resting baseline tension, increased both the sensitivity and maximum response of the preparation to NA (Figure 19b, Table 5). Higher concentrations of Bay K 8644 (>0.3μM) induced spontaneous contractions in the lateral saphenous vein.

Effects of A II and Bay K 8644 on α-adrenoceptor antagonist potency

In a previous study in the rabbit lateral saphenous vein, it was reported that A II made responses to the reportedly selective α_2-adrenoceptor agonist, BHT-920, more resistant to prazosin (Schumman & Lues, 1983), suggesting a selective facilitation of α_2-adrenoceptor-mediated responses. The effects of A II (and of Bay K 8644) on the antagonism produced by α-adrenoceptor antagonists against responses to NA were therefore examined.

As mentioned previously both prazosin and rauwolscine non-competitively antagonised responses to NA in this preparation under normal experimental conditions (Figures 8,9 Table 1). A II (0.05μM) reduced the potency of the selective α_1-adrenoceptor antagonist prazosin, particularly on the lower portion of the CCRC to NA (Figure 20). The log agonist-concentration ratio for NA, in the presence of 0.1μM prazosin, measured at the 25% level of the maximum response was significantly reduced, although this was less marked at the 50 and 75% levels

(Table 4). Although the pA_2 value for prazosin, in the presence of A II, was reduced from 8.19 (8.67-7.72) to 7.55 (7.84-7.26), the slope of the Schild plot was still significantly different from unity, 0.81 (0.68-0.93). In contrast to the marked effects on prazosin antagonism in the lateral saphenous vein, A II (0.05μM) was without effect on the antagonism produced by the selective α_2-adrenoceptor antagonist, rauwolscine (Figure 21, Table 4). Bay K 8644 (0.03μM) had a pronounced effect on the ability of prazosin to antagonise responses to NA (Figure 22). The log agonist-concentration ratio value for NA in the presence of prazosin (0.1μM), measured at all levels of the maximum response to NA was significantly reduced (Table 4).

Lateral saphenous vein after α_2-adrenoceptor isolation

After isolation of postjunctional α_2-adrenoceptors, both A II and Bay K 8644 markedly enhanced responses to NA (Figures 23, 24, Table 5). This was associated with an increase in both the sensitivity and maximum response of the preparation to NA. It is interesting to note that this facilitatory action of A II was observed with either 0.05μM or 5nM A II, the latter concentration producing a small contraction in only 2 of 6 preparations. The potentiation of α_2-adrenoceptor-mediated responses produced by A II, was reversed by the angiotensin receptor antagonist, saralasin (0.01 - 0.1μM) (Figure 25). Neither concentration of saralasin alone, had an effect on the residual response to NA (Figure 25a). Interestingly, despite the fact that both concentrations of saralasin abolished the contractile response to A II (0.05μM) (Fig, 18b), only the higher concentration of the antagonist was associated with a complete reversal of the facilitatory action of A II (Figure 25b,c). After attempted isolation of postjunctional α_1-adrenoceptors in the lateral saphenous vein, A II (0.05μM) produced only a small leftward shift in the CCRC to NA (Figure 26).

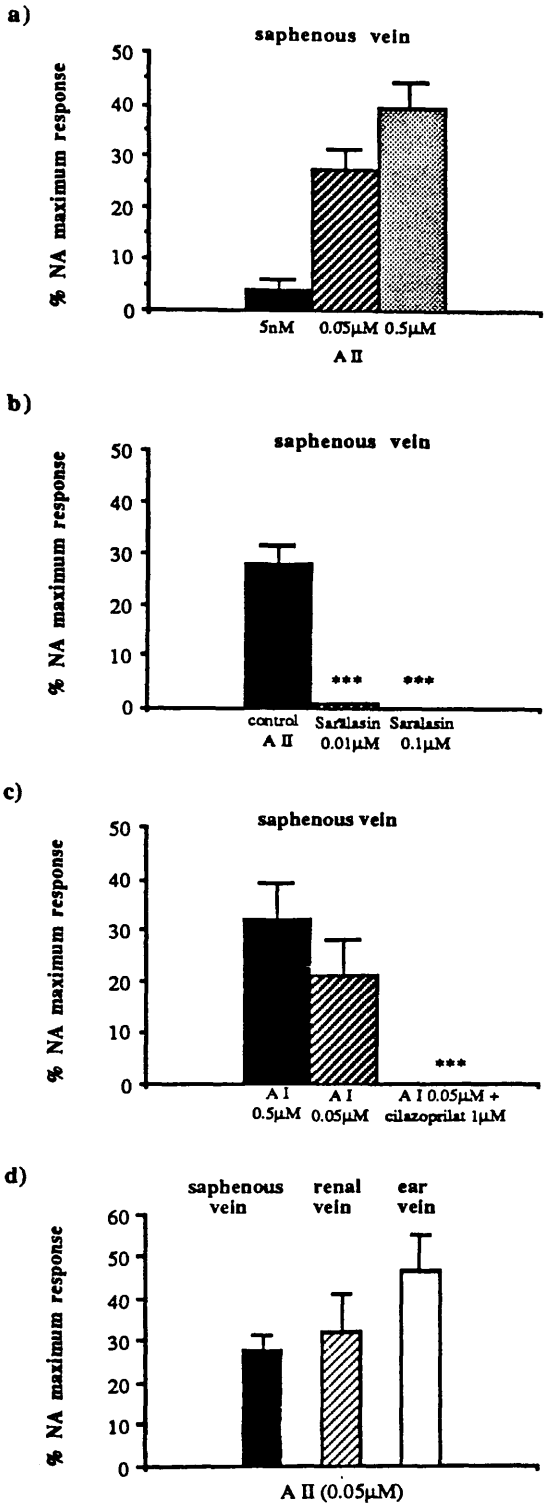


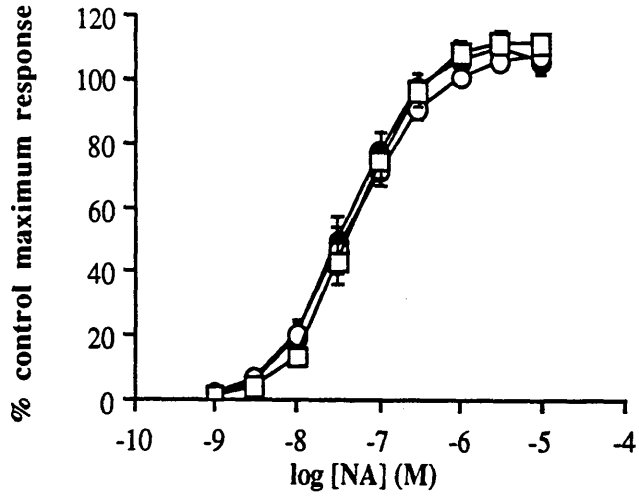
Figure No. 18

The peak contractile responses to angiotensin II and angiotensin I, and the effects of antagonists thereon, in several isolated venous preparations from the rabbit. Results are expressed as a % of the maximum response to NA obtained in each experiment.

Each column represents mean ± s.e.mean of 6 - 28 experimental observations.

***denotes a significant difference in the contractile response in the presence of antagonist, Student's *t* -test $p<0.001$.

a)



b)

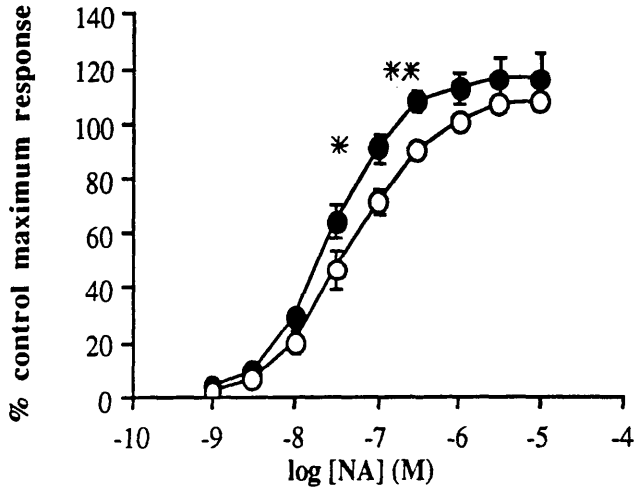


Figure No. 19

Effects **of a)** angiotensin II 0.05μM (●) and 0.5μM (□) and of **b)** Bay K 8644 0.03μM (●) on responses to NA (○) in isolated lateral saphenous vein after correction for time-related changes.

Each point represents mean ± s.e.mean (n = 6 - 10). Statistically significant differences between responses to NA in the absence and presence of Bay K 8644 are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

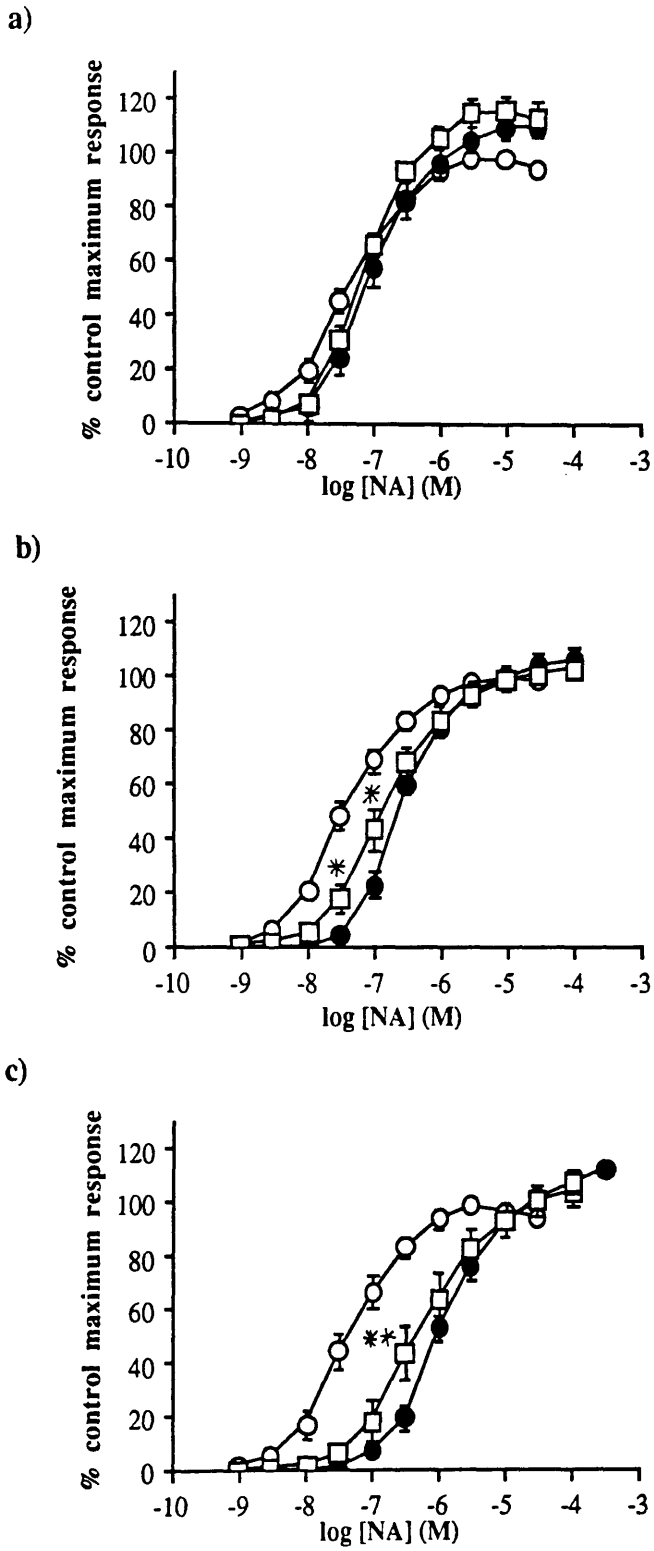
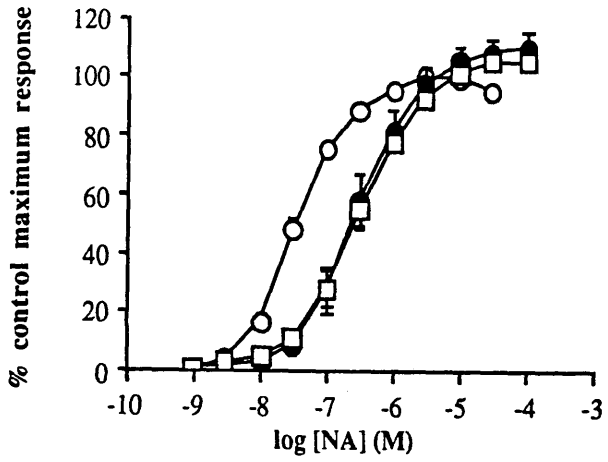


Figure No. 20

Effects of prazosin in the absence (●) and in the presence (□) of angiotensin II (0.05μM) **a)** 0.01μM **b)** 0.1μM and **c)** 1μM on responses to NA (○) in the rabbit isolated lateral saphenous vein.

Each point represents mean ± s.e.mean (n = 5 - 6). Statistically significant differences between responses to NA in the presence of prazosin alone and in combination with A II, are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

a)



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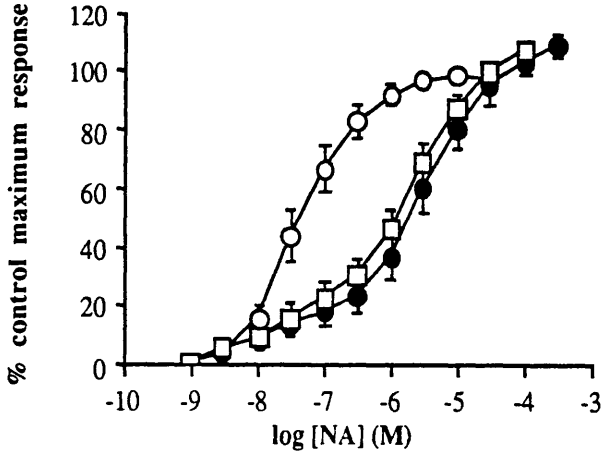


c)

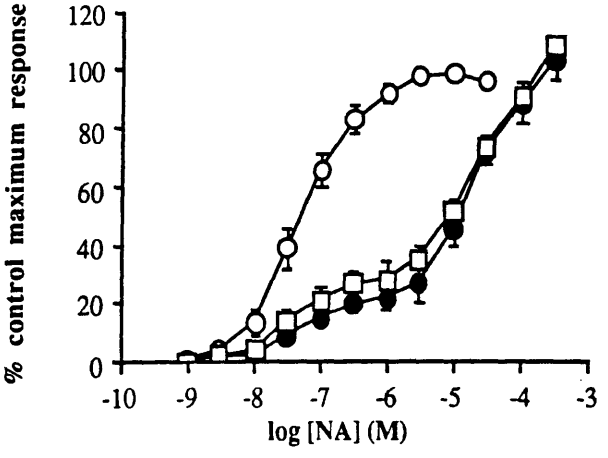


Figure No. 21

Effects of rauwolscine in the absence (●) and in the presence (□) of angiotensin II (0.05μM) **a)** 0.05μM **b)** 0.5μM and **c)** 2.5μM on responses to NA (○) in the rabbit isolated lateral saphenous vein.

Each point represents mean ± s.e.mean (n = 4 - 5).

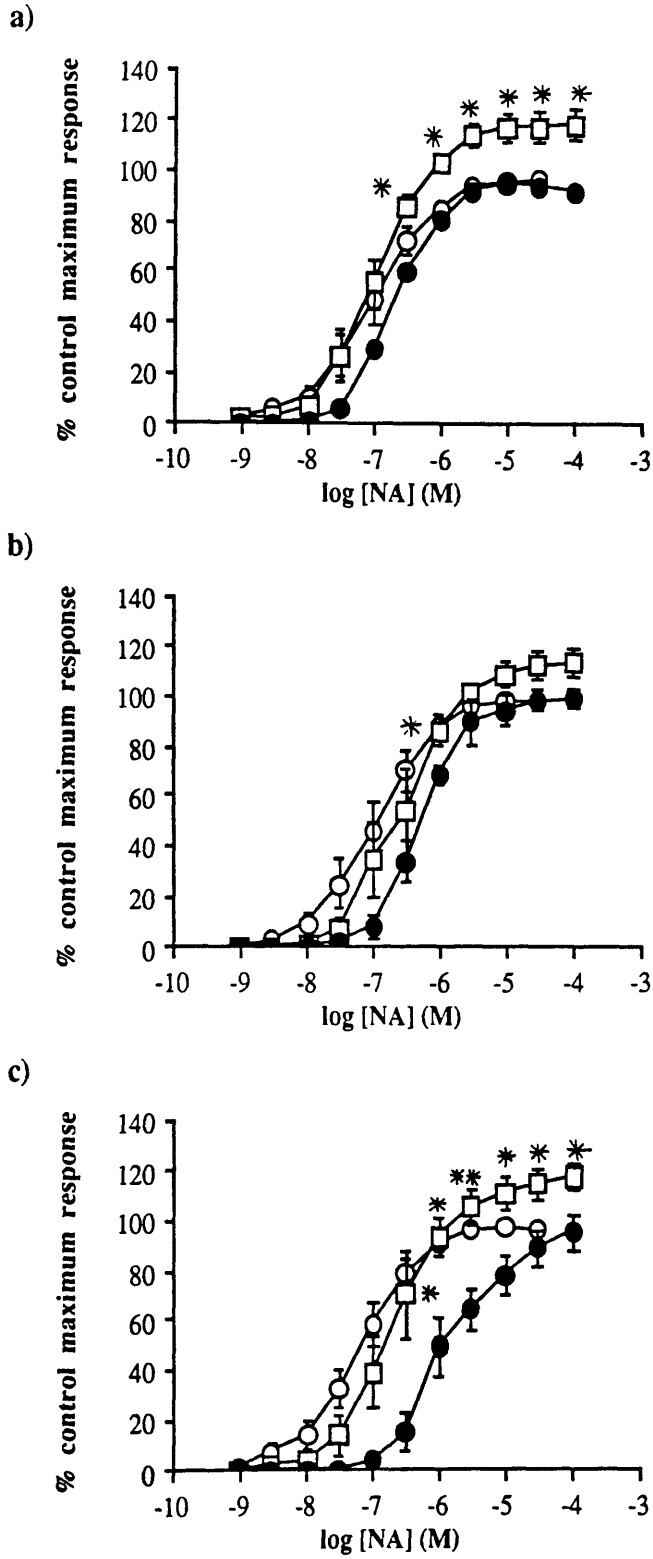


Figure No. 22

Effect of prazosin in the absence (●) and in the presence (□) of Bay K 8644 (0.03μM) **a)** 0.01μM **b)** 0.1μM and **c)** 1μM on responses to NA (○) in the rabbit isolated lateral saphenous vein.

Each point represents mean ± s.e.mean (n = 5). Statistically significant differences between responses to NA in the presence of prazosin alone and in combination with Bay K 8644 are represented by; *$p<0.05$, **$0.01<p<0.001$, ***$0.001>p$ Student's *t*- test.

Antagonist	*25%*	*50%*	*75%*
prazosin (0.1μM) (n = 12)	0.81 ± 0.07	0.69 ± 0.07	0.64 ± 0.10
prazosin (0.1μM) with A II (0.05μM) (n = 7)	0.60 ± 0.09[a]	0.57 ± 0.08	0.59 ± 0.09
prazosin (0.1μM) with Bay K 8644 (0.03μM) (n = 5)	0.49 ± 0.08[b]	0.35 ± 0.09[b]	0.33 ± 0.08[b]
rauwolscine (0.5μM) (n = 5)	1.47 ± 0.14	1.70 ± 0.12	1.85 ± 0.09
rauwolscine (0.5μM) with A II (0.05μM) (n = 5)	1.38 ± 0.18	1.73 ± 0.08	1.86 ± 0.07

Table 4

log agonist concentration-ratio values for NA measured at the 25%, 50% and 75% level of the maximum response for NA in the presence of prazosin (0.1μM) or rauwolscine (0.05μM) and the effects of A II (0.05μM) and Bay K 8644 (0.03μM) thereon.

[a] denotes a significant difference in the value for prazosin (0.1μM) calculated at the 25% level of the maximum response to NA, in the presence of A II, compared to control. $p<0.05$

[b] denotes a significant difference in the value for prazosin (0.1μM) calculated at the 25%, 50% and 75% levels of the maximum response to NA in the presence of Bay K 8644 compared to control. $p<0.05$ Student's *t*- test.

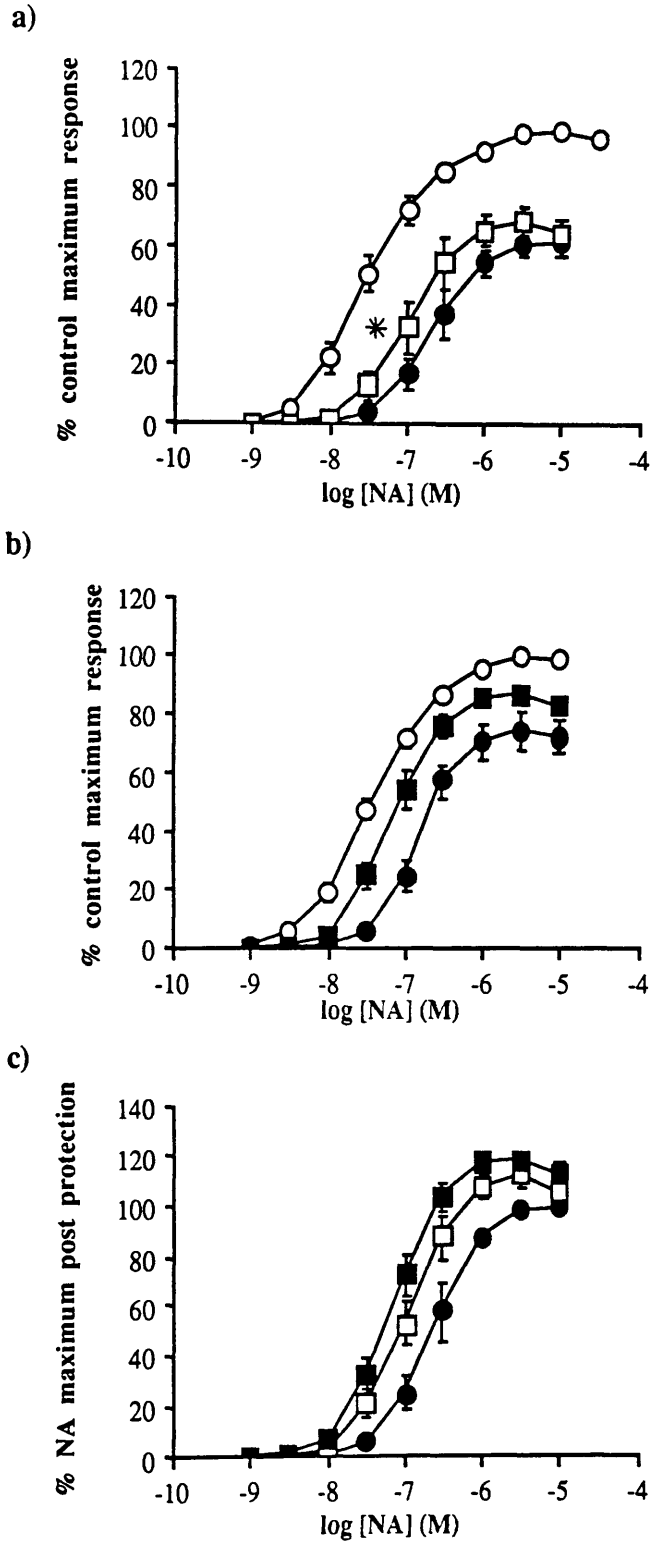
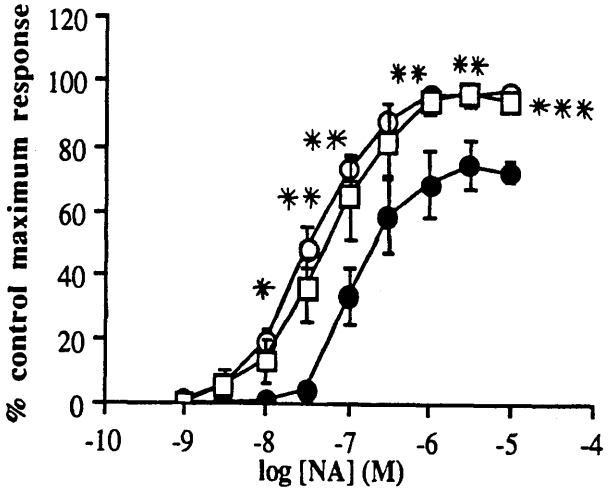


Figure No. 23

Effects of angiotensin II **a)** (5nM) (□) and **b)** (0.05μM) (■) on responses to NA (●) after isolation of postjunctional α_2-adrenoceptors. Responses are expressed as a % of the original maximum response to NA before (○) (**a** and **b**) or after (●) (**c**) the protection protocol.

Each point represents mean ± s.e.mean (n = 6 - 13). All points on the NA CCRC in the presence of A II, after α_2-adrenoceptor isolation (**b** and **c**), are statistically significantly different from those in the absence of A II, $p<0.05$, Student's *t*- test.

a)



b)

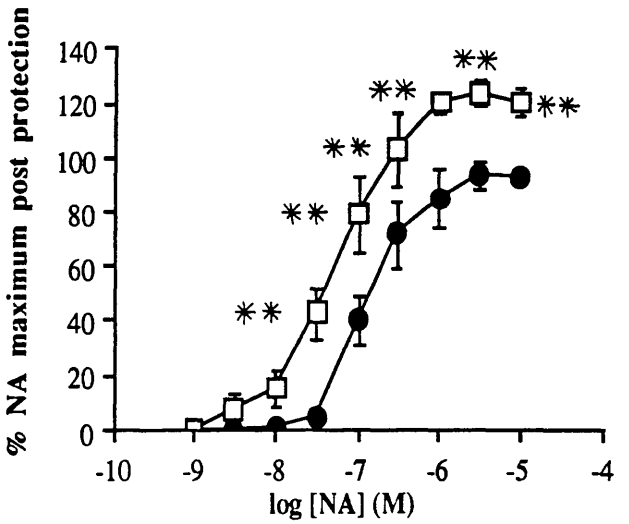


Figure No. 24

Effects of Bay K 8644 (0.03μM) (□) on responses to NA (●) after isolation of postjunctional α_2-adrenoceptors in rabbit isolated lateral saphenous vein. Results are expressed as a % of **a)** the original maximum to NA (○) or **b)** as a % of the maximum response after the protection protocol.

Each point represents mean ± s.e.mean (n = 6). Statistically significant differences between responses to NA in the absence and presence of Bay K 8644 are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

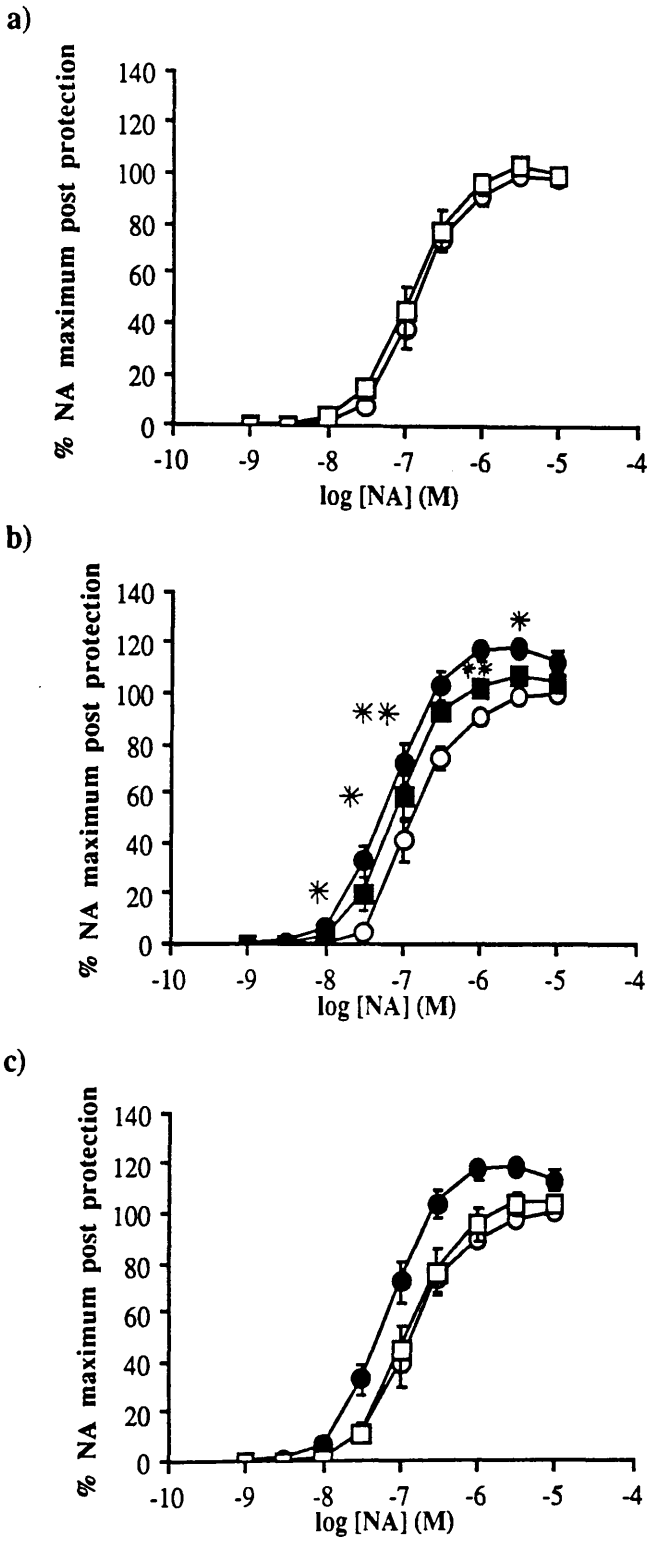


Figure No. 25

Effects of **a)** saralasin, 0.1μM (□) alone and of **b)** saralasin, 0.01μM (■) and **c)** 0.1μM (□), on A II (0.05μM) induced potentiation (●) of responses to NA after isolation of postjunctional α_2-adrenoceptors in rabbit isolated lateral saphenous vein. Results are expressed as a % of the maximum response to NA following the protection protocol (○). Each point represents mean ± s.e.mean (n = 6). Statistically significant differences between responses to NA in the absence and presence of A II and saralasin (0.01μM) alone (**b**) are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

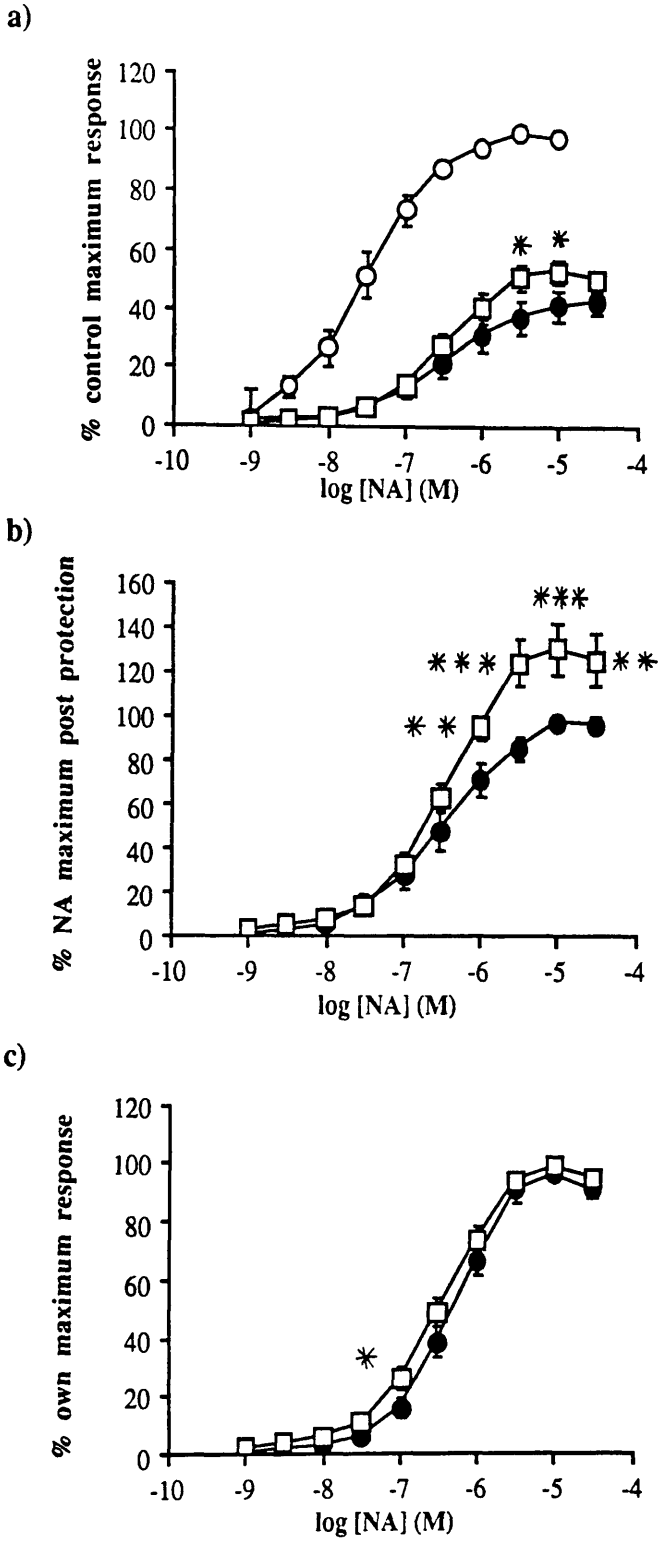


Figure No. 26

Effects of angiotensin II 0.05μM (□) on responses to NA (●) after attempted isolation of postjunctional α_1-adrenoceptors in rabbit isolated lateral saphenous vein. Responses are expressed as % of **a)** the original NA maximum response (○) **b)** the maximum response after the protection protocol or **c)** the individual maximum response for each CCRC.

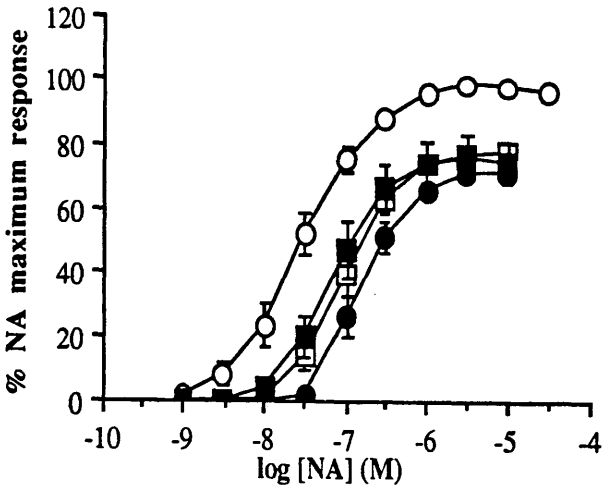
Each point represents mean ± s.e.mean (n = 6). Statistically significant differences between responses to NA in the absence and presence of A II, after receptor protection are represented by; *$p<0.05$, **$0.01<p<0.001$,*** $p<0.001$, Students' *t*- test.

The physiological precursor of A II, angiotensin I (A I) (0.05 - 0.5μM), produced a concentration-dependent contraction in the lateral saphenous vein (Fig 18c). This contraction was again transient, but was characterised by a slower onset and longer duration, than equivalent sized responses to A II, returning to baseline after approximately 12-15 mins. A I mimicked the effects of A II, by producing a leftward shift in the NA CCRC and an increase in the maximum response to NA after α_2-adrenoceptor isolation (Figure 27). Again, however, there was difficulty in reversing the facilitatory action of an angiotensin peptide with an appropriate antagonist. The angiotensin-converting enzyme inhibitor cilazoprilat (1μM) abolished contractile responses to A I (Fig 18c), but this was not associated with complete reversal of its facilitatory action (Figure 28b). Cilazoprilat (0.1μM) had no effect alone, on responses to NA mediated via postjunctional α_2-adrenoceptors in the rabbit isolated lateral saphenous vein (Figure 28a).

Other contractile agents

The effects of A II were also examined against a non α-adrenoceptor agonist. Bradykinin produced reproducible concentration-dependent contractions in the lateral saphenous vein (Figure 29a). These responses were unaffected by A II (0.05μM) (Figure 29b). α,β-methylene ATP (3μM), in a similar manner to A II, produced a transient contraction, equivalent to 52.0 ± 5.5% (n = 5) of the original NA maximum response, in the isolated lateral saphenous vein. The presence of this agent was associated with a small, but significant, leftward shift in the CCRC to NA after isolation of postjunctional α_2-adrenoceptors (Figure 30b,c). α,β-methylene ATP (3μM) was without effect on responses to NA when the full α-adrenoceptor complement was present (Figure 30a).

a.)



b.)

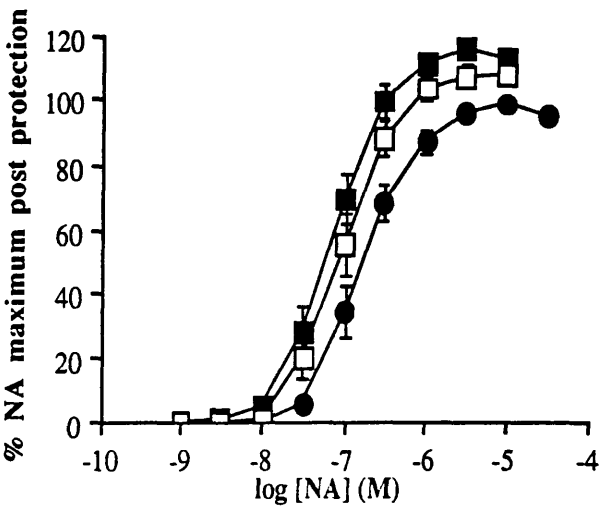


Figure No. 27

Effect of Angiotensin I, 0.5μM (■) and 0.05μM (□) on responses to NA (●) after isolation of postjunctional α_2-adrenoceptors in rabbit isolated lateral saphenous vein. Results are expressed as a % of the maximum response to NA a) before (○) or b) after (●) the protection protocol. Each point represents mean ± s.e.mean (n = 5). All responses to NA in the presence of A II, after isolation of postjunctional α_2-adrenoceptors, were significantly different from those in the absence of A I, $p<0.05$, Student's *t*-test.

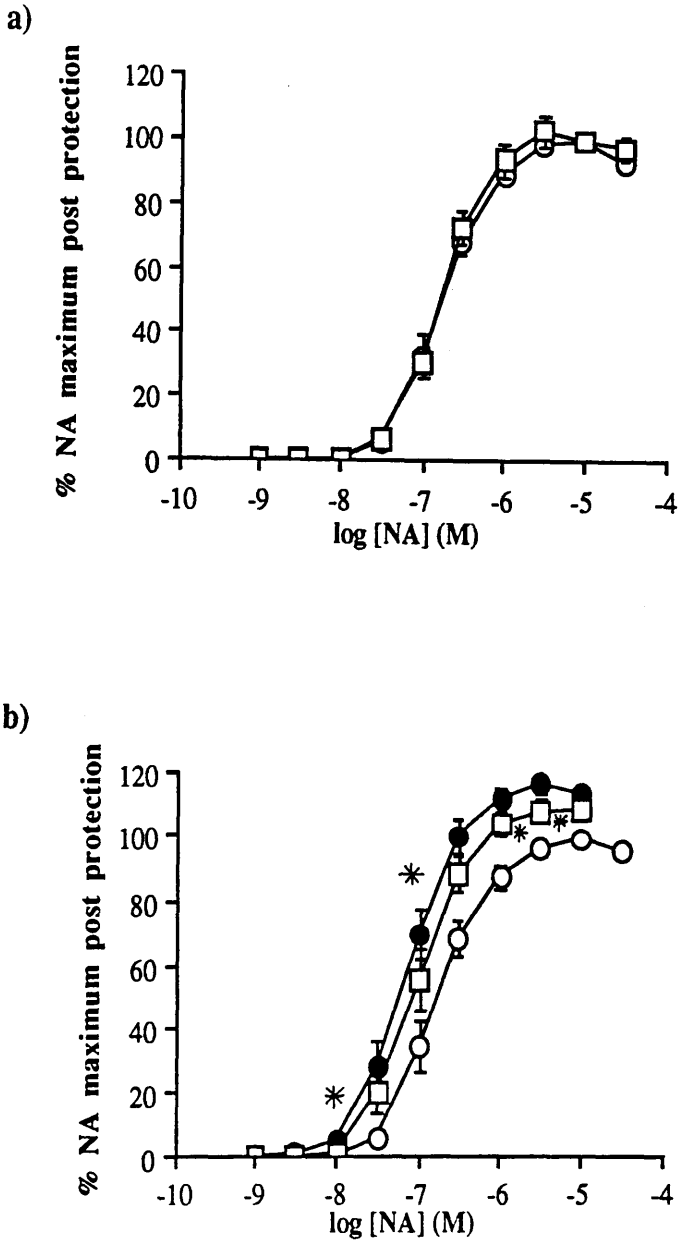
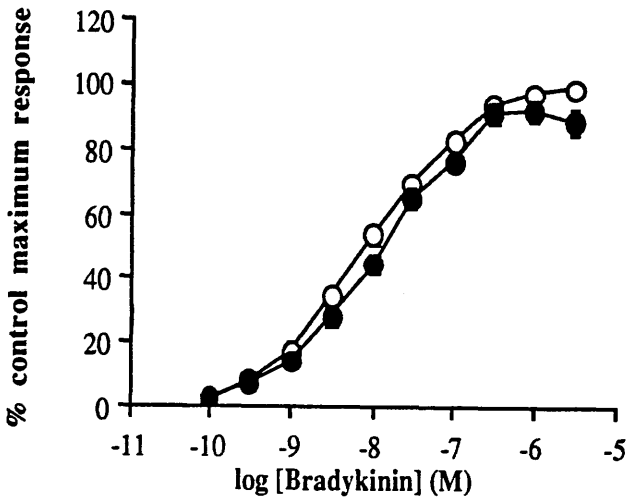


Figure No. 28

a) Effects of 1μM cilazaprilat (□) on responses to NA alone and **b)** on the potentiation of NA responses, after isolation of postjunctional α_2-adrenoceptors, produced by 0.05μM A I (●) in rabbit isolated lateral saphenous vein. Results are expressed as a % of the maximum response to NA (○) after the protection protocol.

Each point represents mean ± s.e.mean (n = 6). Statistically significant differences between responses to NA in the absence and presence of A I and cilazaprilat are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

a)



b)

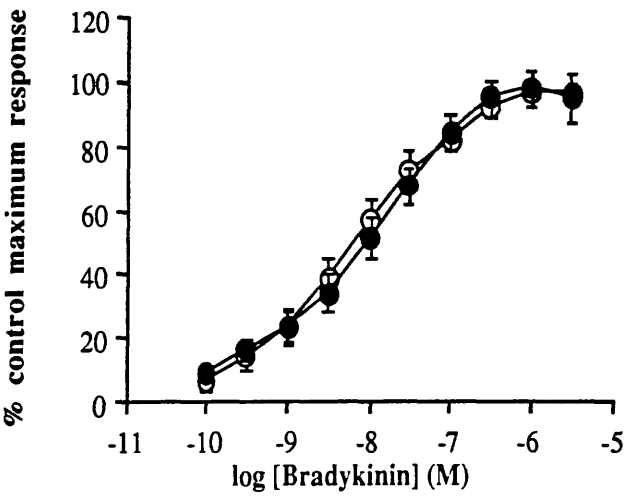


Figure No. 29

a) Reproducibility of consecutive CCRC's to bradykinin in rabbit isolated lateral saphenous vein. (○) represents the 1st, while (●) respresents the 2nd CCRC. **b)** The effects of A II (0.05μM) (●) on responses to bradykinin (○) in this preparation.

Each point represents the mean ± s.e.mean (n = 6).

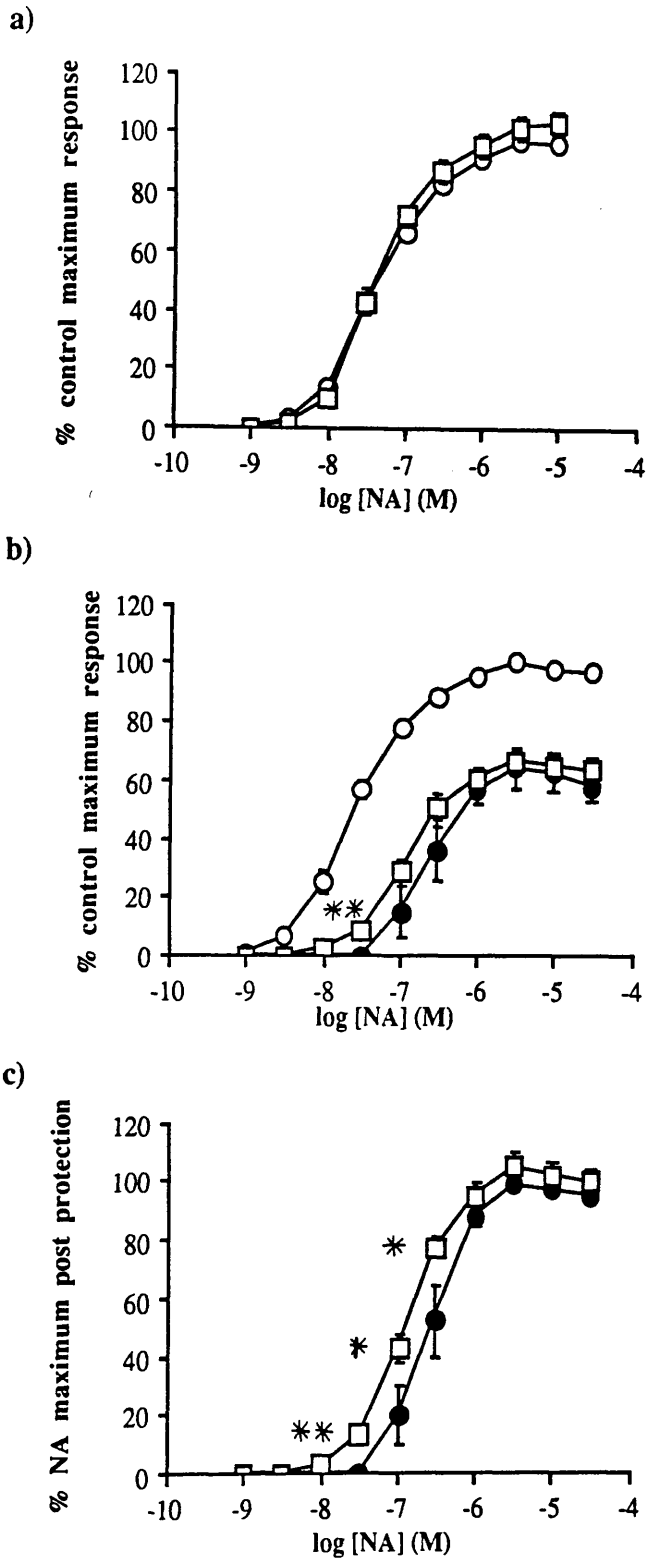


Figure No. 30

Effect of α,-β-methylene ATP (0.03μM) (□) on responses to NA **a)** under normal experimental conditions (○) or **b)** and **c)** after isolation of postjunctional α_2-adrenoceptors (●). Results are expressed either as a % of the maximum response to NA (○) prior to (a and b) or after the protection protocol (●) (c).

Each point represents the mean ± s.e.mean (n = 5 - 8). Statistically significant differences in responses to NA after receptor protection, in the presence and absence of α,β-methylene ATP are represented by; *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

Influence of A II and Bay K 8644 on voltage-operated and receptor-operated calcium channels in rabbit isolated lateral saphenous vein.

The superficial similarity of the facilitatory action of both A II and Bay K 8644, on α_2-adrenoceptor mediated responses to NA in the isolated lateral saphenous vein, prompted the study of their actions on responses to extracellular Ca^{2+} mediated by voltage-operated or receptor-operated calcium channels in this preparation.

The exchange of normal Krebs' for nominally Ca^{2+}-free high K^+(65mM) Krebs', induced a small contraction in most preparations (equivalent in size to between 5-10% of the maximum response observed upon the readdition of 2.5mM Ca^{2+}). Subsequently, consecutive Ca^{2+} CCRC's were superimposable (pD_2 for Ca^{2+} - 3.49 ± 0.07 and 3.48 ± 0.08, n = 10; for 1st and 2nd CCRC's respectively, Figure 31a). A II (0.05μM) caused a significant rightward displacement of the Ca^{2+} CCRC (pD_2 for Ca^{2+} - 3.49 ± 0.07 and 3.29 ± 0.07, n = 9; $p<0.05$. Figure 32a) without altering the maximum response. In marked contrast, Bay K 8644 (0.03μM) failed to alter the sensitivity of the depolarised preparation to Ca^{2+} (pD_2 - 3.47 ± 0.06 and 3.46 ± 0.06, n = 8) but significantly increased the contractile response to all concentrations of Ca^{2+} (Figure 32a).

After isolation of postjunctional α_2-adrenoceptors, NA (30μM) produced a small transient contraction (7.7 ± 1.9 %, n = 9), which returned to baseline within 10 mins., in nominally Ca^{2+}-free Krebs'. Subsequently consecutive CCRC's to the re-addition of Ca^{2+} were reproducible (Figure 31b). A II (0.05μM) produced a small increase in the maximum response to the re-addition of Ca^{2+}, although this was not associated with a change in sensitivity of the preparation to Ca^{2+}. In contrast Bay K 8644 increased both the sensitivity and the maximum response to

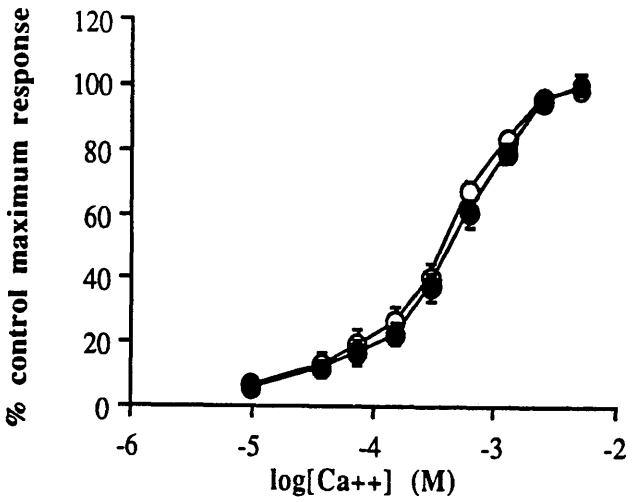
the re-addition of Ca^{2+}, upon stimulation of postjunctional α_2-adrenoceptors with NA.

Effects of A II and Bay K 8644 on responses to NA in the rabbit isolated left renal vein

The rabbit isolated left renal vein contains a homogeneous population of postjunctional α_1-adrenoceptors (Daly *et al.*, 1988c). Consecutive CCRC's to NA in this preparation were reproducible with time (Figure 33). A II (0.05μM) produced a transient contraction in the left renal vein, which was equivalent in size to that seen in the lateral saphenous vein (Figure 18d). This concentration of A II was without effect on responses to NA (Figure 33, Table 5). In contrast, Bay K 8644 (0.03μM), which had no effect alone on resting baseline tension, increased the sensitivity and the maximum response of the preparation to NA (Figure 33, Table 5).

It has previously been shown that reducing the receptor reserve in the rabbit femoral artery increased the facilitatory effect of A II on responses to NA mediated via α_1-adrenoceptors (Purdy & Weber, 1988). Therefore, the left renal vein was exposed to phenoxybenzamine (1nM) for 30 mins to reduce the receptor reserve in this preparation. This produced an average 42.0 ± 9.3 % (range 17-75%, n = 5) reduction in the maximum response to NA, although there was no change in the sensitivity of the preparation to NA (pD_2 for NA, 5.91 ± 0.04 and 5.92 ± 0.11, before and after treatment with phenoxybenzamine respectively, n = 6). In two further preparations, phenoxybenzamine abolished all responses and in another failed to reduce the maximum response. These results have been excluded from the analysis. Even under these conditions, A II (0.05μM) had no effect on the residual response remaining after phenoxybenzamine (Figure 34c, Table 5).

a)



b)

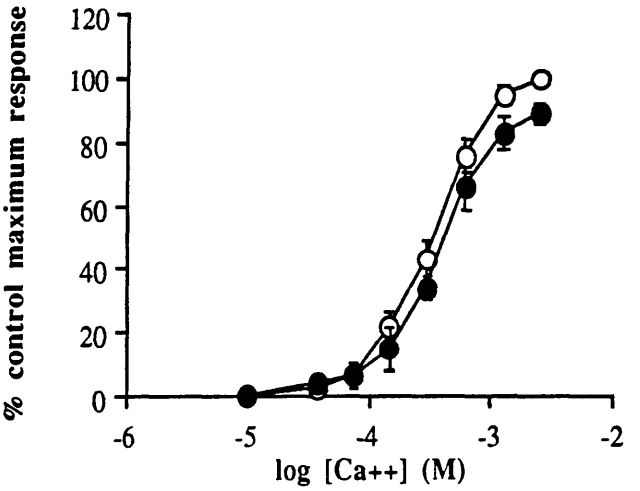
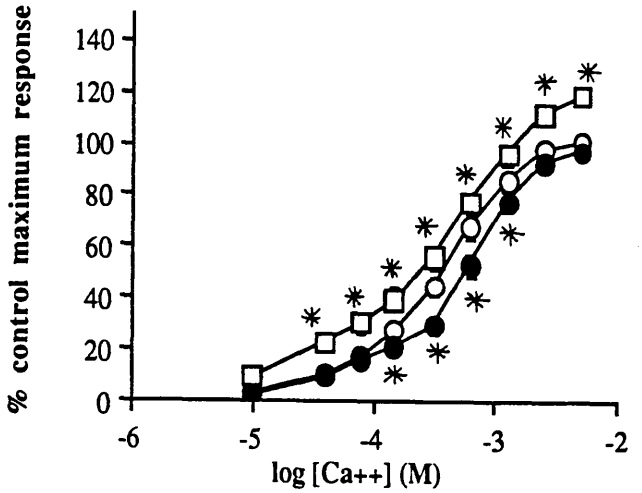


Figure No. 31

Reproducibility of Ca^{2+} CCRC's in rabbit isolated lateral saphenous vein. **a)** in the presence of KCl (65mM) and **b)** in the presence of NA (30μM) after isolation of postjunctional α_2-adrenoceptors. (○) represents the 1st CCRC, while (●) represents the 2nd CCRC.

a)



b)

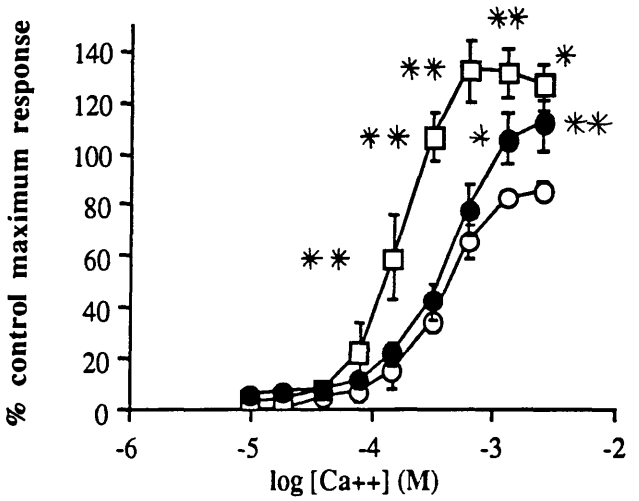


Figure No. 32

Effect of A II (0.05μM) (●) and Bay K 8644 (0.03μM) (□) on responses to the readdition of Ca^{2+} in the presence of **a)** 65mM KCl and **b)** 30μM NA after isolation of postjunctional α_2-adrenoceptors in the rabbit isolated lateral saphenous vein. Results are expressed as a % of the original maximum response to Ca^{2+}.

Each point represents the mean ± s.e.mean (n = 6-8). Statistically significant differences between responses in the absence and presence of A II or Bay K 8644 are represented by *$p<0.05$, ** $0.01<p<0.001$ Student's *t*- test.

Effects of A II and Bay K 8644 on responses to NA in rabbit isolated ear vein

NA produced concentration-dependent contractions in the isolated ear vein, a preparation containing predominantly postjunctional α_2-adrenoceptors (Daly *et al.*, 1988b). Unfortunately consecutive CCRC's to NA were not reproducible with time, there being an approximately 30% increase in the maximum response (Figure 35a) although there was no change in sensitivity (pD_2 for NA, 7.82 ± 0.08 and 7.83 ± 0.08 for curves 1 and 2 respectively, n = 11). After correcting for time-related changes A II (0.05μM), which again produced a transient contraction (Figure 18d), was observed to produce a small, but significant, leftward shift in the CCRC to NA (Figure 35b, Table 5). In contrast, Bay K 8644 (0.03μM) did not affect resting tone and had no effect on responses to NA in this preparation (Figure 35c, Table 5).

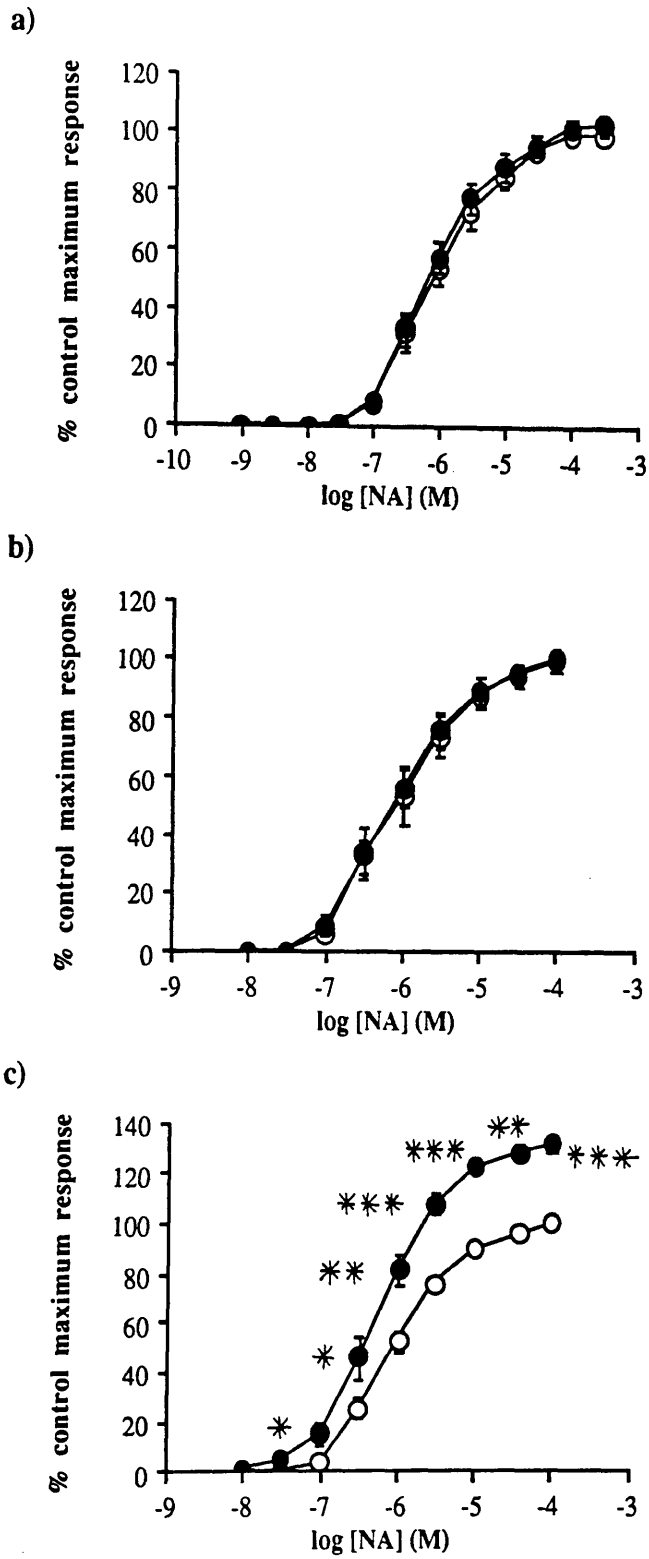


Figure No. 33

a) Reproducibility of responses to NA in the rabbit isolated left renal vein. (○) represents the 1st CCRC to NA while (●) represents the 2nd. **b)** the effect of A II (0.05μM) (●) and **c)** of Bay K 8644 (0.03μM) (●) on responses to NA (○) in the same preparation.

Each point represents the mean ± s.e.mean (n = 5-7). Statistically significant differences between responses in the absence and presence of Bay K 8644 are represented by *p<0.05, **0.01<p<0.001, ***p<0.001, Student's *t*- test.

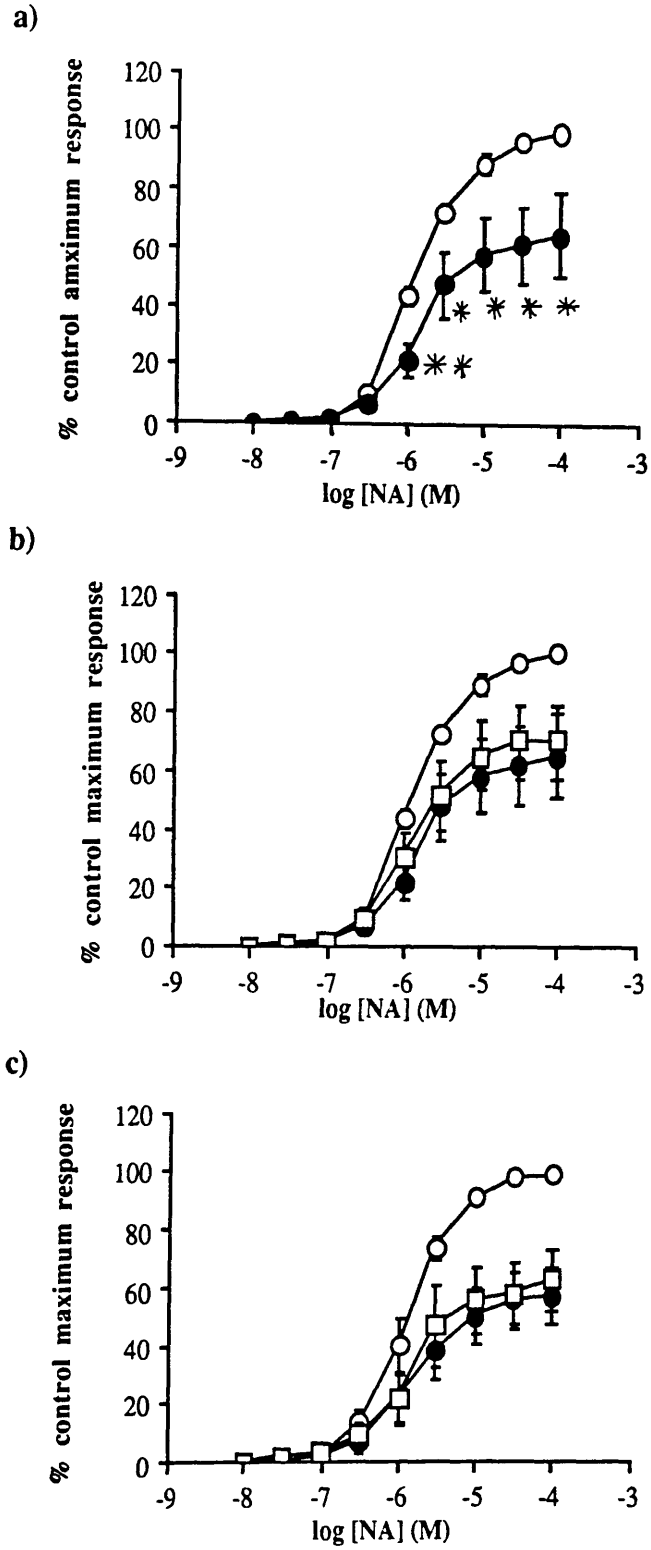
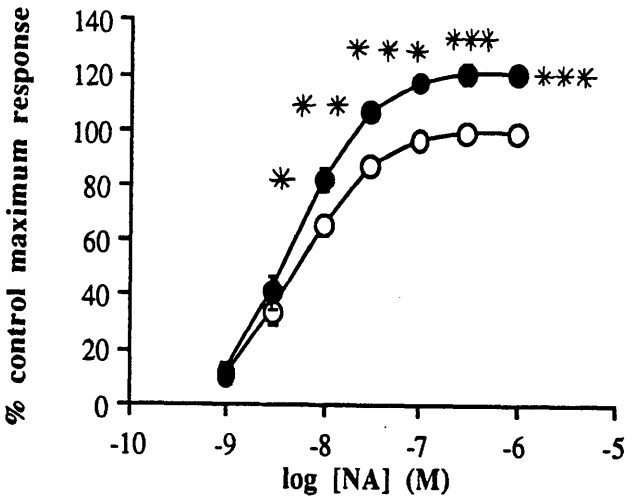


Figure No. 34

a) Effect of phenoxybenzamine (1nM) (●) on responses to NA in the rabbit isolated left renal vein and the subsequent influence of **b)** time (□) and **c)** A II (0.05μM) (□), on the residual response remaining after the irreversible antagonist. Results are expressed as a % of the maximum response to NA (○) prior to phenoxybenzamine. Each point represents the mean ± s.e.mean (n = 5-6). Statistically significant differences between responses in the absence and presence of phenoxybenzamine are represented by *$p<0.05$, Student's *t*- test.

a)



b)

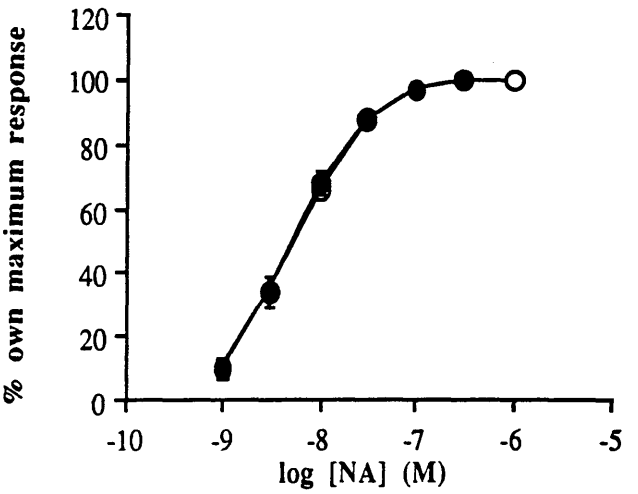
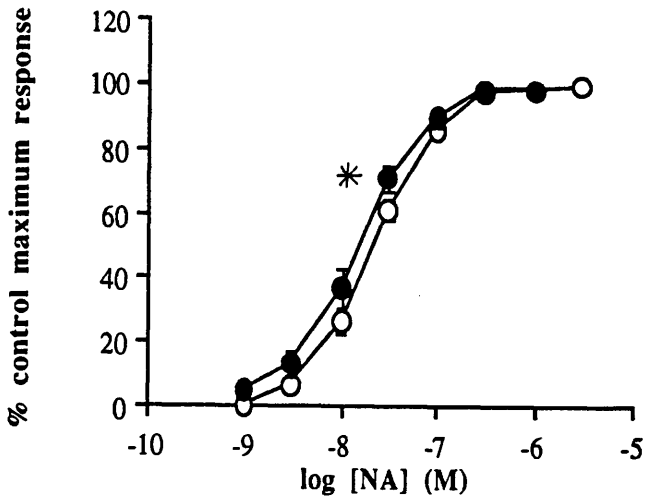


Figure No. 35

Reproducibility of consecutive CCRC's to NA in rabbit isolated ear vein. Results are expressed as a % of **a)** the initial control maximum response to NA or **b)** the maximum response to NA in each individual CCRC. (○) represents the 1st CCRC while (●) represents the 2nd.

Each point represents the mean ± s.e.mean (n = 11). Statistically significant differences between responses to NA in the 1st and 2nd CCRC's are represented by * $p<0.05$, ** $0.01<p<0.001$, ***$p<0.001$ Student's *t*- test.

a)



b)

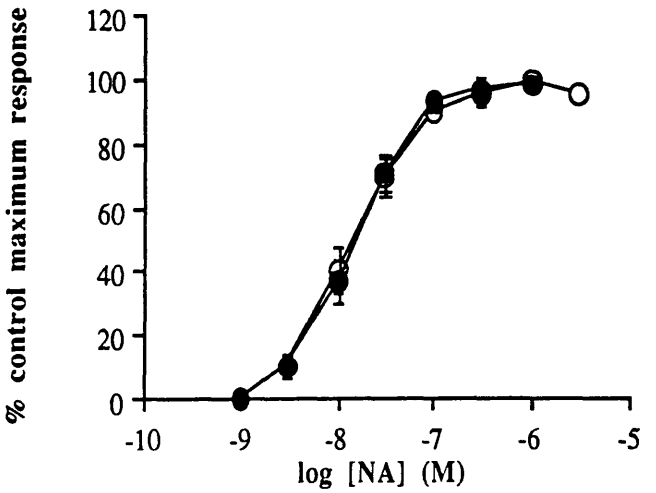


Figure No.36

Effect of **a)** A II (0.05μM) (●) and of **b)** Bay K 8644 (0.03μM) (●) on responses to NA (○) in rabbit isolated ear vein after correction for time related changes.

Each point represents the mean ± s.e.mean (n = 5-11). Statistically significant differences between responses in the absence and presence of A II are represented by *$p<0.05$, Student's *t*- test.

preparation	*pD_2 value*		*E_{max}*	
	control	*A II*	*control*	*A II*
LSV (n = 10)	7.31 ± 0.08	7.40 ± 0.10	1	1.05 ± 0.05
LSVRP (n = 13)	6.83 ± 0.06	7.18 ± 0.08***	1	1.18 ± 0.03***
LRV (n = 5)	6.12 ± 0.19	6.12 ± 0.11	1	1.02 ± 0.04
LRVP (n = 5)	5.76 ± 0.16	5.85 ± 0.17	1	0.93 ± 0.05
EV	7.62 ± 0.07	7.80 ± 0.31*	1	1.03 ± 0.07
	control	*Bay K 8644*	*control*	*Bay K 8644*
LSV (n = 5)	7.28 ± 0.13	7.53 ± 0.10*	1	1.22 ± 0.05**
LSVRP (n = 6)	6.81 ± 0.16	7.23 ± 0.20***	1	1.25 ± 0.05***
LRV (n = 7)	6.06 ± 0.08	6.24 ± 0.10*	1	1.30 ± 0.03***
EV	7.82 ± 0.09	7.79 ± 0.05	1	1.09 ± 0.11

Table 5

pD_2 values (with 95% confidence limits) and E_{max} values as compared to the control maximum responses to NA, in the rabbit isolated lateral saphenous vein (LSV), lateral saphenous vein after receptor protection using rauwolscine (1μM) and phenoxybenzamine (0.3μM) (LSVRP), left renal vein (LRV), left renal vein post treatment with phenoxybenzamine (1nM) (LRVP) and the ear vein (EV). Differences between control and treated preparations were considered statistically significant if $p<0.05$ for either paired or unpaired observations - Student's *t*-test and are denoted by: * $0.01<p<0.05$, ** $0.01<p<0.001$, *** $0.001<p$.

Alpha-adrenoceptors in the rabbit isolated distal saphenous artery - influence of angiotensin II

The difficulty in demonstrating postjunctional α_2-adrenoceptors, while acute in isolated venous preparations, has been even more difficult for arterial vessels *in vitro* (McGrath *et al.*, 1989). Having successfully demonstrated postjunctional α_2-adrenoceptors in the lateral saphenous vein, the α-adrenoceptor population, and the effects of contractile agents thereon, was studied in the corresponding arterial preparation, the distal saphenous artery.

Agonist Potencies

The α-adrenoceptor agonists NA, phenylephrine, Sgd 101/75 and UK-14304 produced concentration-dependent contractions in the rabbit isolated distal saphenous artery. The rank order of potencies for these agonists was as follows: NA > phenylephrine = UK-14304 > Sgd 101/75. Based upon the maximum contractions, NA and phenylephrine can be classed as full agonists, while UK-14304 and Sgd 101/75 are partial agonists compared to NA (Figure 37). The pD_2 and E_{max} values for each agonist are given in Table 6a.

Reproducibility of responses to α-adrenoceptor agonists

Consecutive CCRC's to NA were reproducible in the rabbit isolated distal saphenous artery (pD_2 for NA: 7.19 ± 0.09 and 7.17 ± 0.1, n = 6, for curves 1 and 2 respectively) (Figure 38a). Likewise, two CCRC's to phenylephrine were superimposable (pD_2 for phenylephrine, 6.54 ± 0.05 and 6.50 ± 0.08, n = 5) (Figure 38b). Unfortunately, consecutive CCRC's to the partial α_1-adrenoceptor agonist Sgd 101/75 were characterised by a small increase in the maximum

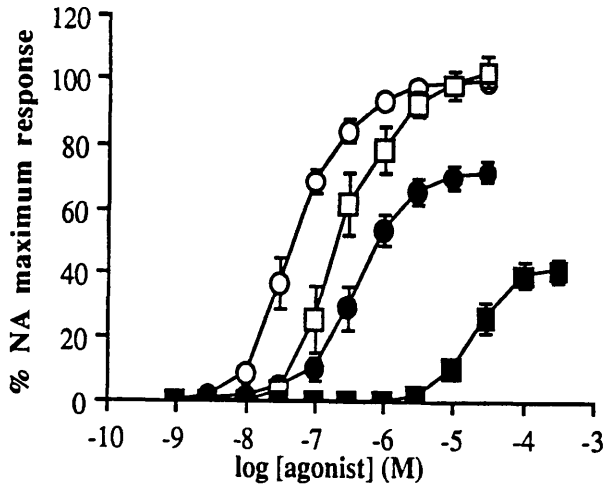
response and sensitivity of the preparation to the agonist (Figure 39a). These time related changes in responses to Sgd 101/75 were therefore taken into consideration when examining the effects of antagonists or A II, against responses to the agonist. In addition, there was a small, time-dependent increase in the maximum response to UK-14304 in this preparation, although this was not associated with a change in sensitivity to the agonist (pD_2 for UK-14304: 6.61 ± 0.10 and 6.47 ± 0.12, n = 7, for the 1st and 2nd CCRC's respectively) (Figure 39b).

Effects of antagonists on responses to α-adrenoceptor agonists

As shown in Figure 40, the α_1-adrenoceptor antagonist prazosin produced a concentration-dependent, rightward, parallel displacement of the NA CCRC. The pA_2 value for prazosin was 8.57 and the slope of the Schild plot was not significantly different from unity, indicating competitive antagonism (Table 6b). In addition, 0.1μM prazosin produced an approximately 25-fold rightward shift in the CCRC to the agonists phenylephrine (Figure 41a), Sgd 101/75 (Figure 41b) and UK-14304 (Figure 41c).

A relatively high concentration of the selective α_2-adrenoceptor antagonist rauwolscine (1μM) produced only a 2-fold rightward shift in the CCRC to NA (Figure 40b). This concentration of rauwolscine was without effect on responses to either phenylephrine (Figure 41a) or the partial agonist Sgd 101/75 (Figure 41b). In contrast however, rauwolscine (1μM) produced a non-parallel rightward displacement of the CCRC to UK-14304 (Figure 41c). This rightward shift was more apparent against the lower portion of the CCRC to the reportedly selective α_2-adrenoceptor agonist. The log agonist concentration-ratio value at the level of 25 % of the maximum response for UK-14304 in the presence of 1μM rauwolscine was significantly greater than that calculated at the 75 % level (0.54 ± 0.08

a)



b)

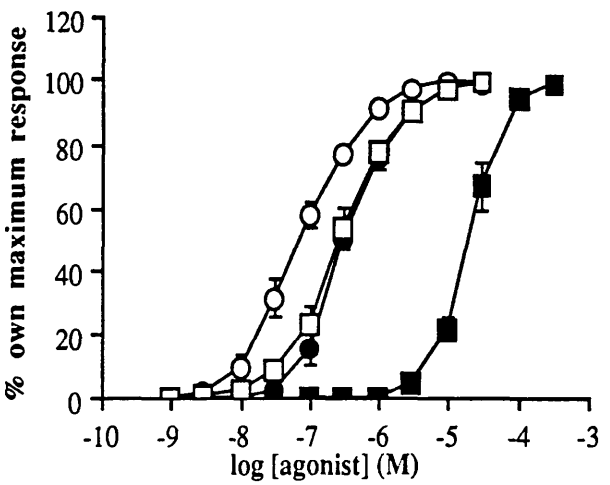
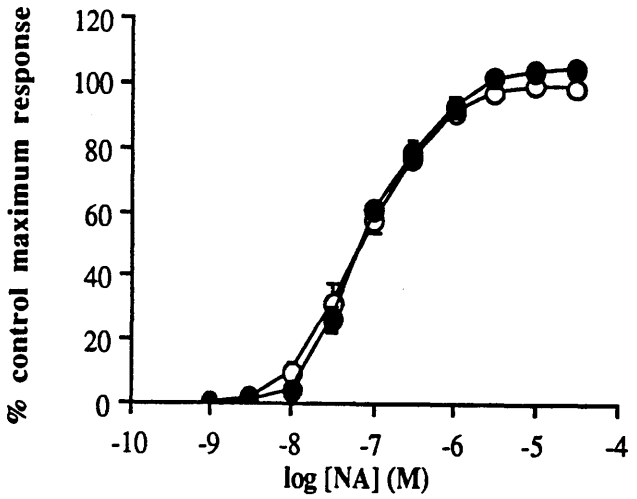


Figure No. 37

Concentration-dependent responses to the α-adrenoceptor agonists NA (○), UK-14304 (●), phenylephrine (□) and Sgd 101/75 (■) in rabbit isolated distal saphenous artery. Results are expressed as a % of **a)** the maximum response to NA in each preparation or **b)** the maximum response to each individual agonist.

Each point represents the mean ± s.e.mean (n = 5 - 7).

a)



b)

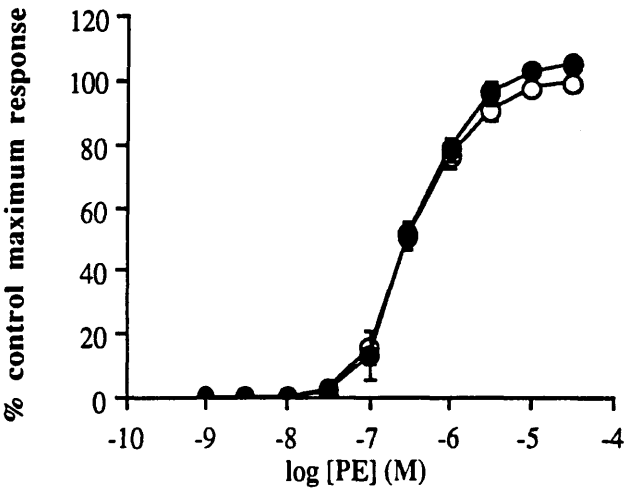


Figure No. 38

Reproducibility of consecutive CCRC's to the α-adrenoceptor agonists **a)** NA and **b)** phenylephrine (PE) in rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to each agonist in the 1st CCRC(○). (●) represents the 2nd CCRC.

Each point represents the mean ± s.e.mean (n = 5 - 6).

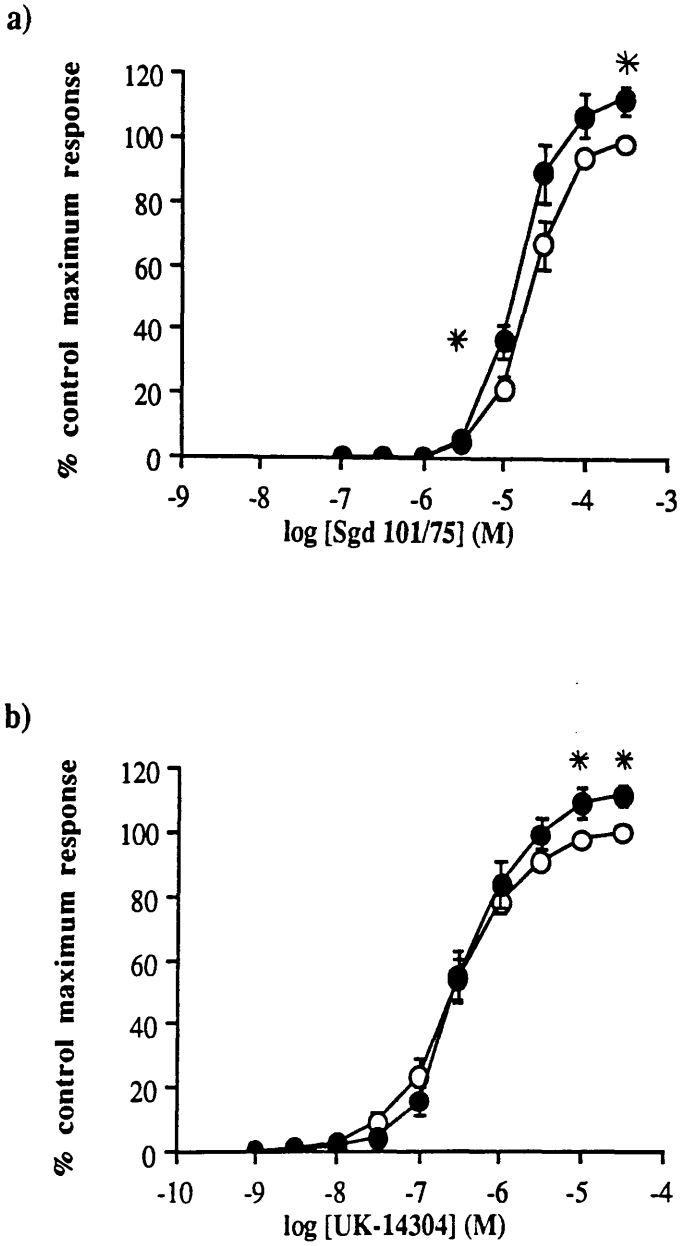


Figure No. 39

Reproducibility of consecutive CCRC's to the α-adrenoceptor agonists a) Sgd 101/75 and b) UK-14304 in rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to each agonist in the 1st CCRC(○). (●) represents the 2nd CCRC.

Each point represents the mean ± s.e.mean (n = 5 - 7). Statistically significant differences between the 1st and 2nd CCRC's are represented by *$p<0.05$ Student's *t*- test.

compared to 0.32 ± 0.10 for the 25 and 75% levels respectively, n = 7, $p < 0.05$, Student's *t*- test). It should be noted however, that for all agonists, prazosin (0.1μM) was a more potent antagonist of responses than rauwolscine (1μM).

Effects of A II on responses to α-adrenoceptor agonists

A II (0.05μM) produced a transient contraction, which returned to baseline after 12-15 mins, in the rabbit isolated distal saphenous artery. This response was equivalent to 37.8 ± 4.8 (n = 13) of the maximum response to NA in each preparation. A II had no effect on subsequent responses to either NA (Figure 42a) or phenylephrine (Figure 42b, Table 7). There was however, a small but significant leftward shift in the CCRC to the α_1-adrenoceptor partial agonist Sgd 101/75 in the presence of A II (0.05μM) (Figure 42c, Table 7).

In contrast to the relatively small effects on responses to the aforementioned agonists, A II (0.05μM) produced a marked increase in the sensitivity of the preparation to UK-14304. Since there was no change in the maximum response, this resulted in a change in the slope of the CCRC. The magnitude of the displacement produced by A II for the threshold concentration of UK-14304, was approximately 300-fold; 0.01μM in the absence of A II compared to 0.01nM in the presence of A II (Figure 43a, Table 7). A representative trace recording of the effect of A II (0.05μM) on responses to UK-14304 is shown in Figure 44. A 10-fold lower concentration of A II (5nM) produced a contraction equivalent to 6.0 ± 2.1 (n = 6) of the maximum response to NA. This contraction was characterised by a slower onset of response than that to 0.05μM A II, and in addition the response in two of the six preparations did not return completely to baseline. 5nM A II also uncovered a previously unseen component of the response to UK-14304, although the size of the "uncovered response" was smaller than that seen with 0.05μM A II (Figure 43b). The potentiation of responses to UK-14304 produced by A II, was

compared to 0.32 ± 0.10 for the 25 and 75% levels respectively, n = 7, $p < 0.05$, Student's *t*- test). It should be noted however, that for all agonists, prazosin (0.1μM) was a more potent antagonist of responses than rauwolscine (1μM).

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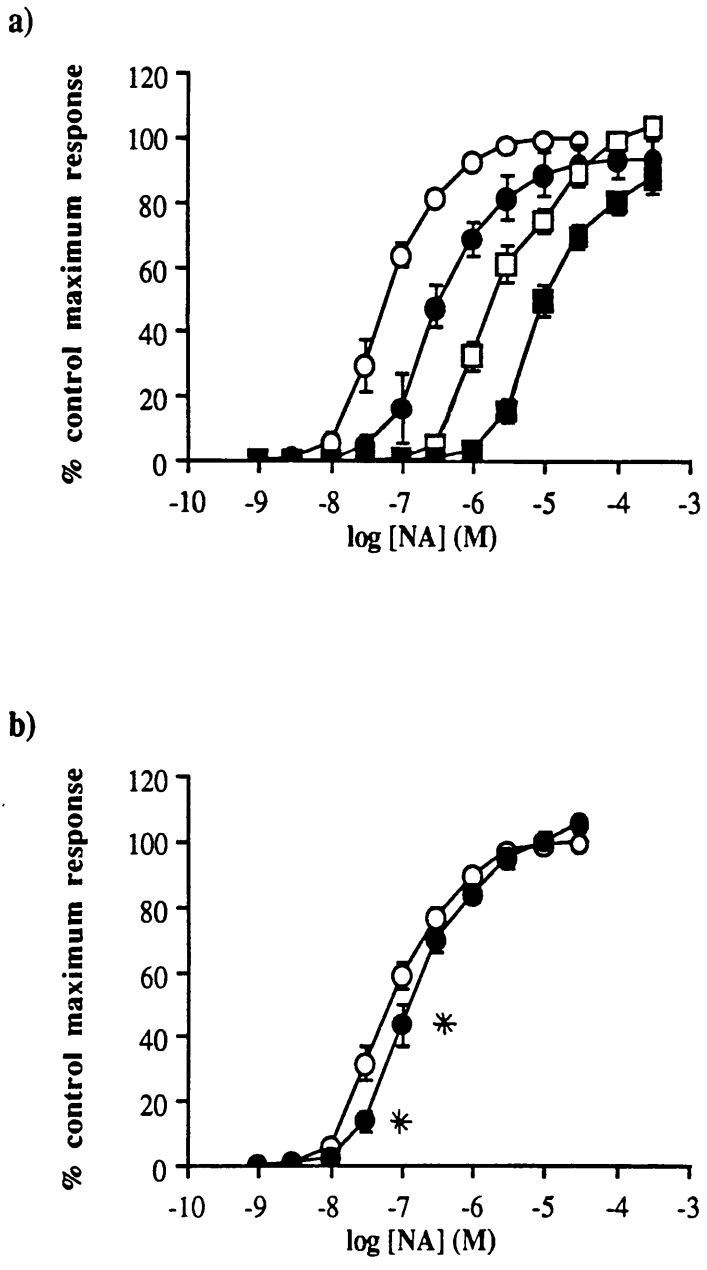
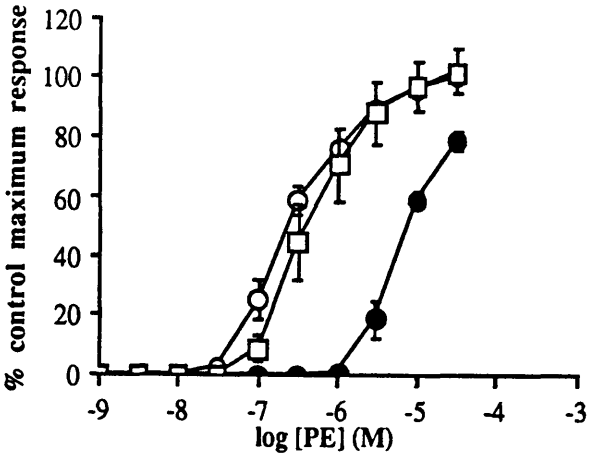


Figure No. 40

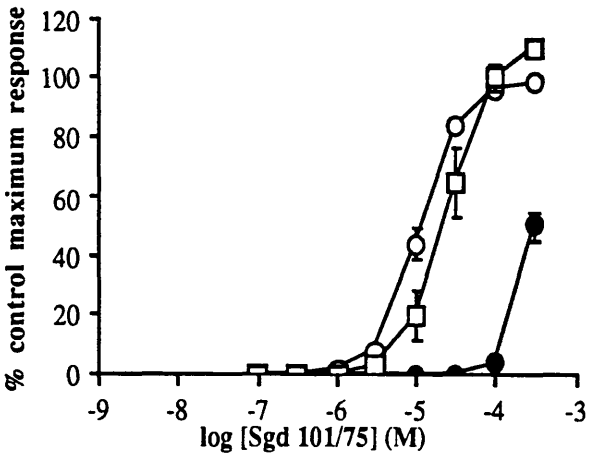
Effect of the α-adrenoceptor antagonists **a)** prazosin 0.01μM (●), 0.1μM (□) and 1μM (■) and of **b)** rauwolscine (1μM) (●) on responses to NA (○) in rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to NA (○).

Each point represents the mean ± s.e.mean (n = 5 - 6). Statistically significant differences between responses in the absence and presence of rauwolscine (1μM) are represented by *$p<0.05$ Student's *t*- test.

a)



b)



c)

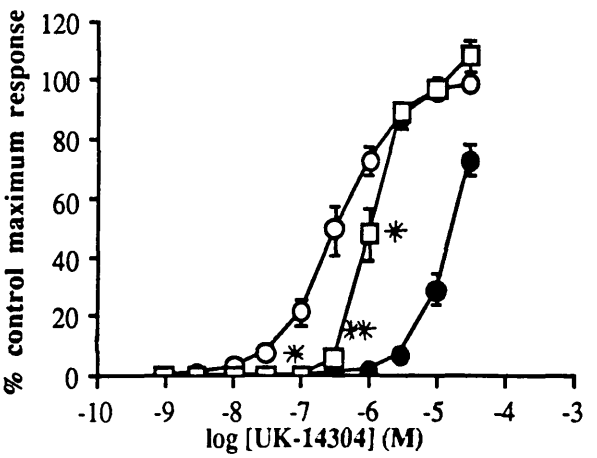


Figure No. 41

Effect of the α-adrenoceptor antagonists, prazosin (0.1μM) (●) and rauwolscine (1μM) (□) on responses to **a)** phenylephrine **b)** Sgd 101/75 and **c)** UK-14304 in rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to each agonist (○).

Each point represents the mean ± s.e.mean (n = 5 - 7). Statistically significant differences between responses in the absence and presence of rauwolscine are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

a)	*Agonist*	*pD_2*	*E_{max}*
	NA (n = 6)	7.19 ± 0.09	1
	phenylephrine (n = 4)	6.54 ± 0.05	0.99 ± 0.06
	Sgd 101/75 (n = 5)	4.69 ± 0.05	0.42 ± 0.04
	UK 14304 (n = 7)	6.42 ± 0.11	0.72 ± 0.03

b)	*Antagonist*	*pA_2*	*Slope*
	prazosin (0.01 - 0.1μM)	8.57 (8.81 - 8.34)	0.98 (0.87 - 1.12)

Table 6

a) List of pD_2 values (with 95% confidence limits) and E_{max} values for α-adrenoceptor agonists; and of **b)** pA_2 value with the slope of the Schild plot (with 95% confidence limits) for prazosin against responses to NA in rabbit isolated lateral saphenous vein. All experiments were carried out in the presence of propranolol (1μM) and cocaine (10μM). The pA_2 value was determined from a regression analysis of the log agonist concentration ratio from 12 individual observations.

reversed by the angiotensin receptor antagonist, saralasin (1μM) (Figure 45). This concentration of saralasin had no effect on responses to UK-14304 alone (Figure 45a)

Effects of A II on α-adrenoceptor antagonist potencies

Under normal experimental conditions, prazosin (0.1μM) was a more potent antagonist than rauwolscine (1μM) against all α-adrenoceptor agonists tested. In the presence of A II however, the potency of these two antagonists against responses to UK-14304 was reversed in the lower portion of the CCRC to UK-14304 (Figure 46). Prazosin (0.1μM) was ineffective against the "uncovered response" to low concentrations of UK-14304 in the presence of A II, although it continued to displace the upper portion of the CCRC. In marked contrast, rauwolscine (1μM) prevented the facilitatory action of A II on responses to UK-14304.

A II (0.05μM) also had a profound effect on the ability of prazosin to antagonise responses to the endogenous ligand NA (Figure 47). This was particularly true for the lower portion of the CCRC to NA, such that the log agonist concentration-ratio at the levels of 25 and 50% of the NA maximum response, in the presence of prazosin (0.1μM), were significantly reduced from control values obtained in the absence of A II (Table 8).

A II (0.05μM) was without effect on the rightward shift of responses to phenylephrine produced by 0.1μM prazosin (Figure 48a). A II appeared to make responses to the partial agonist Sgd 101/75 slightly more resistant to the antagonistic action of prazosin (0.1μM) (Figure 48b). However A II (0.05μM) alone produced a small leftward shift in the CCRC to Sgd 101/75 (Figure 42c).

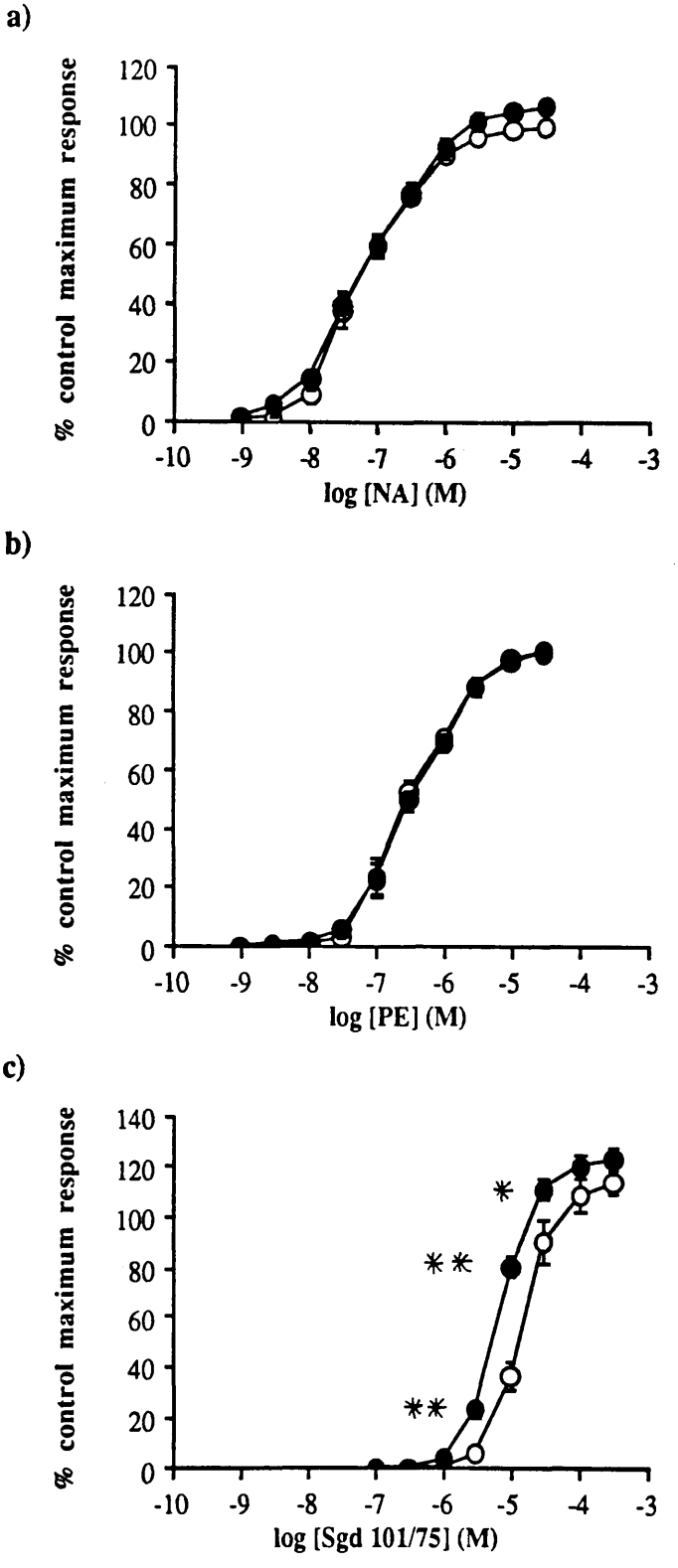


Figure No. 42

Effect of A II (0.05μM) (●) on responses to the α-adrenoceptor agonists **a)** NA **b)** phenylephrine and **c)** Sgd 101/75 in rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to each agonist after correction for time-related changes (○).

Each point represents the mean ± s.e.mean (n = 5 - 6). Statistically significant differences between responses in the absence and presence of A II (0.05μM) are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

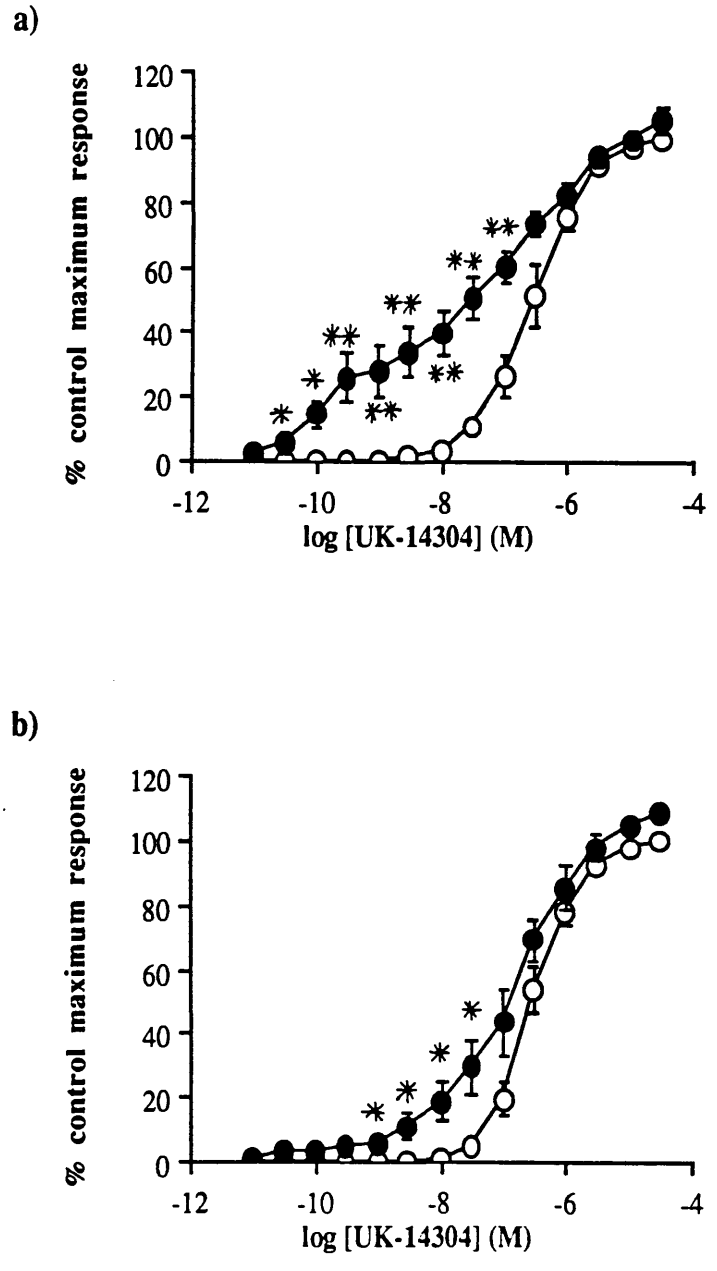


Figure No. 43

Effects of A II (●) **a)** 0.05μM and **b)** 5nM on responses to UK-14304 (○) in rabbit isolated distal saphenous artery. Results are expressed as a % of the initail maximum response to UK-14304.

Each point respresents the mean ± s.e.mean (n = 6 - 8). Statistically significant differences between responses in the absence and presence of A II (0.05μM) are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

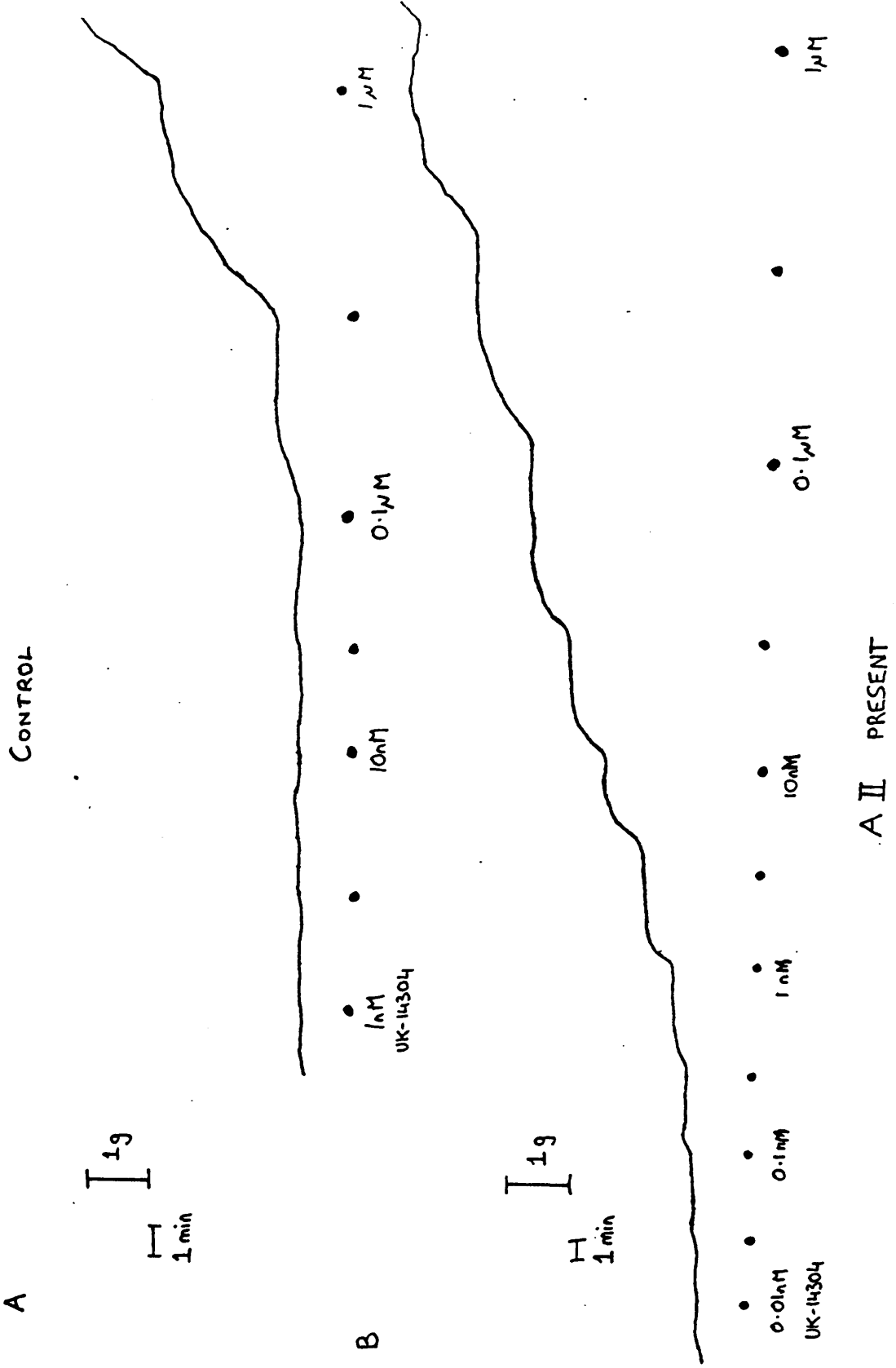
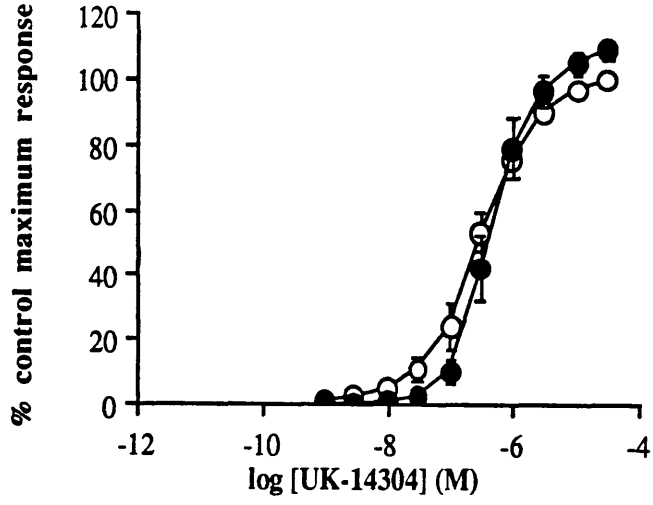


Figure No. 44

Representative trace recording of the effect of A II (0.05μM) on UK-14304 induced contractions (cumulative addition; approximately 3 fold increments in concentration) of the rabbit isolated distal saphenous artery. a) control and b) in the presence of A II. For clarity responses to higher concentrations of UK-14304 are not shown, although a maximum response was achieved in both situations.

a)



b)

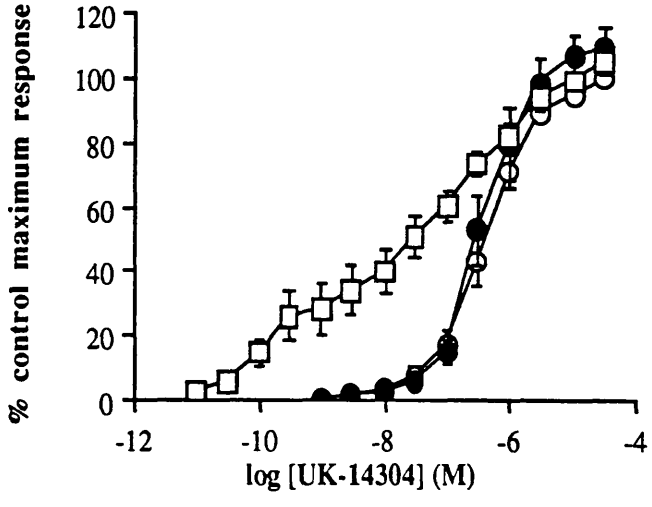


Figure No. 45

Effects of saralasin (1μM) (●) **a)** alone and **b)** on A II (0.05μM) induced potentiation (□) of responses to UK-14304 in rabbit isolated distal saphenous artery. Results are expressed as a % of the control maximum response to UK-14304 (○).

Each point represents the mean ± s.e.mean (n = 5).

agonist	*control* pD_2	*A II present* pD_2
NA (n = 6)	7.25 ± 0.09	7.17 ± 0.10
phenylephrine (n = 5)	6.56 ± 0.08	6.54 ± 0.10
UK-14304 (n = 7)	6.59 ± 0.12	7.78 ± 0.36**
Sgd 101/75 (n = 5)	4.84 ± 0.06	5.16 ± 0.03**

Table 7

List of pD_2 values (with 95% confidence limits) for α-adrenoceptor agonists in the absence and presence of A II (0.05μM) in rabbit isolated distal saphenous artery. **denotes a significant difference between responses in the absence and presence of A II (0.05μM), $p<0.01$, Student's *t*- test.

When this effect was taken into consideration, prazosin (0.1μM) produced similar rightward displacements in the absence and presence of A II (0.05μM). The log agonist concentration ratios values were approximately 1.44 and 1.46 respectively, (unpaired observations, n = 5).

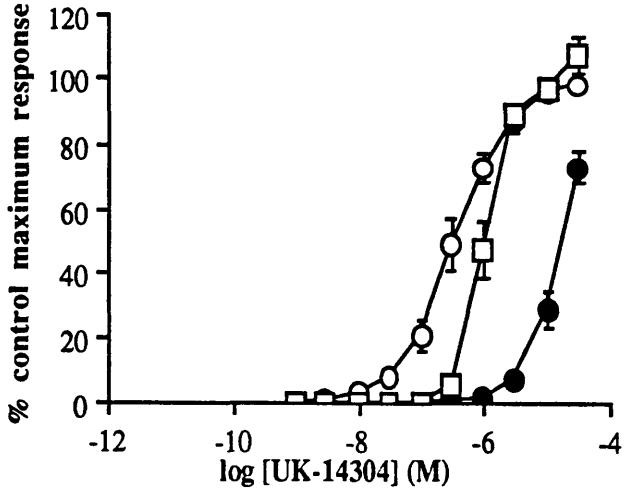
Attempted isolation of postjunctional α_2 -adrenoceptors

The previous results suggested that postjunctional α_2-adrenoceptors could be demonstrated in the rabbit isolated distal saphenous artery under appropriate experimental conditions. An attempt was therefore made to isolate a homogeneous population of postjunctional α_2-adrenoceptors in this preparation using the receptor protection protocol outlined in Figure 4. Phenoxybenzamine (0.3μM) virtually abolished all responses to NA in this preparation (Figure 49a). The rationale employed therefore, was to prevent phenoxybenzamine binding to α_2-adrenoceptors by masking these receptors with 1μM rauwolscine. This concentration of rauwolscine was considered to be without effect at α_1-adrenoceptors, since it was ineffective against responses to phenylephrine in this preparation.

Agonist responses

The inclusion of rauwolscine (1μM) prior to, and during, the incubation period with phenoxybenzamine (0.3μM), did not result in part of the response to NA being "spared" (Figure 49b). The maximum response to NA was reduced to a similar degree by phenoxybenzamine alone and by the combination of rauwolscine and phenoxybenzamine (% reduction of NA maximum response: 97.4 ± 1.2 and 95.8 ± 2.3, respectively). If however, the preparations were exposed to A II (5nm - 0.05μM) after the combination of rauwolscine (1μM) and phenoxybenzamine

a)



b)

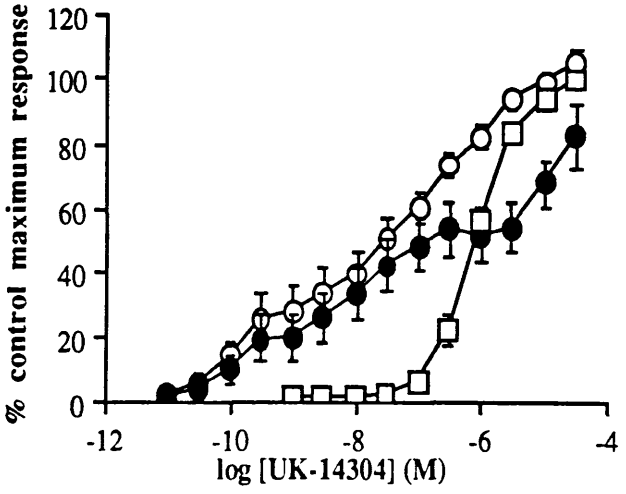


Figure No. 46

Effect of the α-adrenoceptor antagonists prazosin (0.1μM) (●) and rauwolscine (1μM) (□) on responses to UK-14304 (○) in **a)** the absence and **b)** the presence of A II (0.05μM) in the rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to UK-14304 obtained under normal experimental conditions (**a**) or in the presence of A II (**b**).

Each point represents the mean ± s.e.mean (n = 7).

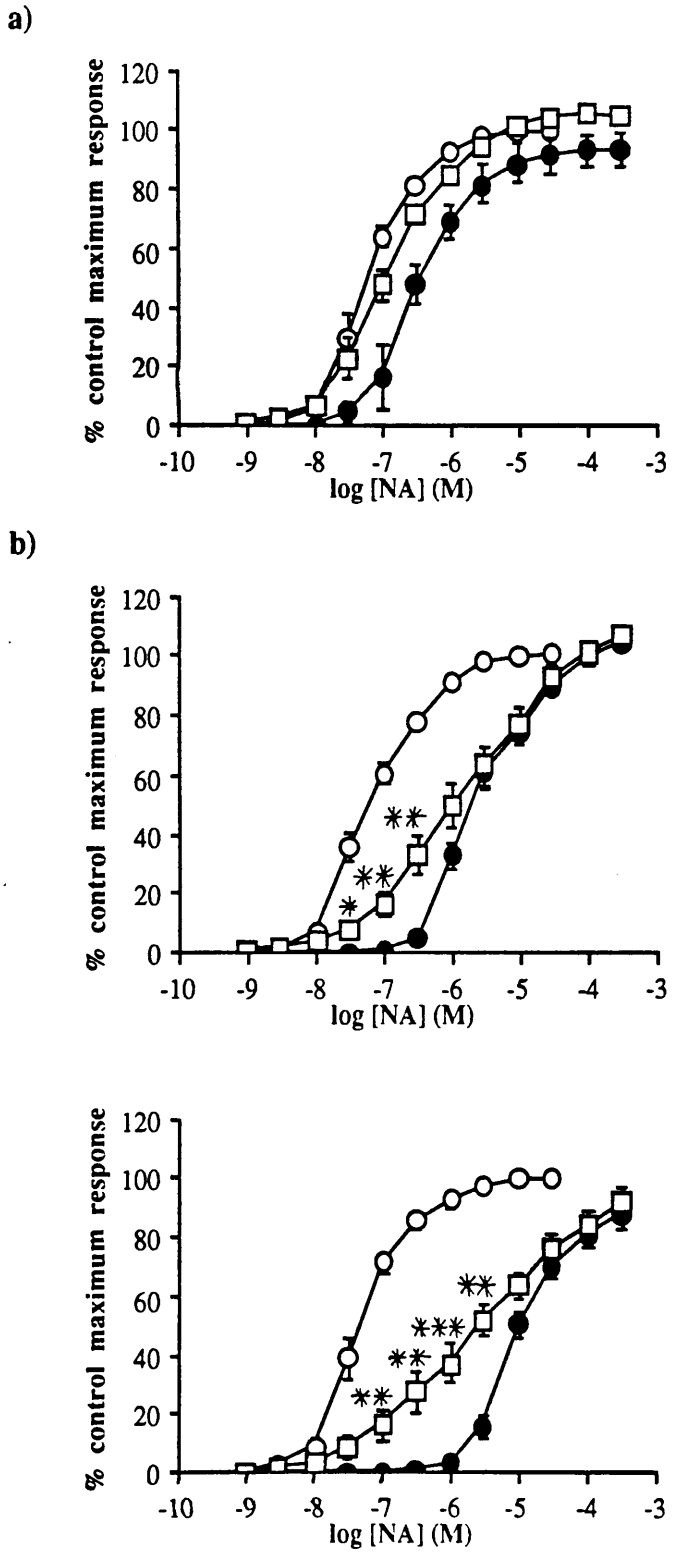
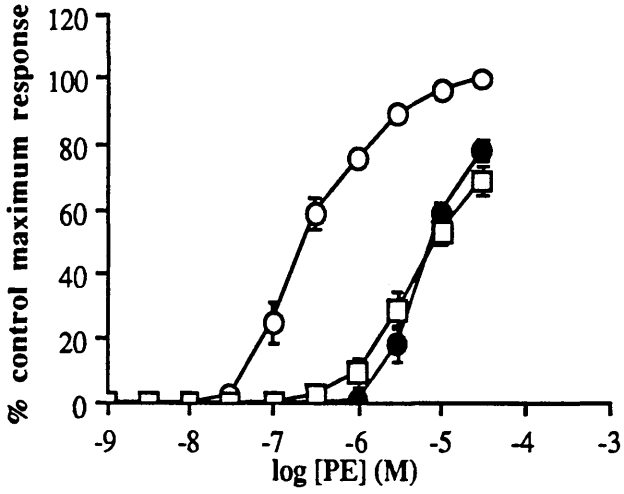


Figure No. 47

Effects of prazosin in the absence (●) and in the presence (□) of A II 0.05μM **a)** 0.01μM **b)** 0.1μM and **c)** 1μM on responses to NA (○) in the rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to NA. Each point represents the mean ± s.e.mean (n = 5 - 6). Statistically significant differences between responses in the presence of prazosin alone and in combination with A II are represented by *p<0.05, **0.01<p<0.001, ***p<0.001, Student's *t*- test.

a)



b)

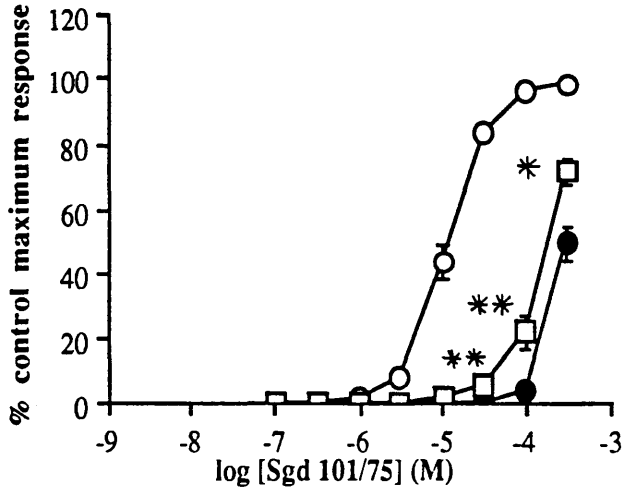


Figure No. 48

Effect of prazosin (0.1μM), in the absence (●) and in the presence (□) of A II 0.05μM on responses to **a)** phenylephrine (○) and **b)** Sgd 101/75 (○) in rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response obtained for each agonist.

Each point represents the mean ± s.e.mean (n = 5). Statistically significant differences between responses in the presence of prazosin alone and in combination with A II are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

Antagonist	*25%*	*50%*	*75%*
prazosin (0.1μM) (n = 6)	1.55 ± 0.06	1.57 ± 0.08	1.63 ± 0.06
prazosin (0.1μM) with A II (0.05μM) (n = 6)	1.08 ± 0.09[a]	1.32 ± 0.06	1.67 ± 0.07

Table 8

Log agonist concentration-ratio values for NA in the presence of prazosin (0.1μM) alone or in combination with A II (0.05μM) calculated at the levels of 25%, 50% and 75% of the maximum response to NA.

[a] represents a significant difference for the log agonist concentration ratio value for NA calculated at the 25% level of the maximum response in the presence of A II.

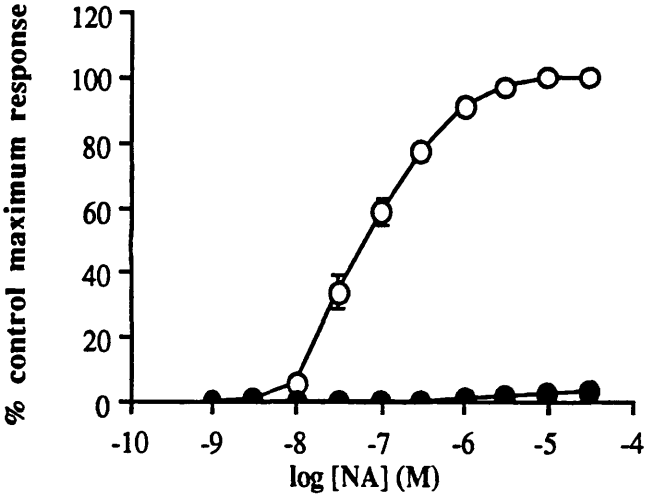
(0.3μM), concentration-dependent responses to NA were observed. The maximum response after attempted isolation of postjunctional α_2-adrenoceptors was increased from 4.2 ± 2.3 to 35.1 ± 2.9 % (n = 11) of the initial maximum response to NA (Figure 50a) in the absence and presence of A II (0.05μM) respectively. Similarly, A II (5nM) allowed the expression of responses to NA equivalent to 23.3 ± 3.3 % (n = 9) of the initial maximum response after treatment with the combination of rauwolscine and phenoxybenzamine (Figure 50b).

A II was also found to be a necessary prerequisite for the expression of responses to the α-adrenoceptor agonists phenylephrine and UK-14304 after the protection protocol (Figure 51a). In the presence of A II all agonists, NA, phenylephrine and UK-14304, produced concentration-dependent contractions with pD_2 values of 6.66 ± 0.29 (NA), 5.15 ± 0.07 (phenylephrine, estimated value) and 8.36 ± 0.31 (UK-14304) respectively (Figure 51b). Under these circumstances the relative potency of the agonists was: UK-14304 > NA > phenylephrine in contrast to the potency ratio of NA > phenylephrine = UK-14304 seen under normal experimental conditions (see Figure 37).

Effects of α-adrenoceptor antagonists

As shown in Figure 52, responses to NA in the presence of A II, after attempted isolation of postjunctional α_2-adrenoceptors, were resistant to prazosin (0.1μM) (pD_2 values for NA; 6.66 ± 0.29 and 6.42 ± 0.18 in the absence and presence of prazosin (0.1μM) respectively, n = 5), but susceptible to rauwolscine (1μM), which produced an approximately 100-fold rightward displacement of the CCRC to NA (Figure 52a). Furthermore, the combination of prazosin (0.1μM) and rauwolscine (1μM) was no more effective at antagonising responses to NA than rauwolscine (1μM) alone (Figure 52b).

a)



b)

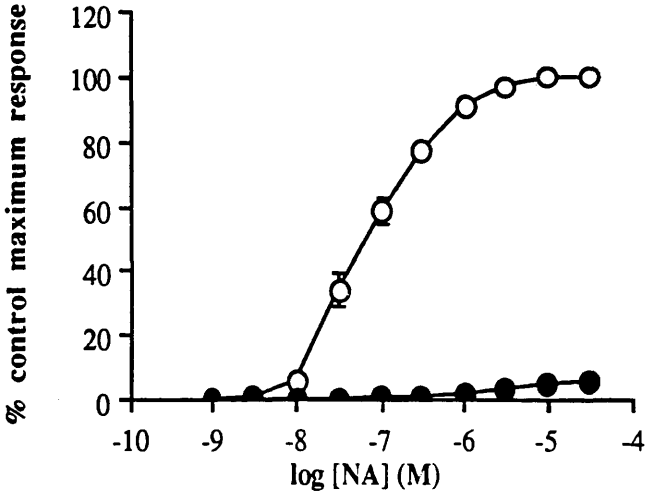
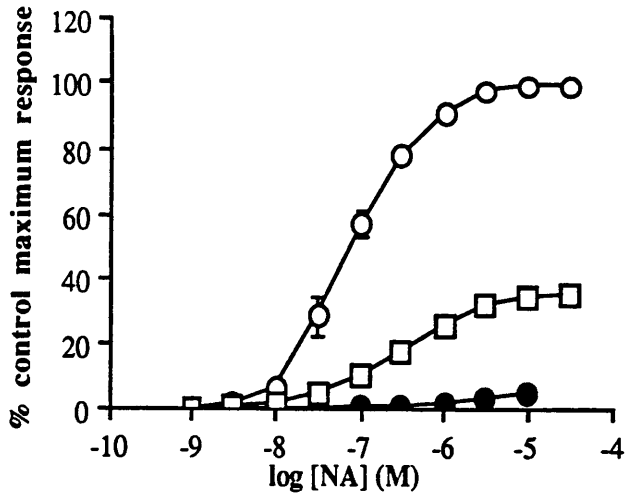


Figure No. 49

Effect of prior incubation with phenoxybenzamine (0.3μM) (●) on responses to NA (○) in **a)** the absence and **b)** the presence of rauwolscine (1μM) in rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to NA.

Each point represents the mean ± s.e.mean (n = 6). All points on the CCRC to NA in the presence of either phenoxybenzamine alone or in combination with rauwolscine are significantly different from control, $p<0.05$, Student's *t*- test

a)



b)

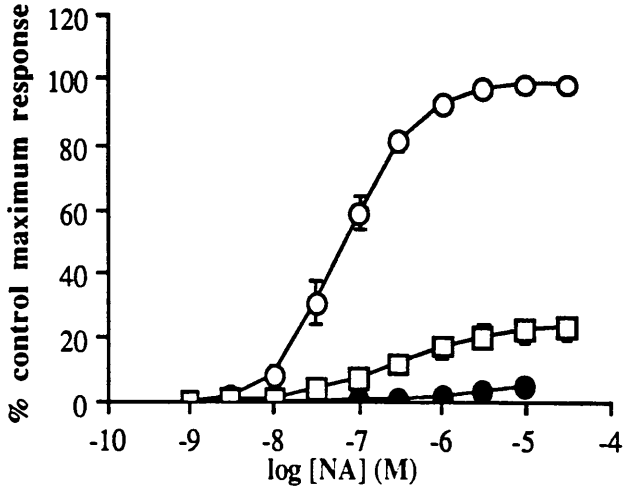
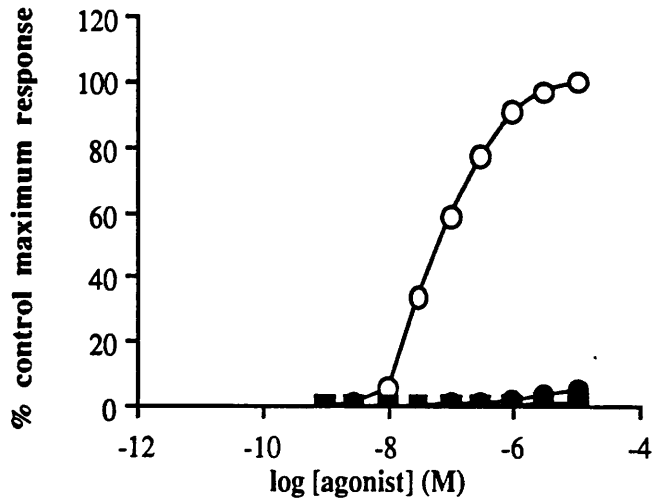


Figure No. 50

Effects of **a)** A II (0.05μM) (□) and **b)** A II (5nM) (□) on responses to NA after attempted isolation of postjunctional α_2-adrenoceptors (●) in rabbit isolated distal saphenous artery. Responses are expressed as a % of the maximum response to NA (○) prior to the protection protocol.

Each point represents the mean ± s.e.mean (n = 9 - 11). All responses to NA in the presence of A II after the protection protocol are significantly different from those after the protection protocol alone, $p<0.05$, Student's *t*- test.

a)



b)

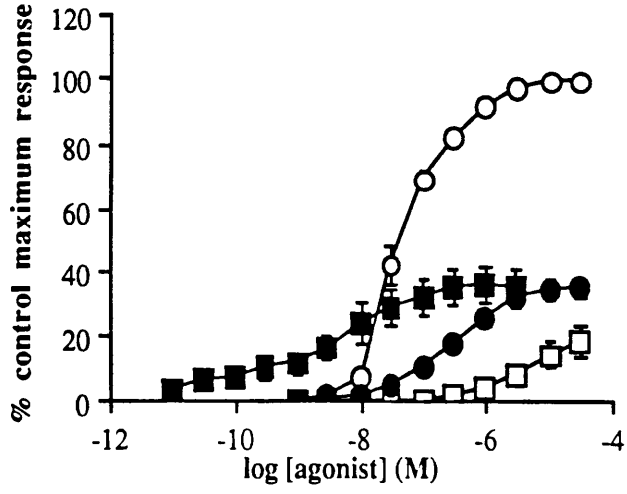
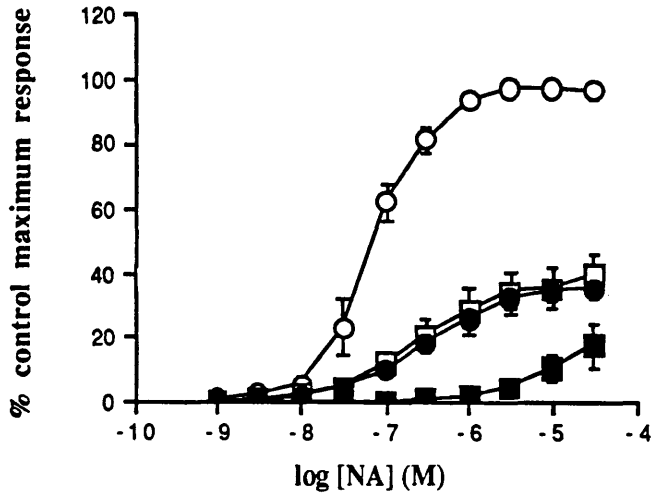


Figure No. 51

Responses to the α-adrenoceptor agonists NA (●), phenylephrine (□) and UK-14304 (■) in a) the absence and b) the presence of A II (0.05μM) after attempted isolation of postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to NA (○) prior to the protection protocol.

Each point represents the mean ± s.e.mean (n = 6 - 11).

a)



b)

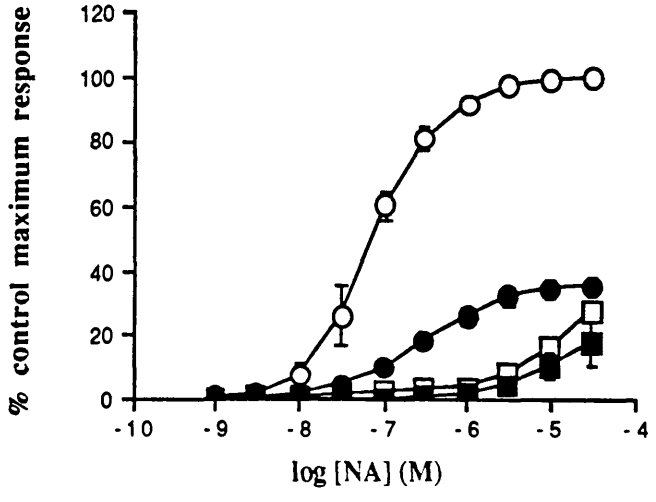


Figure No. 52

Effect of a) prazosin (0.1μM) (□) and rauwolscine (1μM) (■) alone and of **b)** rauwolscine (1μM) (■) alone and in combination with 0.1μM prazosin (□) on responses to NA, in the presence of A II (0.05μM), after attempted isolation of postjunctional α_2-adrenoceptors (●) in rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to NA (○) prior to the protection protocol.

Each point represents the mean ± s.e.mean (n = 5 - 11).

Effects of other contractile agents on responses to UK-14304 in rabbit isolated distal saphenous artery

Bay K 8644

As demonstrated in the present study, A II and Bay K 8644 enhance α_2-adrenoceptor mediated responses in the rabbit isolated lateral saphenous vein. In addition, Bay K 8644 has been reported to be a necessary stimulus for the expression of α_2-adrenoceptors in the canine saphenous artery (Sulpizio & Hieble, 1987). The influence of Bay K 8644 on responses to UK-14304 in the rabbit isolated distal saphenous artery was therefore examined

Bay K 8644 (3nM - 0.3μM), without influencing resting baseline tension, significantly increased responses to UK-14304 in this preparation. This enhancement was associated with an approximately 25% increase in the maximum response and a two-fold leftward displacement of the CCRC to UK-14304 and was maximal with the lowest concentration of Bay K 8644 employed (3nM) (Figure 53). Despite the potentiation produced by Bay K 8644, there was no increase in the threshold concentration of UK-14304 for contraction, which was approximately 0.01μM in the absence and presence of Bay K 8644.

Angiotensin I

The physiological precursor of A II, angiotensin I (A I) (0.5μM) produced a transient contraction in the distal saphenous artery, equivalent to 55.7 ± 6.7% (n = 7) of the maximum response to NA. This contraction was characterised by a slower onset and longer duration than equivalent sized responses to A II, and in two of seven preparations the contraction did not completely return to baseline. The

remaining residual contraction to A I was not greater than 5% of the maximum response to NA in either experiment. A I mimicked the effects of A II by producing a marked increase in the sensitivity of the distal saphenous artery to UK-14304, with a small increase in the maximum response (Figure 54a). The threshold concentration for contraction to UK-14304 was increased from approximately 0.01μM to 0.01nM in the presence of A I. A high concentration of the angiotensin-converting enzyme inhibitor captopril (1μM) had no effect on responses to UK-14304 alone (Figure 54b). This concentration of captopril reduced, but did not completely abolish, the contractile response to A I (0.5μM). A peak response equivalent to 19.7 ± 8.8% (n = 5) of the maximum response to NA was obtained in the presence of the ACE inhibitor. In addition, captopril did not reverse the facilitatory action of A I on responses to UK-14304 (Figure 54c).

Phenylephrine

A concentration of phenylephrine selective for α_1-adrenoceptors (10nM), produced a sustained contraction equivalent to 13.8 ± 2.0% (n = 5) of the maximum response to UK-14304 in the rabbit isolated distal saphenous artery. Subsequently, exposure of the preparation to UK-14304, resulted in concentration-dependent contractions to this agonist superimposed upon the phenylephrine-induced tone. The sensitivity of the preparation to UK-14304 was markedly increased in a manner analogous to that seen with A II, with the threshold sensitivity of the preparation to UK-14304 being increased from approximately 0.01μM to 0.03nM in the presence of the α_1-adrenoceptor agonist (Figure 55).

U46619

The thromboxane A_2 mimetic agent, U46619 (1nM) produced a sustained

contraction, equivalent to 24.2 ± 8.1% (n = 5) of the maximum response to UK-14304 in the rabbit isolated distal saphenous artery. Again, in the presence of a contractile agent, the preparation was exquisitely sensitive to UK-14304. Responses to the α_2-adrenoceptor agonist were observed at concentrations as low as 0.01nM, with no change in the maximum response, resulting in a flattening of the CCRC to UK-14304 (Figure 56a).

Potassium Chloride (KCl)

KCl (17-20mM) produced a sustained contraction equivalent to 12.8 ± 3.8% (n = 4) of the maximum response to UK-14304 in this preparation. Responses to UK-14304 were significantly enhanced when superimposed on top of depolarisation-induced tone (pD_2 values for UK-14304 were 6.39 ± 0.11 and 6.89 ± 0.04 in the absence and presence of tone respectively, $p<0.05$, Student's *t*- test, n = 4) (Figure 56b). This enhancement of responses however, was not associated with a marked increase in the threshold sensitivity of the preparation to UK-14304, as seen with A II, phenylephrine or U46619. In only one of four preparations, was the threshold concentration for contraction for UK-14304 less than 1nM.

5-Hydroxytryptamine (5-HT)

5-HT (0.01μM-0.3μM) produced a sustained contraction equivalent to 17.9 ± 6.1% (n = 5) of the maximum response to UK-14304 in the rabbit isolated distal saphenous artery. Inducing tone with this agent was not associated with a significant enhancement or "uncovering" of responses to UK-14304 (Figure 56c).

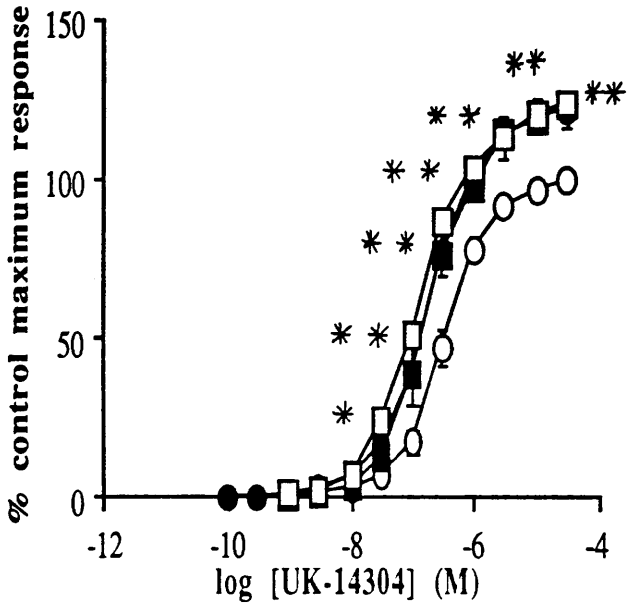


Figure No. 53

Effect of Bay K 8644 3nM (●), 0.03μM (□) and 3μM (■) on responses to UK-14304 (○) in rabbit isolated distal saphenous artery. Results are expressed as a % of the original maximum response to UK-14304 (○). Each point represents the mean ± s.e.mean (n = 5). Statistically significant differences between responses in the absence and presence of Bay K 8644 are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

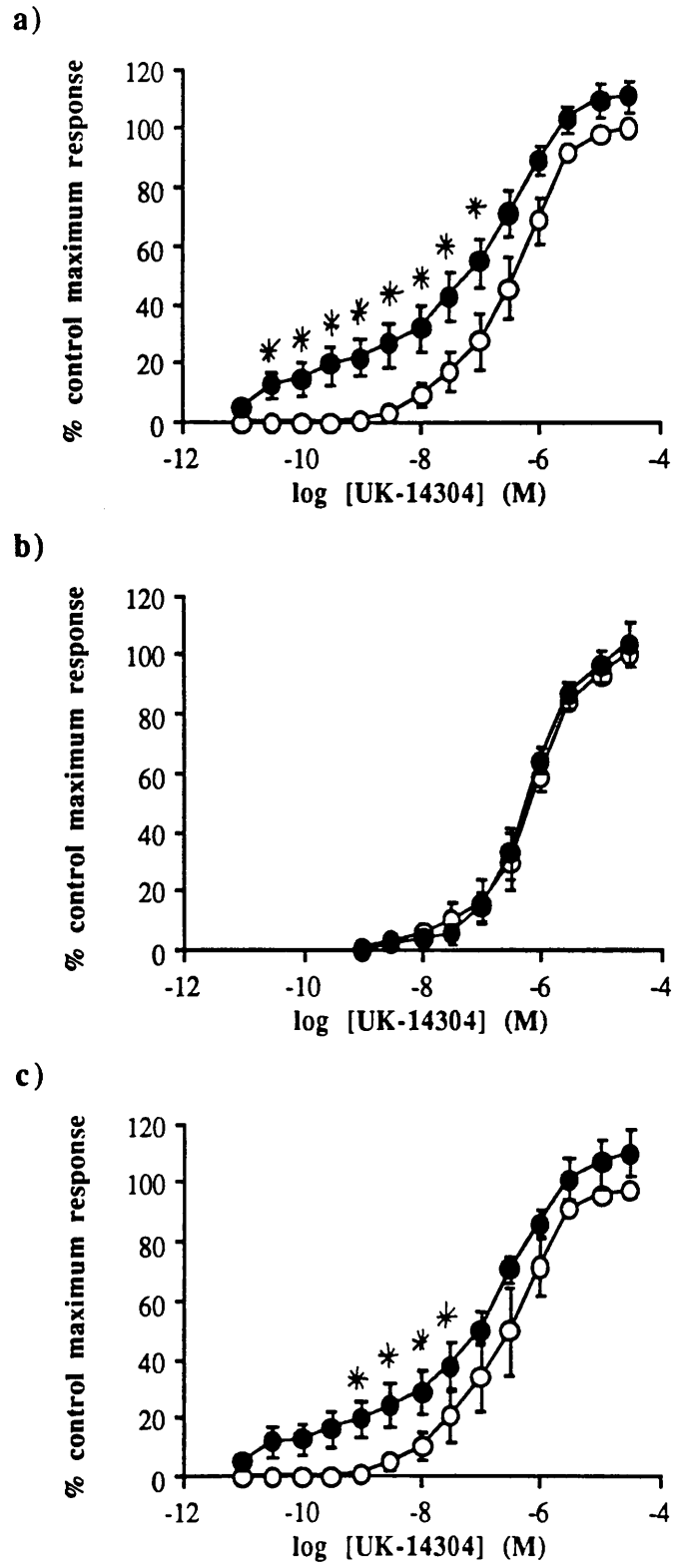


Figure No. 54

Effect of **a)** angiotensin I (0.5μM) (●) **b)** captopril (1μM) (●) and **c)** the combination of A I (0.5μM) and captopril (1μM) (●) on responses to UK-14304 (○) in rabbit isolated distal saphenous artery.

Each point represents the mean ± s.e.mean (n = 4 - 7). Statistically significant differences between responses to UK-14304 in the absence and presence of A I or the combination of captopril and A I, are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

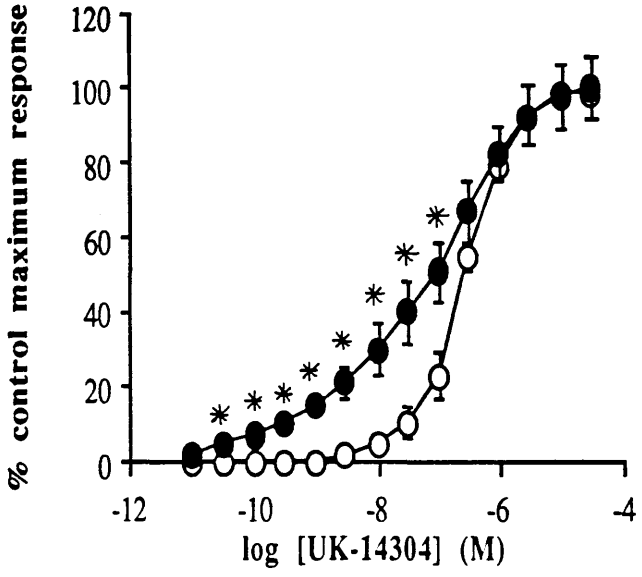


Figure No. 55

Effects of inducing tone with the selective α_1-adrenoceptor agonist phenylephrine on responses to the selective α_2-adrenoceptor agonist UK-14304 (●) in rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to UK-14304 (○).

Each point represents the mean ± s.e.mean (n = 5). Statistically significant differences between responses to UK-14304 in the absence and presence of phenylephrine are represented by *$p<0.05$, Student's *t*- test.

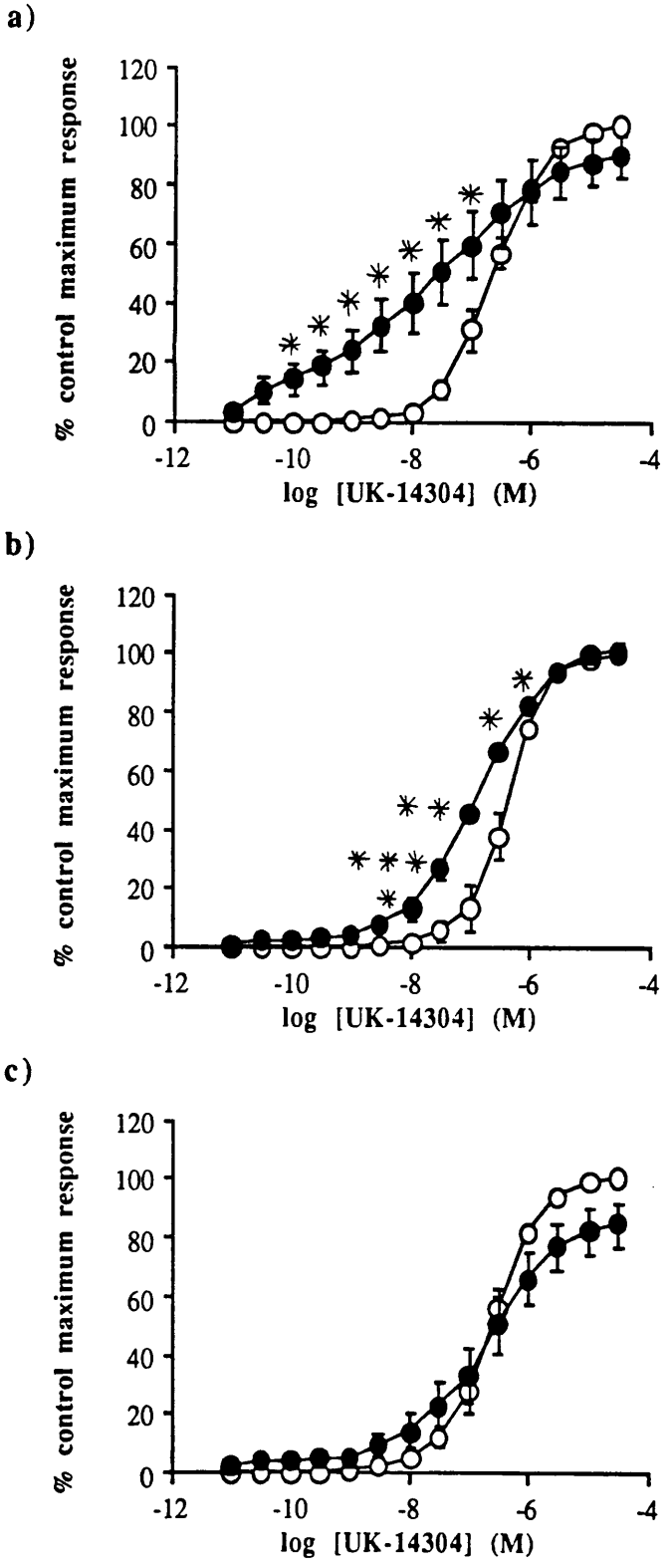


Figure No. 56

Effects of inducing tone with **a)** U46619 **b)** KCl or **c)** 5-HT on responses to UK-14304 (●) in rabbit isolated distal saphenous artery. Results are expressed as a % of the original maximum response to UK-14304 (○).

Each point represents the mean ± s.e.mean (n = 5). Statistically significant differences between control responses and those in the presence of a contractile agent are represented by *$p<0.05$, **$0.01<p<0.001$, ***$p<0.001$, Student's *t*- test.

Effects of A II on responses to UK-14304 in various other arterial preparations from the rabbit

A series of preliminary experiments were carried out in various other arterial preparations from the rabbit to determine the effects of A II (0.05μM) on responses to UK-14304. In these experiments preparations were initially exposed twice to 3μM NA at 30 minute intervals. The response to the second exposure of 3μM NA was assumed to be maximal.

Femoral artery

UK-14304 produced concentration-dependent contractions in the rabbit isolated femoral artery with a maximum response equivalent to 50.8 ± 5.5% of the maximum response to NA. A II (0.05μM) produced a transient response in this preparation, the peak of which was equivalent to 77.1 ± 3.4% of the NA maximum. This contraction did not completely return to baseline. Therefore a residual response equivalent to 9.3 ± 3.3 % of the NA maximum persisted prior to the onset of a 2nd CCRC to UK-14304. This concentration of A II significantly potentiated responses to UK-14304 at all points of the CCRC, producing an increase in the maximum response and sensitivity of the preparation to the agonist. This increase in sensitivity was particularly marked in the lower portion of the CCRC to UK-14304 (the mean log agonist concentrations required to produce 25% of the maximum response to UK-14304 were 6.84 ± 0.21 and 7.52 ± 0.16, in the absence and presence of A II respectively, n = 5, $p<0.05$ Student's *t*-test) (Figure 57a). Perhaps more importantly however, the threshold concentration for contraction for UK-14304 was reduced from approximately 0.01μM under control conditions to 0.03nM in the presence of A II. This resulted in the shape of the CCRC to UK-14304 becoming distinctly biphasic.

Superior Mesenteric artery

No responses were observed upon exposure of the superior mesenteric artery to UK-14304. A II (0.05μM) produced a transient response equivalent to 26.9 ± 8.9% (n = 5) of the NA maximum response in this preparation, however even in the presence of A II this tissue remained unresponsive to UK-14304 (Figure 57b).

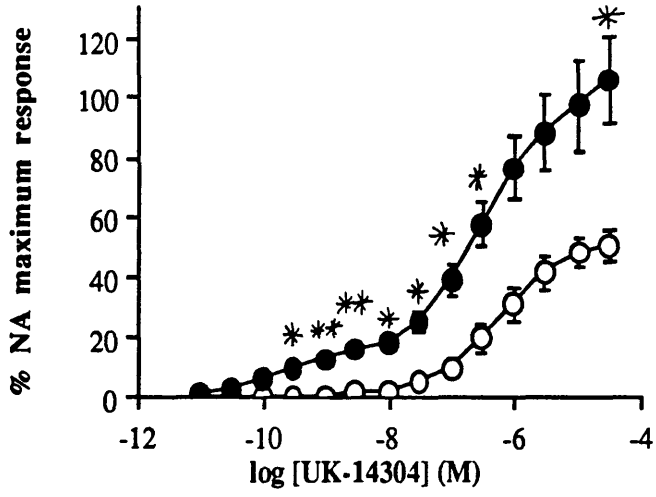
Central ear artery

UK-14304 produced concentration-dependent contractions in the rabbit isolated central ear artery with a maximum response equivalent to 71.4 ± 9.4 % of the maximum response to NA (n = 5). A II (0.05μM) was virtually inactive in this preparation producing a response equivalent to only 1.9 ± 0.5 % of the NA maximum. This concentration of A II did not significantly influence responses to UK-14304 (pD_2 values for UK-14304 were 6.74 ± 0.16 and 6.72 ± 0.15 in the absence and presence of A II respectively, n = 5) (Figure 58a)

Renal artery

UK-14304 produced concentration-dependent contractions in the renal artery with a maximum response equivalent to 19.9 ± 5.1% of the NA maximum response (n = 5). A II (0.05μM) produced a transient contraction in this preparation which was equivalent to 57.1 ± 12.9% of the maximum response to NA. In the presence of A II, responses to UK-14304 were significantly increased in size, however this was not associated with a change in sensitivity of the preparation to the agonist (estimated pD_2 values for UK-14304 were 5.44 ± 0.09 and 5.61 ± 0.19, in the absence and presence of A II respectively, n = 5) or in the threshold concentration for contraction which was approximately 10μM on each occasion (Figure 58b).

a)



b)

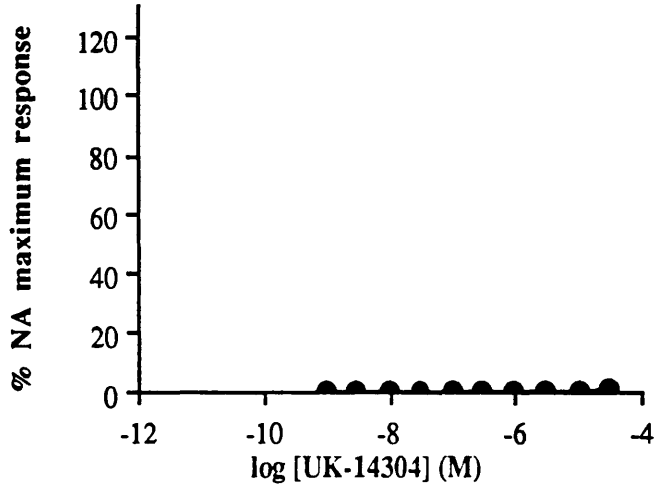
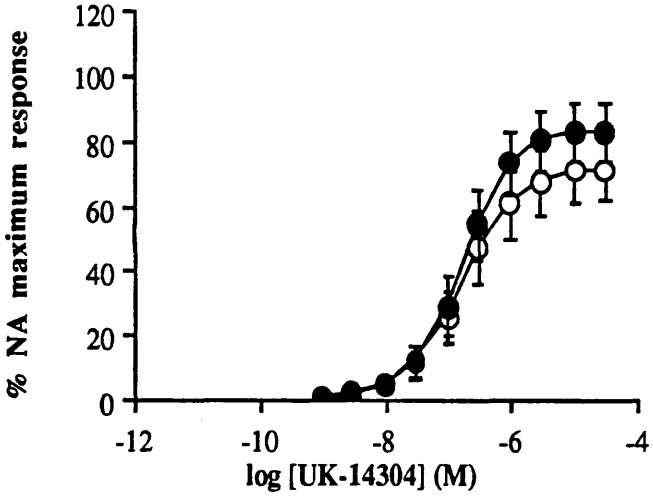


Figure No. 57

Effects of A II (0.05μM) (●) on responses to UK-14304 (○) in rabbit isolated **a)** femoral artery and **b)** superior mesenteric artery. Results are expressed as a % of the maximum response to NA obtained in each preparation.

Each point represents the mean ± s.e.mean (n = 4 - 5). Statistically significant differences between responses in the absence and presence of A II (0.05μM) are represented by *$p<0.05$, **$0.01<p<0.001$, ***$0.001<p$, Student's *t*- test.

a)



b)

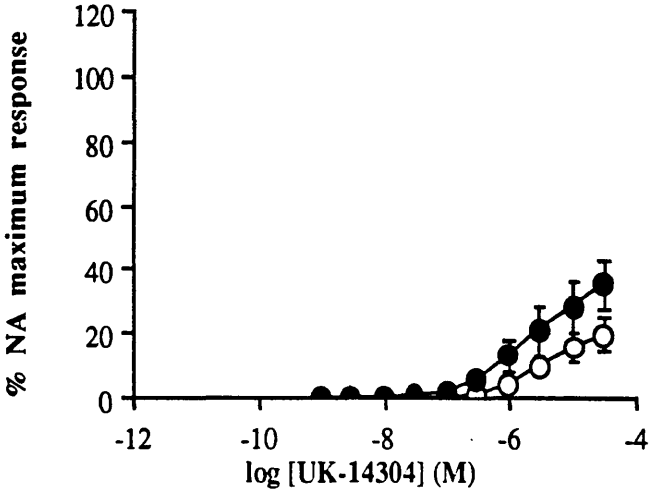


Figure No. 58

Effect of A II (0.05μM) (●) on responses to UK-14304 (○) in rabbit isolated **a)** central ear artery and **b)** renal artery. Results are expressed as a % of the maximum response to NA obtained in each preparation.
Each point represents the mean ± s.e.mean (n = 5).

Effects of nifedipine on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors in rabbit isolated blood vessels

In parallel with the subdivision of postjunctional α-adrenoceptors was an examination of the Ca^{2+} sources utilised for contraction upon stimulation of each subtype. From whole animal studies it has been suggested that postjunctional α_2-adrenoceptor-mediated responses are more susceptible to the action of calcium channel blockers than are those to α_1-adrenoceptor stimulation (Van Meel *et al.*, 1981), although this contention has been debated by other groups (O'Brien *et al.*, 1985, Nichols & Ruffolo, 1988). This prolonged disagreement reflects a limitation of studies carried out in whole animals, where bolus injections of 'selective' agonists invariably do not reflect "equilibrium" responses. In addition, clarification from *in vitro* experiments has not been forthcoming due to the lack of suitable isolated vascular preparations which have populations of postjunctional α_2-adrenoceptors. Therefore, having demonstrated a method for isolating homogeneous populations of α_2-adrenoceptors in an isolated arterial and venous preparation from the rabbit, the effect of a calcium channel blocker, nifedipine, was examined on responses to NA. For comparison, the effect of nifedipine against responses to NA mediated by postjunctional α_1-adrenoceptors in the rabbit isolated left renal vein was also attempted.

Lateral saphenous vein

Responses to NA were not reproducible with time when the full receptor complement was present in the rabbit isolated lateral saphenous vein (Figure 59a). There was a 17 ± 3 % increase in the maximum response to the agonist in the 2nd CCRC, although this was not associated with a change in the sensitivity of the preparation to NA (pD_2 value for NA, 7.73 ± 0.27 and 7.65 ± 0.17 for the 1st and

2nd CCRC's respectively, n = 5). Nifedipine (0.01-0.1μM) was apparently without effect on responses to NA under these conditions (Figure 59b). This agent did however prevent the time-related increase in response to NA seen in the 2nd CCRC. Therefore after correction for time-related changes, each concentration of nifedipine significantly reduced responses to NA (Figure 59c, Table 9). This reduction in the magnitude of the response to NA produced by nifedipine was not associated with a change in the sensitivity of the preparation (Table 9).

After isolation of postjunctional α_2-adrenoceptors, responses to NA were reproducible with time. Nifedipine concentration-dependently reduced the magnitude of the responses to NA mediated via postjunctional α_2-adrenoceptors (Figure 60a). This effect was maximal with 0.1μM nifedipine. Again this reduction in the maximum response was not associated with a change in sensitivity of the preparation to NA (Figure 60b, Table 9).

KCl produced concentration-dependent contractions in the rabbit isolated lateral saphenous vein. Nifedipine (0.1μM) markedly reduced responses to the depolarising agent, although contractions were still apparent with higher concentrations of KCl (>40mM) (Figure 63a). These contractions were abolished by the subsequent addition of prazosin (0.1μM).

Distal saphenous artery

Nifedipine (0.01-1μM), at all concentrations employed, significantly reduced the sensitivity of the rabbit isolated distal saphenous artery to NA when the full α-adrenoceptor complement was present (Figure 61a, Table 9), although a reduction in the maximum response was observed only in the presence of the highest concentration (1μM) of the calcium channel blocker.

After isolation of postjunctional α_2-adrenoceptors, responses to NA were observed only in the presence of A II (0.05μM). Nifedipine (0.01-1μM) had no effect on the size of the contraction produced by A II (responses to A II (0.05μM) were equivalent to 22.7 ± 4.9 and 18.8 ± 4.3 % of the maximum response to NA in the absence and presence of nifedipine (0.1μM) respectively, n = 4), but produced a concentration-dependent depression of the maximum response to NA under these conditions (Figure 61b, Table 9). This reduction in the maximum response was not associated with a change in the sensitivity of the preparation to the agonist (Table 9)

KCl produced concentration-dependent contractions in this preparation with a maximum response equivalent to 77.9 ± 4.9 (n = 5) of the maximum response to NA. These responses were virtually abolished by nifedipine (0.1μM) (Figure 63b). Again any residual response remaining after treatment with nifedipine could be abolished by adding prazosin (0.1μM) to the bathing medium.

Left renal vein

In a preparation containing a homogeneous population of postjunctional α_1-adrenoceptors, the rabbit isolated left renal vein, nifedipine (0.1μM) produced a small but significant reduction in the maximum response to NA with no change in the sensitivity of the preparation to the agonist (Figure 62, Table 9). Interestingly, exposure of this preparation to KCl resulted in only very small contractile responses, equivalent to only 18.0 ± 6.5 of the maximum response to NA. Again these responses were virtually abolished upon exposure to nifedipine (0.1μM) (Figure 63c).

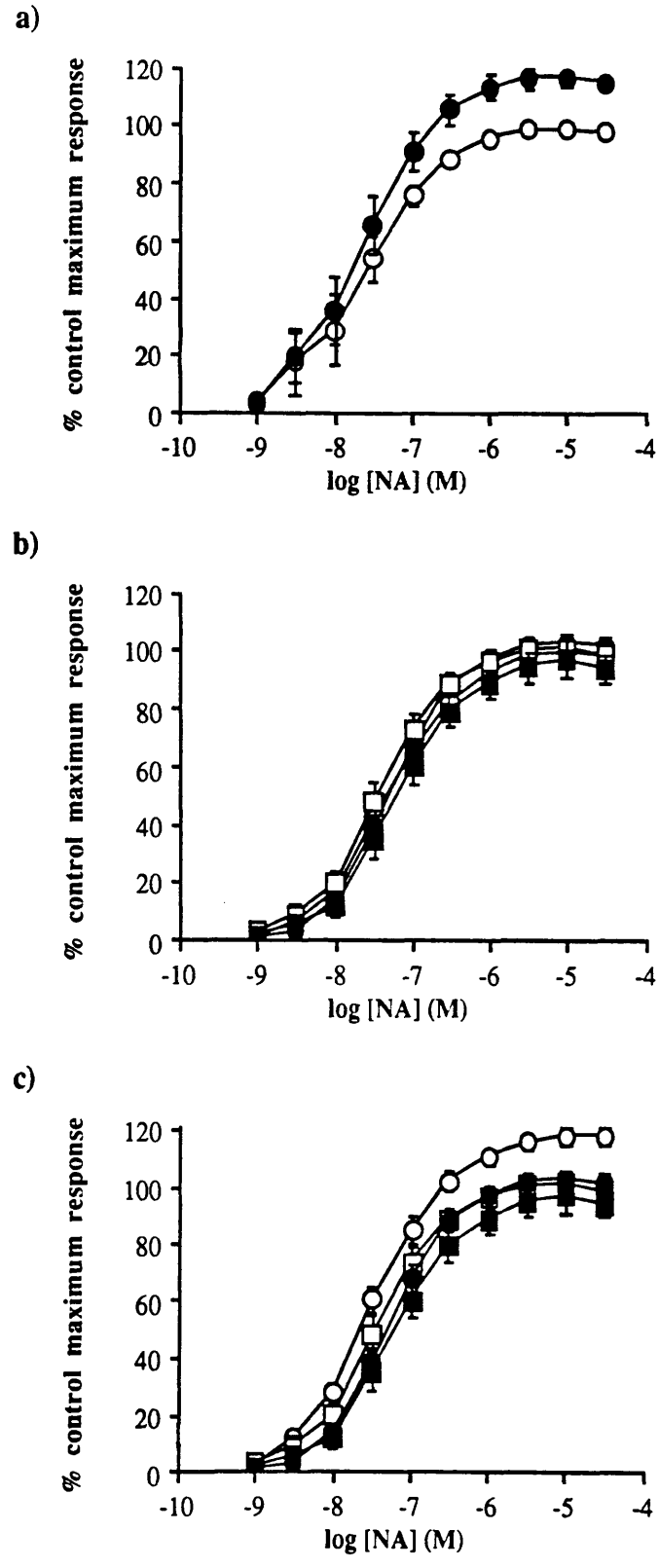


Figure No. 59

The effect of **a)** time (●) and of **b)** and **c)** nifedipine 0.01μM (●), 0.1μM (□) and 1μM (■) on responses to NA (○) in rabbit isolated saphenous vein. Results are expressed as either a % of the control maximum response (**a** and **b**) or as a % of the maximum response after correction for time-related changes (**c**).

Each point represents the mean ± s.e.mean (n = 4 - 5).

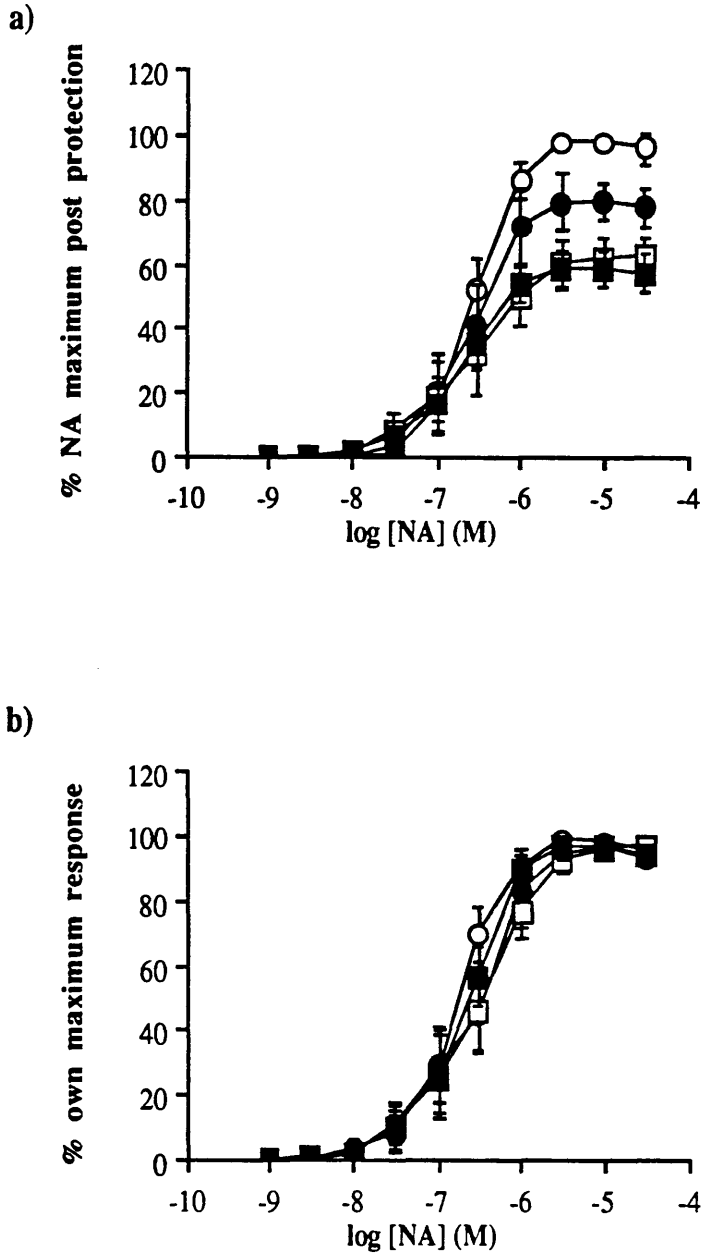
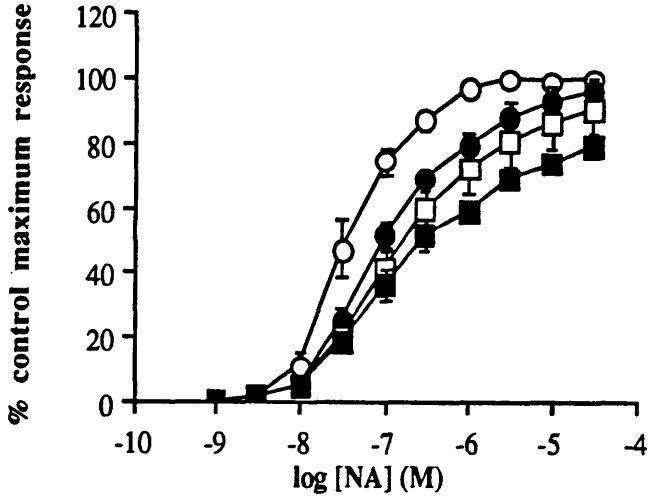


Figure No. 60

Effect of nifedipine 0.01μM (●), 0.1μM (□) and 1μM (■) on responses to NA (○) after isolation of postjunctional α_2-adrenoceptors in rabbit isolated lateral saphenous vein. Results are expressed as a % of either **a)** the control maximum response after the protection protocol or **b)** the maximum response in each individual CCRC.

Each point represents the mean ± s.e.mean (n = 4 - 5).

a)



b)

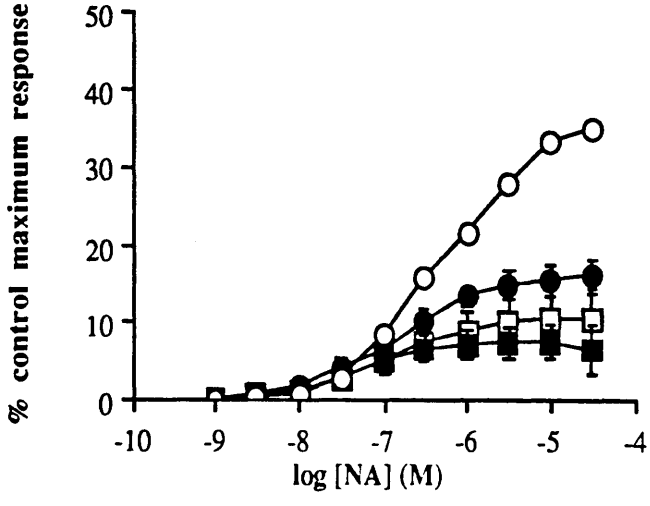
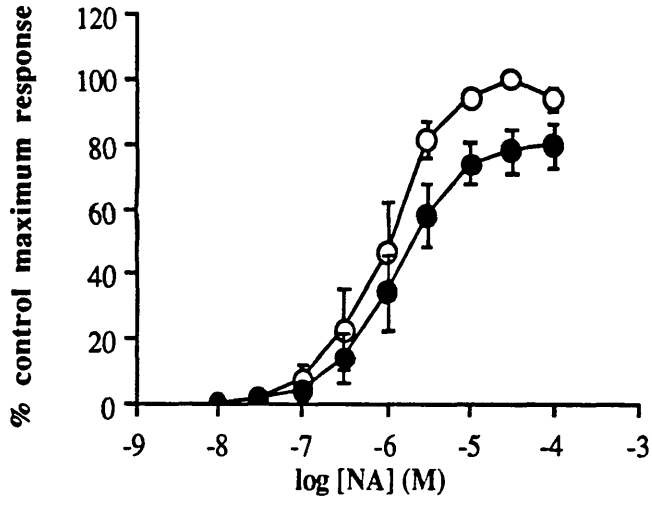


Figure No. 61

Effect of nifedipine 0.01μM (●), 0.1μM (□) and 1μM (■) on responses to NA (○) **a)** prior to and **b)** after isolation of postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery. Responses are expressed as a % of the control maximum response. Responses in figure **b** were obtained in the presence of A II (0.05μM).

Each point represents the mean ± s.e.mean (n = 4 - 5).

a)



b)

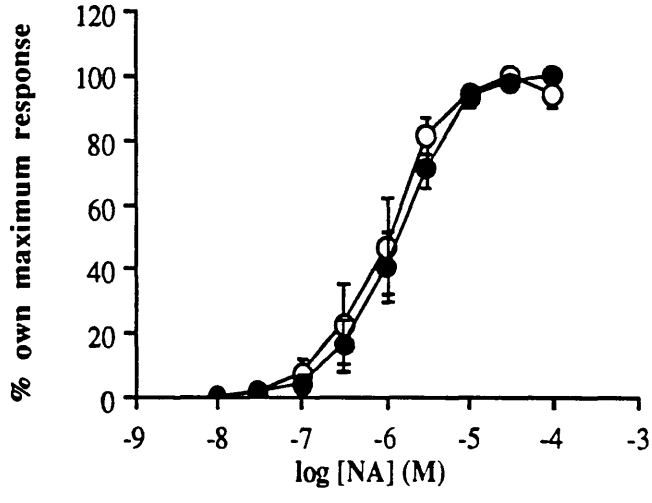


Figure No. 62

Effect of nifedipine (0.1μM) (●) on responses to NA (○) in the rabbit isolated left renal vein. Responses are expressed as a % of **a)** the control maximum response or **b)** of the maximum response in each individual CCRC.

Each point represents the mean ± s.e.mean (n = 4).

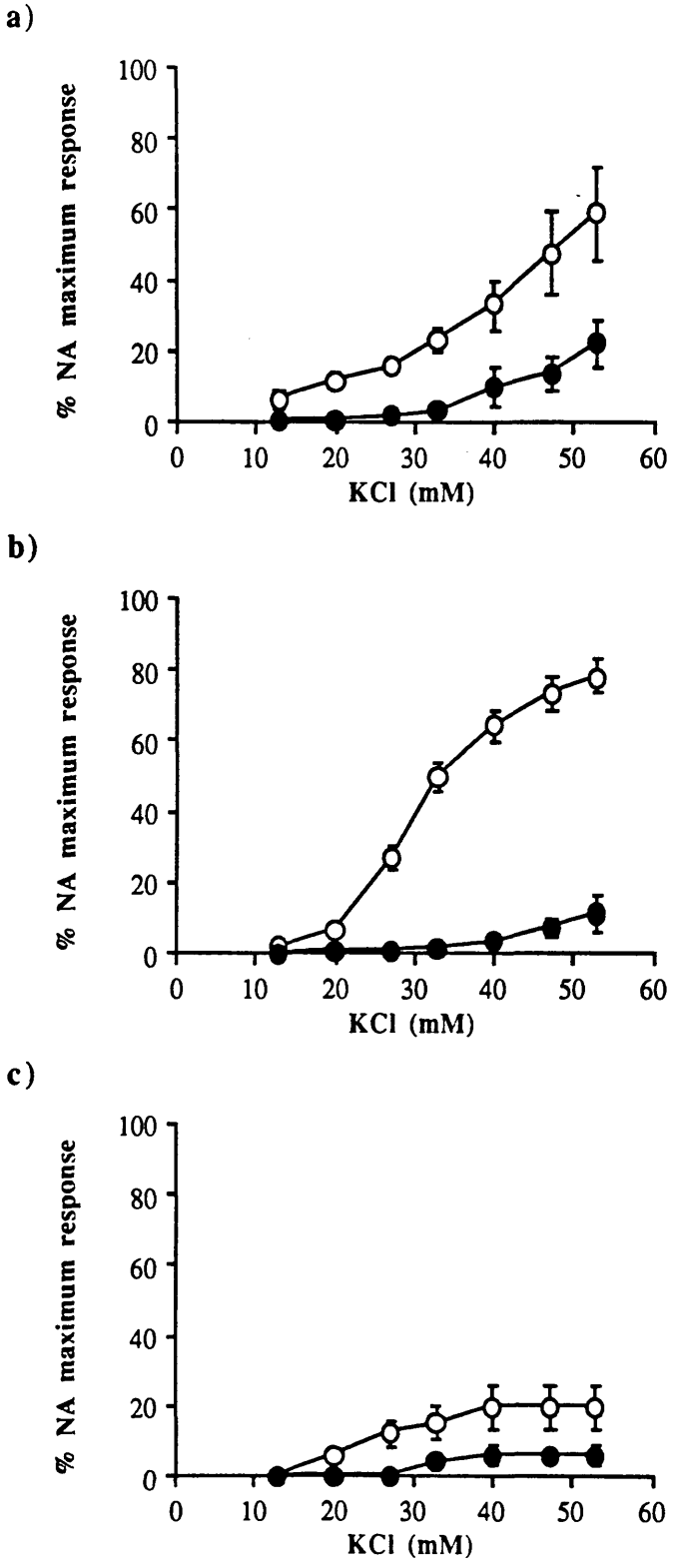


Figure No. 63

Effects of nifedipine (0.1μM) (●) on responses to KCl (○) in rabbit isolated **a)** lateral saphenous vein **b)** distal saphenous artery and **c)** left renal vein. Responses are expressed as a % of the maximum response to NA in each preparation.

Each point represents the mean ± s.e.mean (n = 3 - 6). All responses in the presence of nifedipine (0.1μM) are significantly different from control responses, $p<0.05$, Student's *t*- test.

preparation	*pD_2 value*		*E_{max}*	
	control	*Nifedipine*	*control*	*Nifedipine*
LSV (n = 5)	7.47 ± 0.07	7.46 ± 0.09	1	0.86 ± 0.04**
LSVRP (n = 4)	6.66 ± 0.17	6.53 ± 0.24	1	0.63 ± 0.05**
DSA (n = 5)	7.45 ± 0.15	6.88 ± 0.05*	1	0.91 ± 0.07
DSARP (n = 4)	6.66 ± 0.29	6.95 ± 0.16	1	0.30 ± 0.11*
LRV (n = 4)	6.07 ± 0.20	5.90 ± 0.16	1	0.80 ± 0.07*

Table 9

pD_2 values (with 95% confidence limits) and E_{max} values for NA in the presence of 0.1μM nifedipine as compared to the control values corrected for time-related changes in responses, in the rabbit isolated lateral saphenous vein (LSV), lateral saphenous vein after receptor protection using rauwolscine (1μM) and phenoxybenzamine (0.3μM) (LSVRP), distal saphenous artery (DSA), DSA after receptor protection with rauwolscine (1μM) and phenoxybenzamine (0.3μM) in the presence of A II (0.05μM) (DSARP) and the left renal vein (LRV).

Differences between control and treated preparations were considered statistically significant if $p<0.05$ for either paired or unpaired observations - Student's *t*-test and are denoted by: * $0.01<p<0.05$, ** $0.01<p<0.001$.

Sympathetic neurovascular transmission in the rabbit isolated distal saphenous artery

The fact that prazosin was found to be more effective at blocking responses to sympathetic nerve stimulation than it was at blocking equivalent sized responses to exogenous NA in whole animals, led to the suggestion that postjunctional α_1-adrenoceptors were preferentially innervated, while α_2-adrenoceptors were located extrajunctionally responding to circulating catecholamines (McGrath, 1982). However, some relatively clear cut examples of innervated postjunctional α_2-adrenoceptors have been demonstrated in a number of isolated venous preparations (eg. Flavahan *et al.*, 1984, Docherty & Hyland, 1985). Having demonstrated prazosin-resistant responses to NA in the rabbit isolated distal saphenous artery under appropriate conditions, the α-adrenoceptors involved in mediating the contractile response to sympathetic nerve stimulation in this preparation were examined. In the course of these experiments it was also found necessary to take into account the role of a purinergic component of the response to sympathetic nerve stimulation.

Responses to sympathetic nerve stimulation

Electrical field stimulation of ring segments of rabbit isolated distal saphenous artery resulted in frequency-dependent contractile responses which were reproducible for three frequency-response curves (FRC's) (Figure 64). With a stimulation duration of 1second, 4Hz was found to be the threshold frequency for contraction of this preparation. Subsequent two-fold increments in the frequency of stimulation resulted in a graded increase in the size of contractile response observed up to the highest frequency applied, 64Hz, which did not produce a maximum response. These contractions were characterised by a rapid rise to a peak response

approximately 5 seconds after the onset of electrical stimulation, which then returned to baseline over the period of 15-60 seconds. Contractile responses to a longer duration of electrical stimulation, 10 seconds, produced a larger peak response for a given frequency, which subsequently returned to baseline after 20-60 seconds. All responses to sympathetic nerve stimulation at any frequency or duration were sensitive to tetrodotoxin (0.1μM) (n = 3).

Effects of antagonists on responses to sympathetic nerve stimulation

The possible involvement of three receptor systems, postjunctional α_1- and α_2-adrenoceptors and purinoceptors, in mediating the end-organ contractile response to sympathetic nerve stimulation in the rabbit isolated distal saphenous artery, was examined using three appropriate antagonists, prazosin (0.1μM) selective for α_1-adrenoceptors, rauwolscine (1μM) selective for α_2-adrenoceptors and α,β-methylene ATP (3μM), a desensitising agent selective for purinoceptors. All experiments were carried out in the absence of either cocaine or propranolol.

Prazosin (0.1μM) markedly reduced contractile responses to sympathetic nerve stimulation at all frequencies in the FRC (Figure 65a), leaving only a small residual contractile response to nerve stimulation (for 64Hz this was equivalent to 17.2 ± 2.3 % of the initial control response). In contrast, rauwolscine (1μM) produced an enhancement of responses to electrical field stimulation in this preparation. This was particularly marked for lower frequencies or if a longer stimulation period was used (Figure 65b). A potentiation of nerve-mediated responses was also observed in the presence of α,β-methylene ATP (3μM) at all frequencies of the FRC (Figure 65c). This agent produced a transient contraction of the preparation equivalent to 113.9 ± 9.5 (n = 15) of the response to nerve stimulation at 64Hz, which returned to baseline after approximately 12-18 mins. No

contractions were subsequently observed upon re-exposure of the preparation to α,β-methylene ATP (3μM).

Since no antagonist on its own abolished responses to sympathetic nerve stimulation, the antagonists were tested in combination. As shown in figure 66a the combination of prazosin (0.1μM) and α,β-methylene ATP (3μM) was no more effective at inhibiting responses than prazosin alone. It should be noted however, that the potentiation of responses produced by α,β-methylene ATP (3μM) was no longer apparent in the presence of prazosin. In contrast, rauwolscine (1μM) continued to enhance responses to sympathetic nerve stimulation at all frequencies even after blockade of postjunctional α_1-adrenoceptors with prazosin (0.1μM) (Figure 66b). With the combination of rauwolscine (1μM) and α,β-methylene ATP (3μM) contractile responses were significantly increased from control values, although these two agents together did not produce a greater potentiation than that seen either antagonist alone (Figure 66c).

Prazosin (0.1μM) virtually abolished responses remaining after the combination of rauwolscine (1μM) and α,β-methylene ATP (3μM) (Figure 67c). Similarly, α,β-methylene ATP (3μM) had a marked inhibitory effect on responses remaining after the combination of prazosin (0.1μM) and rauwolscine (1μM) (Figure 67b). These results therefore confirm an involvement for both α_1-adrenoceptors and purinoceptors in the end-organ contractile response to sympathetic nerve stimulation in this preparation. Such a role for postjunctional α_2-adrenoceptors was more equivocal since rauwolscine (1μM) reduced but did not abolish the small residual responses persisting after the combination of prazosin (0.1μM) and α,β-methylene ATP (3μM) (Figure 67a).

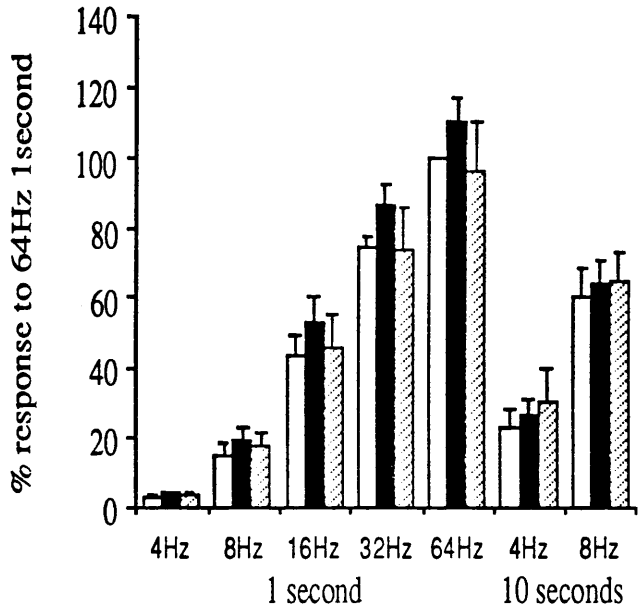


Figure No. 64

Reproducibility of consecutive frequency-response curves in rabbit isolated distal saphenous artery. Results are expressed as a % of the response to 64Hz 1second from the 1st FRC. The 1st FRC is represented by solid bars, the 2nd by closed bars and the 3rd by hatched bars.

Each point represents the mean ± s.e.mean (n = 5).

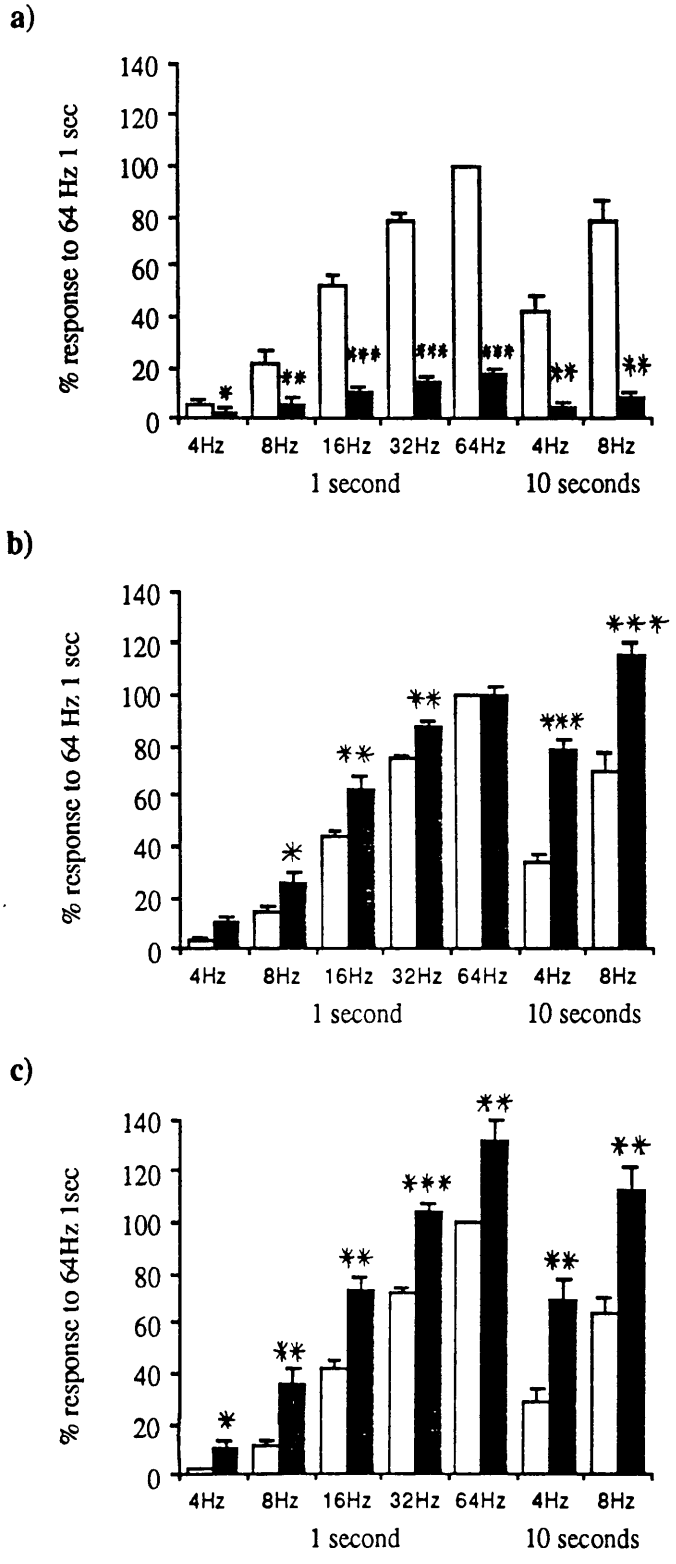


Figure No. 65

Effect of **a)** prazosin (0.1μM) (solid bars) **b)** rauwolscine (1μM) (solid bars) and **c)** α,β-methylene ATP (3μM) (solid bars) on responses to sympathetic nerve stimulation in rabbit isolated distal saphenous artery. Results are expressed as a % of the response to 64Hz 1second, obtained in the 1st FRC, which is represented by open bars.

Each point represents the mean ± s.e.mean (n = 5 - 6). Statistically significant differences between control responses and those in the presence of antagonist are represented by *p<0.05, **0.01<p<0.001, ***p<0.001, Student's *t*- test.

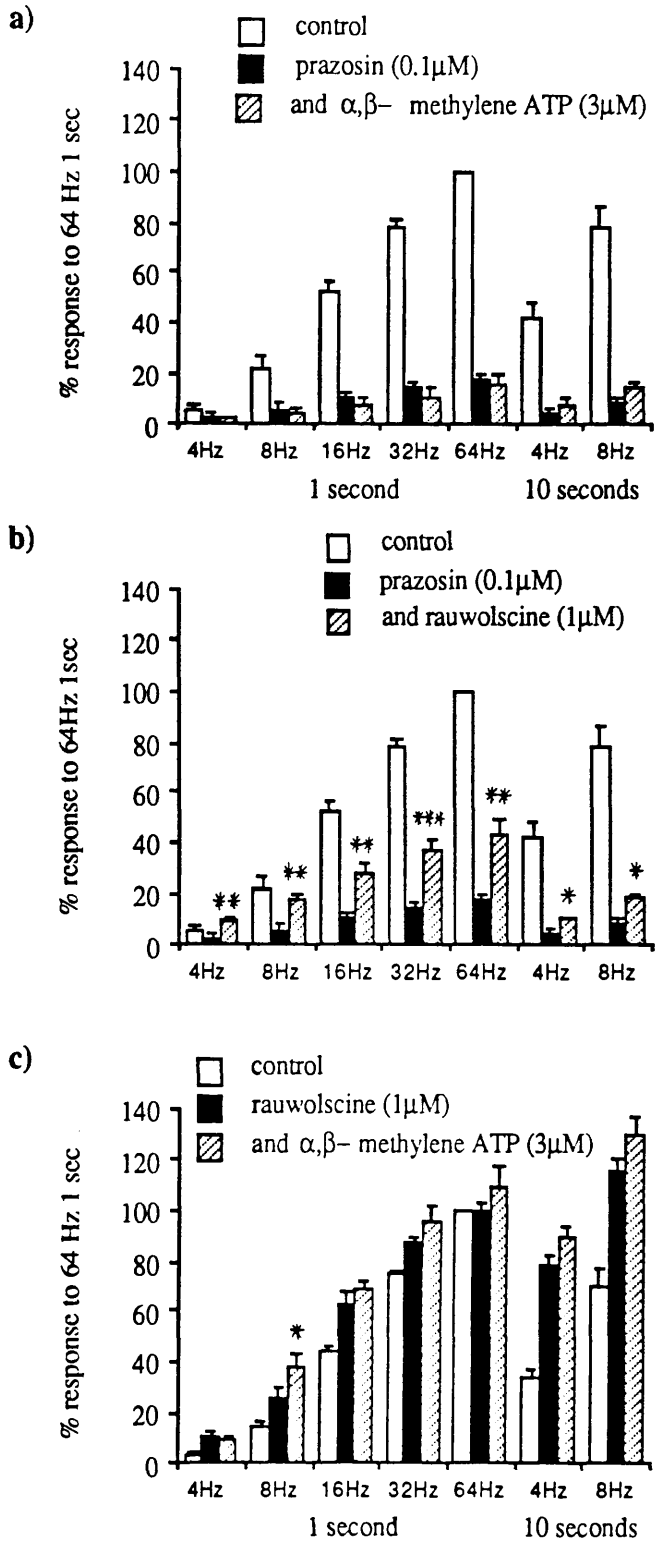


Figure No. 66

Effect of the sequential administration of **a)** prazosin (0.1μM) and α,β-methylene ATP (3μM) **b)** prazosin (0.1μM) and rauwolscine (1μM) and **c)** rauwolscine (1μM) and α,β-methylene ATP (3μM) on responses to sympathetic nerve stimulation in rabbit isolated distal saphenous artery. Results are expressed as a % of the response to 64Hz 1second obtained in the 1st FRC (control). Each point represents the mean ± s.e.mean (n = 5 - 7). Statistically significant differences between responses in the presence of prazosin alone and in combination with rauwolscine (**b**) are represented by *p<0.05, **0.01<p<0.001, ***p<0.001, Student's *t*- test.

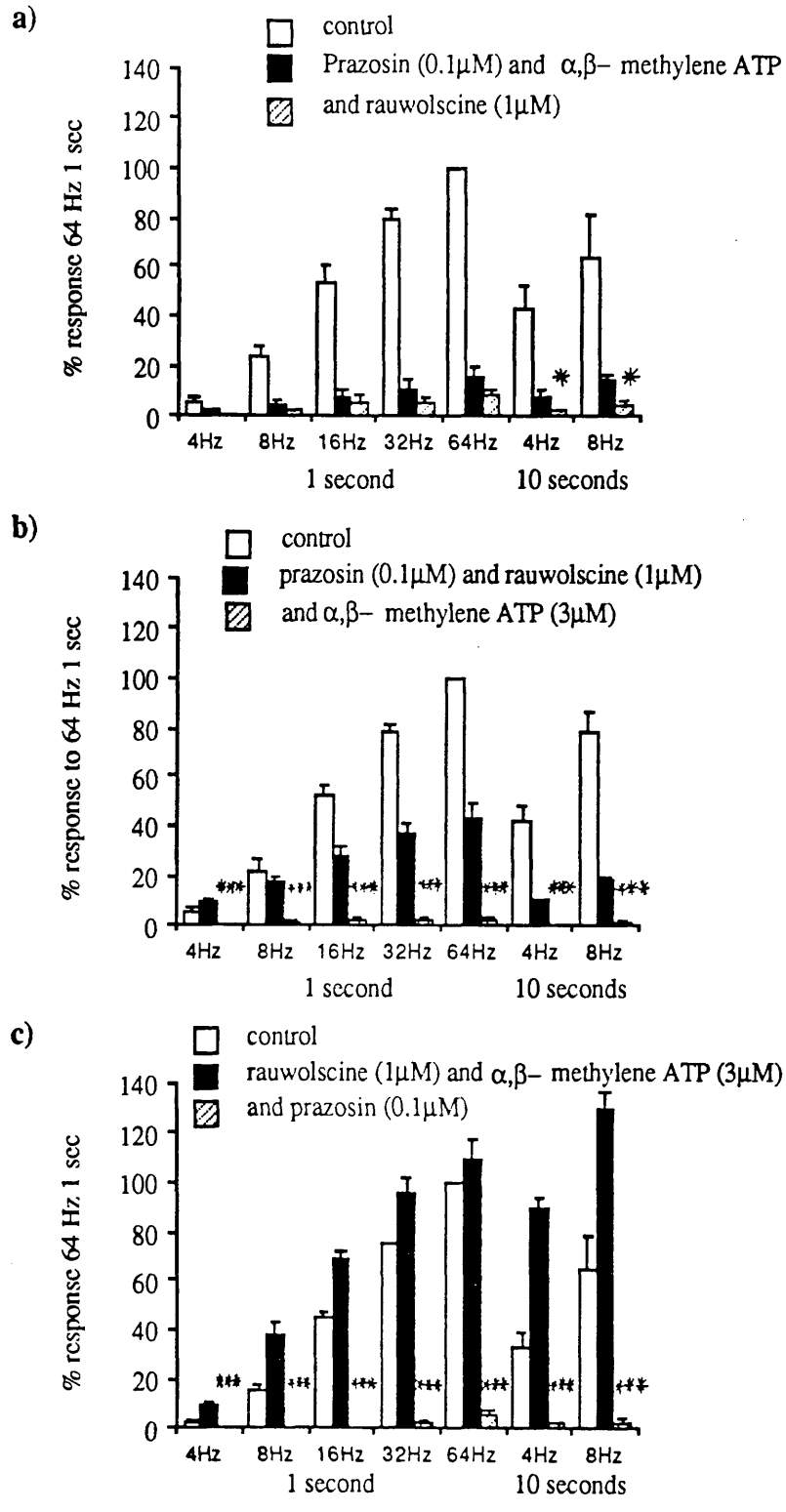


Figure No. 67

Effect of the sequential administration of **a)** rauwolscine (1μM) after the combination of prazosin (0.1μM) and α,β-methylene ATP (3μM) **b)** α,β-methylene ATP (3μM) after prazosin (0.1μM) and rauwolscine (1μM) and **c)** prazosin (0.1μM) after rauwolscine (1μM) and α,β-methylene ATP (3μM) on responses to sympathetic nerve stimulation in rabbit isolated distal saphenous artery. Results are expressed as a % of the response to 64Hz 1second obtained in the 1st FRC (control).

Each point represents the mean ± s.e.mean (n = 3).

Effects of A II on responses to sympathetic nerve stimulation in rabbit isolated distal saphenous artery

A II has been demonstrated to produce an enhancement of responses to sympathetic nerve stimulation in a wide variety of vascular preparations (for review see Westfall, 1977). This effect has largely been ascribed to a prejunctional effect at enhancing transmitter release rather than a postjunctional action. However the present study has demonstrated an interaction betwen A II and responses to NA mediated via postjunctional α_2-adrenoceptors in the rabbit isolated lateral saphenous vein and distal saphenous artery. Therefore the effects of A II on responses to sympathetic nerve stimulation in the rabbit isolated distal saphenous artery were examined.

A II (0.05μM) produced a transient contraction in this preparation as described previously. This concentration of A II markedly increased reponses to electrical field stimulation of the distal saphenous artery at all points on the FRC (Figure 68). After isolation of purinoceptors in this preparation, with the use of the combination of prazosin (0.1μM) and rauwolscine (1μM), A II (0.05μM) produced a small but significant potentiation of nerve-mediated responses, particularly on the lower portion of the FRC (Figure 69b). After isolation of postjunctional α_1-adrenoceptors, with the use of the combination of rauwolscine (1μM) and α,β-methylene ATP (3μM), A II (0.05μM) was without effect on the already potentiated response (Figure 69c). In contrast, A II (0.05μM) produced a marked enhancement of responses after attempted isolation of postjunctional α_2-adrenoceptors using the combination of prazosin (0.1μM) and α,β-methylene ATP (3μM) (Figure 69a). Interestingly this potentiated response in the presence of A II was subsequently susceptible to an inhibitory action of 1μM rauwolscine, a concentration which in most other circumstances had potentiated nerve-mediated responses (Figure 70).

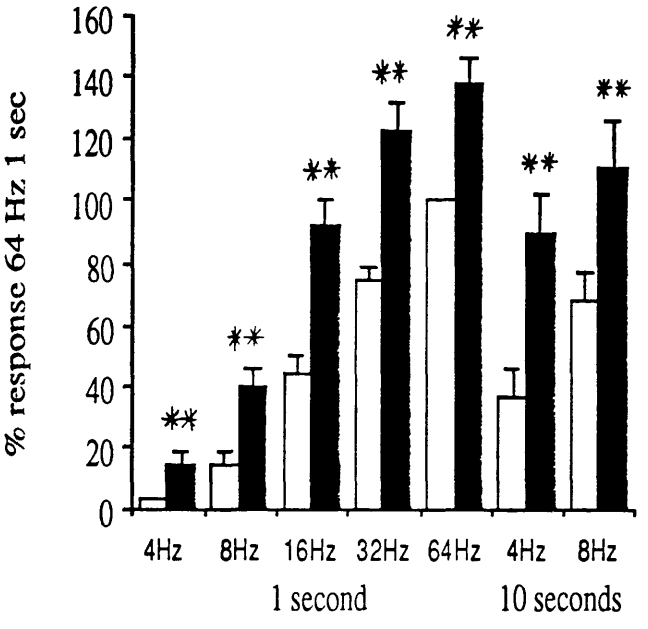


Figure No. 68

Effect of A II (0.05μM) on responses to sympathetic nerve stimulation in rabbit isolated distal saphenous artery. Results are expressed as a % of the response to 64Hz 1second from the 1st FRC. The 1st FRC is represented by open bars, the 2nd in the presence of A II, by solid bars.

Each point represents the mean ± s.e.mean (n = 7). Statistically significant differences between responses in the absence and presence of A II (0.05μM) are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*-test.

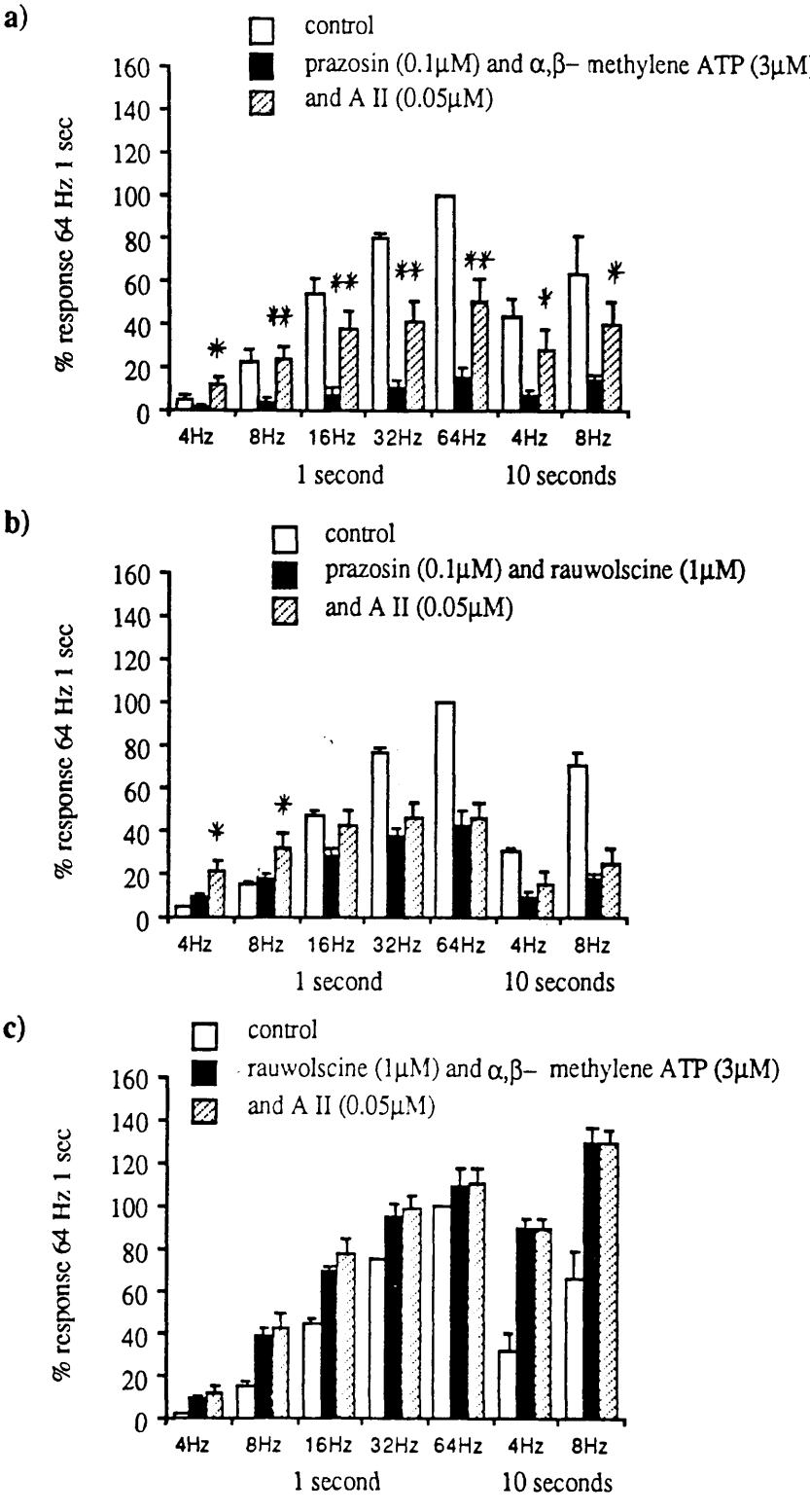


Figure No. 69

Effect of A II (0.05μM) (hatched bars) on the residual response to sympathetic nerve stimulation following treatment with **a)** prazosin (0.1μM) and α,β-methylene ATP (3μM) (solid bars) **b)** prazosin (0.1μM) and rauwolscine (1μM) (solid bars) and **c)** rauwolscine (1μM) and α,β-methylene ATP (3μM) (solid bars) in rabbit isolated distal saphenous artery. Results are expressed as a % of the response to 64Hz 1second obtained in the control FRC (open bars).

Each point represents the mean ± s.e.mean (n = 5 - 7). Statistically significant differences between residual responses in the presence of antagonists alone and in combination with A II (0.05μM) are represented by *$p<0.05$, **$0.01<p<0.001$, Student's *t*- test.

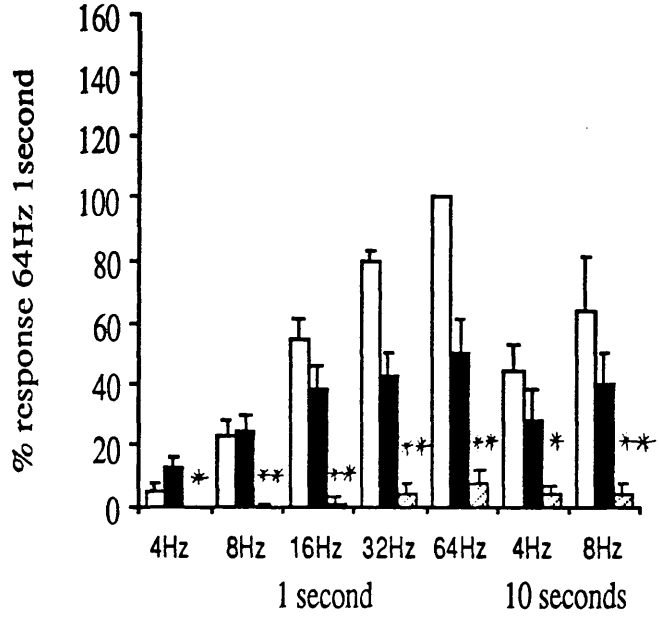


Figure No. 70

Effect of rauwolscine (1μM) (hatched bars) on the response to sympathetic nerve stimulation remaining in the presence of prazosin (0.1μM), α,β-methylene ATP (3μM) and A II (0.05μM) (solid bars). The 1st FRC is represented by open bars, the 2nd in the presence of A II, by closed bars. Each point represents the mean ± s.e.mean (n = 7). Statistically significant differences between responses in the absence and presence rauwolscine (1μM) are represented by *$p<0.05$, **$0.01<p<0.001$, ***$p<0.001$, Student's *t*- test.

DISCUSSION

DISCUSSION

Alpha-adrenoceptors in the rabbit isolated lateral saphenous vein

The rank order of potencies for agonists in this preparation, UK-14304 ≥ NA > phenylephrine strongly indicates a predominance of postjunctional α_2-adrenoceptors over α_1-adrenoceptors in mediating contractile responses, and is similar to those found in previous studies in the rabbit isolated lateral saphenous vein (Schumann & Lues, 1983, Alabaster *et al.*, 1985). This potency order for agonists is also similar to that observed in the rabbit isolated ear vein, a preparation which contains a homogeneous population of postjunctional α_2-adrenoceptors (Daly *et al.*, 1988b). Antagonist potency however, does not give clear support for this hypothesis.

High pA_2 values were obtained against NA for two agents, prazosin and YM 12617, which in other preparations are highly selective for α_1-adrenoceptors (Cambridge *et al.*, 1977, Honda *et al.*, 1985), as well as for the selective α_2-adrenoceptor antagonist rauwolscine (Weitzell *et al.*, 1979). The pA_2 value for rauwolscine, 8.41, is consistent with previously reported values for this antagonist at prejunctional α_2-adrenoceptors in rat anococcygeus (McGrath, 1984) and rabbit vas deferens (Lattimer & Rhodes, 1985) and at postjunctional α_2-adrenoceptors in the rabbit ear vein (Daly *et al.*, 1988b). However, the pA_2 value obtained for prazosin, 8.19, is intermediate between its reported value at postjunctional α_1-adrenoceptors in other rabbit blood vessels; aorta 8.85 (Honda *et al.*, 1985), pulmonary artery 8.76 (Vizi *et al.*, 1986) and its reported pA_2 value at prejunctional α_2-adrenoceptors in the pulmonary artery, 6.3 (Kaposci *et al.*, 1987). Similarly, the pA_2 value for YM 12617, 8.36, is less than its reported value at postjunctional α_1-adrenoceptors in rabbit aorta (10.0) but greater than its value at

prejunctional α_2-adrenoceptors in rat vas deferens (6.4) (Honda *et al.* 1985).

Another interesting feature of the antagonism produced by α-adrenoceptor antagonists in this preparation, is that the slope of the Schild plot for each antagonist is consistently less than unity, indicating non-competitive antagonism. In a concurrent study, this deviation from unity could not be attributed to incomplete blockade of neuronal uptake; 30μM cocaine had no greater effect than 10μM (used in the present study) on the CCRC to NA, nor could it be attributed to an intact extraneuronal uptake system, a feature which is of limited importance in this preparation (Daly *et al.*, 1988a). These results could be interpreted as evidence for a novel α-adrenoceptor mediating contraction in this preparation, with some properties of both postjunctional α_1- and α_2-adrenoceptors, similar to the prazosin-sensitive α_2-adrenoceptor described by Neylon & Summers (1985). Alternatively, since the antagonists employed are known to possess varying degrees of selectivity for α_1- and α_2-adrenoceptors, responses to NA may be the resultant of an action on a mixed population of both postjunctional α_1- and α_2-adrenoceptors. Evidence in favour of the latter possibility is provided by examining the slope of the CCRC to NA in the presence of rauwolscine in greater detail.

Increasing concentrations of the selective α_2-adrenoceptor antagonist, rauwolscine, produce a consistent rightward displacement only in the upper portion of the CCRC: a response equivalent in size to approximately 20-30% of the NA maximum was unaffected. In the presence of a concentration of prazosin which produced a 5-10-fold rightward displacement alone, the component of the response to NA which was resistant to rauwolscine was no longer apparent.

It is conceivable that the 'rauwolscine-resistant' component could be a consequence of the small transient contraction observed upon exposure to the

antagonist, reducing the threshold for contraction. Prazosin (0.1μM) not only removed the 'rauwolscine-resistant' component of the response to NA, but also the contraction to rauwolscine. This explanation seems unlikely however, since a component of the response to NA in this preparation remains resistant to another α_2-adrenoceptor antagonist CH 38083, an agent which did not produce a contraction (Daly *et al.*, 1988a). In addition transient contractions produced by prazosin in the rabbit isolated aorta (Cavero *et al.*, 1978) and by yohimbine in the rabbit isolated ear artery (Tayo *et al.*, 1982) are not associated with the appearance of a resistant component of responses to NA.

A much more likely explanation is that prazosin removes the 'rauwolscine-resistant' component by antagonising a component of the response to NA mediated by a population of α_1-adrenoceptors. The presence of both α-adrenoceptors subtypes, with the α_2-adrenoceptor subtype predominating, would explain why the pA_2 value for rauwolscine approximated its known value at α_2-adrenoceptors, while pA_2 values for prazosin and YM 12617 are intermediate between their potency at α_1- and α_2-adrenoceptors. The explanation for the lack of a 'resistant component' of responses to NA in the presence of α_1-adrenoceptor antagonists can be explained by a functional interaction between the α-adrenoceptor subtypes. This concept will be expanded later in the discussion.

Attempted isolation of postjunctional α-adrenoceptors subtypes in rabbit isolated lateral saphenous vein

Phenoxybenzamine (0.3μM), an irreversible α-adrenoceptor antagonist (Furchgott, 1972), virtually abolished responses to NA in the rabbit isolated lateral saphenous vein. The basis for receptor isolation therefore, was to provide conditions whereby any postjunctional α_2-adrenoceptors or postjunctional α_1-adrenoceptors could be selectively protected by the inclusion of a selective

concentration of a suitable antagonist.

Attempted isolation of postjunctional α_2-adrenoceptors

The inclusion of rauwolscine (1μM) prior to and during exposure to phenoxybenzamine, resulted in part of the response to NA being spared from the action of phenoxybenzamine. Subsequently, the residual response to NA was antagonised by rauwolscine (1μM) which produced an approximately 100-fold rightward displacement of the CCRC to NA after the protection protocol, with an estimated -logK_B value of 7.98. This is a similar dissociation constant value to that obtained for rauwolscine against α_2-adrenoceptor-mediated responses in other vascular preparations (Daly *et al.*, 1988b, Lattimer & Rhodes, 1985). In addition, after the protection protocol there was no longer a component of the CCRC to NA which was resistant to rauwolscine. This contrasts with the 'rauwolscine-resistant' component of responses to NA observed when the full receptor complement was present in this preparation. Therefore treatment with phenoxybenzamine removes a component of the response to NA which was rauwolscine-resistant, prazosin-sensitive and therefore likely to have been mediated by postjunctional α_1-adrenoceptors.

The functional removal of postjunctional α_1-adrenoceptors by phenoxybenzamine is further supported by the observations that both prazosin and YM 12617 at the concentration of 0.1μM, which is a concentration 75-1000 times greater than their reported pA_2 value at α_1-adrenoceptors in other preparations (see Daly *et al.*, 1988a), produced less than 3-fold rightward displacements of the residual response to NA after the protection protocol, and therefore were much less effective than against responses to NA than under control conditions.

These results are consistent with a discrete population of postjunctional α_2-adrenoceptors which are preferentially protected by rauwolscine. The low potency for prazosin at this isolated subtype ($pA_2 < 7$) agrees well with that found by Schumann & Lues (1983) in this preparation against BHT-920 induced contractions in the presence of A II ($pA_2 = 6.8$). Furthermore, the low potency of YM 12617 ($pA_2 < 7$) is also consistent with an action at α_2-adrenoceptors (Honda *et al.*, 1985).

Attempted isolation of postjunctional α_1-adrenoceptors

In contrast to the relatively clear-cut observations obtained after receptor protection with rauwolscine, attempted protection of part of the response to NA with the selective α_1-adrenoceptor antagonist YM 12617 (0.1μM) did not yield straightforward results.

Firstly, YM 12617 antagonised the remaining residual response in an apparently non-competitive manner. A 10-fold increase in the concentration of YM 12617 was not associated with a corresponding 10-fold increase in the rightward displacement of the NA CCRC. If only α_1-adrenoceptors had been spared from the action of phenoxybenzamine, YM 12617 might have been expected to be a competitive antagonist. In addition, the estimated $-\log K_b$ value for YM 12617 under these conditions (7.67) was not significantly different from the pA_2 value obtained for this antagonist when the full receptor complement was present; both values are considerably less than that observed at α_1-adrenoceptors in the rabbit isolated aorta (pA_2 10.0) (Honda *et al.*, 1985) or the rabbit isolated renal vein (V. G. Wilson, personal communication); pA_2 approximately 9.5.

Secondly, rauwolscine (2.5μM) produced an enhancement of responses after attempted isolation of postjunctional α_1-adrenoceptors at low concentrations of the CCRC to NA, while inhibiting response to higher concentrations of NA. This potentiation of responses to NA was inhibited by YM 12617 and the combination of the two antagonists produced a greater displacement of the NA CCRC to NA than either antagonist alone. The potentiation of responses produced by rauwolscine is difficult to explain. However since YM 12617 blocked both contractile responses and the potentiation of responses to NA produced by rauwolscine, an agonistic action or interaction with α_1-adrenoceptors for the α_2-adrenoceptor antagonist cannot be excluded. If this were true, it would suggest subtle differences for the population of postjunctional α_1-adrenoceptors in the lateral saphenous vein from that found in other rabbit vascular preparations such as the thoracic aorta, ear artery or renal vein (Daly *et al.*, 1988c).

Finally, the selective α_2-adrenoceptor antagonist CH 38083 (Vizi *et al.*, 1986) effected a marked inhibition of responses following protection with 0.1μM YM 12617, suggesting the presence of postjunctional α_2-adrenoceptors. This inhibition was associated with the appearance of a small resistant component of the response to NA, although this was considerably less than that seen with rauwolscine. Thus after attempted isolation of postjunctional α_1-adrenoceptors, responses are resistant to one α_2-adrenoceptor antagonist, rauwolscine, but susceptible to another CH 38083.

Taken together these observations cannot be reconciled by NA acting on a single population of α_1-adrenoceptors. With the exception of the potentiation produced by rauwolscine, the antagonists inhibited responses to NA, irrespective of subtype specificity. In many ways these results parallel the observations with antagonists against responses to NA under normal conditions. YM 12617 produced non-competitive antagonism under both conditions, a resistant component of

responses to NA persisted in the presence of an α_2-adrenoceptor antagonist and the combination of α_1- and α_2-adrenoceptor antagonists produce a greater shift than either alone. This leads to the conclusion that YM 12617 (0.1μM) failed to isolate a homogeneous population of α_1-adrenoceptors, but rather protected a mixed population of α-adrenoceptors.

Since the concentration of phenoxybenzamine employed abolished responses to NA in the absence of YM 12617, the responses blocked by CH 38083 might arise if limited protection of the α_2-adrenoceptor subtype was afforded by the inclusion of 0.1μM YM 12617 during the incubation period. While there is always the problem of assumed selectivity of an antagonist at any given concentration, CH 38083 (1μM) can be considered selective for α_2-adrenoceptors, since under control conditions, this concentration of CH 38083 was associated with a resistant component of responses to NA (see Daly *et al.*, 1988a). This then questions the selectivity of 0.1μM YM 12617 for α_1-adrenoceptors. This concentration of YM 12617 however is ineffective at postjunctional α_2-adrenoceptors in the canine isolated saphenous vein (Honda *et al.*, 1985) and rabbit isolated ear vein (Daly *et al.*, 1988b) and in addition is virtually inactive against responses to NA after isolation of postjunctional α_2-adrenoceptors in the lateral saphenous vein (present study).

This apparent paradox could be explained if a residual α_2-adrenoceptor component, which survives phenoxybenzamine, but cannot reach threshold for contraction alone, was facilitated by the response mediated by the α_1-adrenoceptors protected by YM 12617. Evidence for such a functional synergistic interaction between α-adrenoceptor subtypes, both in this preparation and in the rabbit distal saphenous artery, will be discussed in greater detail later in the discussion.

Alternatively, it has been suggested that the diversity of chemical structures capable of inhibiting α-adrenoceptors can be taken as evidence that some antagonists produce their effects by attachment to 'extrareceptor' sites not necessarily corresponding to the area of agonist attatchment (see: McGrath, 1982, Ariens & Simonis, 1983). If this were correct for postjunctional α_2-adrenoceptors in the rabbit lateral saphenous vein, it seems possible that YM 12617 may compete with phenoxybenzamine at a critical 'extra-receptor' site, thereby preventing irreversible inactivation, but without hindering the access of the agonist to the receptor. Thus after incubation with 0.1μM YM 12617, contractile responses to NA would result from the co-stimulation of a population of both α_1- and α_2-adrenoceptors.

In conclusion, based upon the receptor protection experiments in the rabbit isolated lateral saphenous vein, with 'selective' concentrations of either α_1- or α_2-adrenoceptor antagonists in combination with the irreversible antagonist phenoxybenzamine, two populations of postjunctional α-adrenoceptors can be identified. One population has the characteristics of an α_2-adrenoceptor (prazosin or YM 12617-resistant, rauwolscine-sensitive), while the other possesses some of the characteristics of an α_1-adrenoceptor (YM 12617-sensitive, rauwolscine-resistant). Although complete isolation of postjunctional α_2-adrenoceptors could be demonstrated, only limited success was achieved in isolating the α_1-adrenoceptor subtype. The aforementioned protocol therefore, allows us to 'manufacture' a preparation containing a homogeneous population of postjunctional α_2-adrenoceptors, which can be studied using the definitive α-adrenoceptor agonist NA (Furchgott, 1972). This overcomes the necessity of using 'selective' agonists alone or in combination with a selective α_1-adrenoceptor antagonist, normally used to study α_2-adrenoceptor-mediated responses in other preparations containing a mixed population of α-adrenoceptors (eg. the canine saphenous vein, see: Flavahan

et al., 1984, Ruffolo & Zeid, 1985).

Comparison of the effects of A II and Bay K 8644 on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors in vascular smooth muscle

Contractile responses to angiotensin II

A II, at all concentrations which were suprathreshold, produced transient contractions in the rabbit isolated lateral saphenous vein, ear vein and left renal vein. Subsequent exposure of these preparations to A II was not associated with a contractile response. The transient, tachyphylactic nature of responses to A II appears to be a feature in most isolated venous and arterial preparations (see: Peach 1977) and has been reported previously for both the rabbit lateral saphenous vein (Schuman & Lues, 1983) and renal vein (Webb, 1982).

Since responses to even low concentrations of A II (threshold for contraction) are not maintained, it is unlikely that this feature is solely a reflection of exposure to an excessively high concentration of A II. Rather, this inability of A II to maintain a contractile response is more probably associated with some consequence of the formation of the drug receptor interaction, such that once formed, there is a subsequent inability to maintain the production of diacylglycerol and therefore continued protein kinase C activation (Danthuluri & Deth, 1986). This process would ultimately limit the ability of A II to produce an influx of extracellular Ca^{2+} into the smooth muscle cell. Interestingly a number of situations exist where tachyphylaxis to A II can be reversed, most notably by inducing tone in isolated vascular preparations with an appropriate agent such as KCl (Nicholas, 1970, Goodfriend & Simpson, 1981, Juul *et al.*, 1987). The presence of tone would therefore explain why responses to infusions of lower concentrations of A II are maintained and reproducible under *in vivo* conditions (Bohr, 1974).

Another puzzling feature of the action of A II *in vitro,* is the difficulty in correlating the concentrations of A II found in plasma with those required to produce a direct vasoconstrictor action in isolated vascular preparations. The plasma concentrations of A II have been estimated to be in the order of 0.1nM in man (Boyd & Peart, 1974) while even in the pithed rat, where the renin-angiotensin system is highly activated, the plasma levels of A II are only in the order of 0.5nM (Grant & McGrath, 1988a). In the vast majority of isolated blood vessels, large or small, venous or arterial, concentrations of A II exceeding 1nM are generally required before contractile responses are observed (Hof *et al.,* 1982, Juul *et al.,* 1987, Schumann & Lues 1983). This apparent paradox between the plasma levels of A II and those required to contract vascular smooth muscle could be reconciled if, as has recently been proposed, the most important source of A II production is within the vascular smooth muscle cell. This would result in the levels of A II at the vascular smooth muscle being largely independent of the levels of A II found in the general circulation (Campbell, 1987, Dzau, 1988, Unger *et al.,* 1989). Validation of this proposal, and determination of 'physiological' levels of A II, would require overcoming the difficulty in measuring the concentration of A II at the level of the vascular smooth muscle cell.

Comparison of the actions of A II and Bay K 8644

ACE inhibitors and Ca^{2+} channel blockers (CCB's) have qualitatively similar effects on α-adrenoceptor-mediated pressor responses in the pithed rat (van Meel *et al.,* 1981, De Jonge *et al.,* 1981, 1982, O'Brien *et al.,* 1985). For example, in the latter study, the authors demonstrated that the second component of the pressor response to the bolus injection of an agonist in the pithed rat, was antagonised in a similar manner by both ACE inhibitors and CCB's, while the first component of the response was relatively unaffected. These observations prompted

the consideration that A II may have been an endogenous facilitator of voltage-operated Ca^{2+} channels. If this were true then we may have expected A II and Bay K 8644 to produce qualitatively similar effects on responses to NA mediated via either postjunctional α_1- or α_2-adrenoceptors *in vitro*.

Upon examination of a number of isolated venous preparations however, a similarity of action for these agents was not apparent. A II produced a potentiation of responses in the rabbit isolated ear vein and in lateral saphenous vein (although only after isolation of postjunctional α_2-adrenoceptors), while Bay K 8644 produced a small but significant enhancement of responses to NA in all the venous preparations attempted with the exception of the ear vein. Therefore, a similar action for these agents was observed only in the lateral saphenous vein.

Even in this preparation, a closer examination of the influence of A II and Bay K 8644 on Ca^{2+}-evoked contractions, revealed marked differences in their effects on voltage-operated Ca^{2+} channels. Bay K 8644 enhanced Ca^{2+}-induced contractions in the presence of a depolarising concentration of KCl, without altering the sensitivity of the voltage-operated Ca^{2+} channel. This observation is consistent with the voltage-operated Ca^{2+} channel facilitatory effect of this agent, first described by Schramm *et al.* (1983) and of the observations of Su *et al.* (1984) in the rat tail artery. In contrast however, A II inhibited the Ca^{2+}-induced contractions mediated via voltage-operated channels, an effect associated with a reduction in channel sensitivity to Ca^{2+}. It is difficult to understand the mechanism of this inhibition, since A II has no reported calcium channel blocking actions.

In contrast, both A II and Bay K 8644 produced an enhancement of responses to the re-addition of Ca^{2+}, after isolation of postjunctional α_2-adrenoceptors, in the presence of NA as a stimulatory agent. Therefore, both these agents can enhance responses to extracellular Ca^{2+} mediated via receptor-operated

Ca^{2+} channels. Since this effect is seen with Bay K 8644, this suggests that at least part of the response subsequent to postjunctional α_2-adrenoceptor stimulation is mediated via voltage-operated Ca^{2+} channels. Some caution is perhaps warranted in assuming that the action of Bay K 8644 and other 1,4-dihydropyridine derivatives is solely on voltage-operated channels, since Bay K 8644 clearly had a greater effect on those Ca^{2+} channels activated by NA than those activated by KCl. In addition, Bay K 8644 has been reported to enhance postjunctional α_2-adrenoceptor-mediated responses in the canine saphenous vein (Eskinder & Gross, 1987), a preparation in which α_2-adrenoceptor stimulation is not associated with changes in membrane potential (Matthews *et al.*, 1984).

To conclude however, since A II and Bay K 8644 have qualitatively different effects on responses to NA in different blood vessels, this clearly denotes that these agents do not act in the same manner, as has been suggested from observations *in vivo*.

Effect of A II on postjunctional α_1 - and α_2 -adrenoceptors

A number of observations in the present study suggest a selective interaction between A II and responses mediated via postjunctional α_2-adrenoceptors. Firstly, A II produced a modest reduction in the potency of prazosin against responses to NA in the lateral saphenous vein. These results are essentially similar to those obtained by Schumann & Lues (1983) in the same preparation, who demonstrated that responses to BHT-920, which under normal circumstances were markedly inhibited by prazosin, became much more resistant to the α_1-adrenoceptor antagonist in the presence of A II. A lack of effect of A II on α_1-adrenoceptor-mediated responses in this preparation is suggested by the absence of an influence of A II on responses to NA in the presence of rauwolscine i.e. the size of the

'rauwolscine-resistant' component is similar in the absence and presence of A II.

Secondly after isolation of postjunctional α_2-adrenoceptors in the rabbit lateral saphenous vein, A II produced an increase in both the maximum response and sensitivity of the preparation to the NA. This effect of A II was mediated through a specific receptor since it could be blocked by the A II receptor antagonist saralasin (Zimmerman & Kraft, 1979). Interestingly, despite the fact that saralasin (0.01μM) virtually abolished contractile responses to A II, a 10-fold higher concentration of antagonist was required to completely reverse the facilitatory action of this peptide. These results represent a dissociation between the concentrations of A II required for a facilitatory action on responses to NA and a direct action on vascular smooth muscle, such that the former can occur with subcontractile concentrations of A II. In accord with this idea, A II (5nM) also produced a consistent facilitation of postjunctional α_2-adrenoceptor-mediated responses, while producing a very small contraction in only 2 of 6 preparations.

It should be noted that the sensitivity of responses to NA after attempted isolation of postjunctional α_1-adrenoceptors in the rabbit lateral saphenous vein was also increased by A II. As discussed previously however, it is likely that pretreatment with 0.1μM YM 12617 and 0.3μM phenoxybenzamine results in the protection of a mixed population of both α-adrenoceptor subtypes. It is therefore not possible to dissociate the potentiation produced by A II under these conditions, from a possible action on postjunctional α_2-adrenoceptors.

Further evidence for a selective interaction between A II and postjunctional α_2-adrenoceptors is provided in the rabbit isolated distal saphenous artery. A II produced a marked increase in the sensitivity in this preparation to the relatively

'selective' α_2-adrenoceptor agonist UK-14304, resulting in the introduction of a previously unseen component of responses to this agonist. These 'uncovered' responses were unaffected by a concentration of prazosin (0.1μM) which is approximately 50 times greater than that corresponding to its pA_2 value at postjunctional α_1-adrenoceptors in this preparation (8.57). In contrast, rauwolscine (1μM) prevented the potentiation of responses to UK-14304 produced by A II. This therefore represents a complete reversal of antagonist potencies in this preparation, such that in the presence of A II, rauwolscine (1μM) is more potent against response to UK-14304 than prazosin (0.1μM), while under normal experimental conditions the converse is true. In the same preparation, A II was without effect on responses to the selective α_1-adrenoceptor agonist, phenylephrine.

Finally, A II produced a small but significant enhancement of responses to NA in the rabbit isolated ear vein, a preparation containing an almost homogeneous population of postjunctional α_2-adrenoceptors (Daly *et al.*, 1988b). In contrast, in the left renal vein, a preparation containing a homogeneous population of postjunctional α_1-adrenoceptors (Daly *et al.*, 1988c), A II was without effect on responses to NA.

Since the presence of a large receptor reserve can attenuate the inhibition of contractile responses produced by calcium channel blockers (Nichols & Ruffolo, 1988) or by modulation of temperature (Flavahan & Vanhoutte, 1986), it is possible that reciprocal facilitatory actions may be masked by the existence of such a feature in a given preparation. However, even after reducing the receptor reserve in the left renal vein with phenoxybenzamine, no potentiation of α_1-adrenoceptor-mediated responses to NA was observed in the presence of A II. These observations fail to corroborate those of Purdy & Weber (1988) who were able to demonstrate a facilitation of α_1-adrenoceptor-mediated responses in rabbit isolated

femoral artery, this effect being particularly marked when the receptor reserve had previously been reduced with benextramine. A number of observations however suggest that postjunctional α_2-adrenoceptors may be present in the rabbit isolated femoral artery. In the present study, A II introduced a component of responses to UK-14304 in this preparation which was previously absent, in an analogous manner to that seen in the distal saphenous artery. In addition, Purdy & Weber found that responses to NA were slightly more resistant to prazosin in the presence of A II after the receptor reserve had been reduced in the rabbit femoral artery. Again this situation is analogous with that seen in the distal saphenous artery, where postjunctional α_2-adrenoceptors can be quite clearly demonstrated.

Taken in isolation, these observations strongly suggest a selective interaction between A II and postjunctional α_2-adrenoceptors. Due to the lack of suitable isolated vascular preparations which have populations of postjunctional α_2-adrenoceptors, no comparative studies have previously been attempted. However A II has been shown to potentiate responses to NA in a number of isolated vascular preparations such as the rat caudal artery (Nicholas, 1970), perfused mesenteric arteries from a number of species (Malik & Najletti, 1976, Chiba & Tsukada, 1986, Panisset & Bourdois, 1968), human digital arteries (Moulds & Worland, 1980) and in canine hindpaw (Zimmerman & Kraft, 1979). It is interesting to note that more recent characterisations of the α-adrenoceptor populations in at least some of these cutaneous preparations, with the realisation of the existence of both postjunctional α_1- and α_2-adrenoceptors on vascular smooth muscle, have revealed responses mediated via postjunctional α_2-adrenoceptors. For example, α_2-adrenoceptor-mediated responses have been observed in human digital arteries (Glusa & Markwardt, 1983, Stevens & Moulds, 1985), in the rat caudal artery or perfused rat tail (Rajanayagam & Medgett, 1987, Templeton *et al.*, 1989) and in the feline mesenteric vascular bed (Lippton *et al.*, 1987). Thus the

potentiation produced by A II may reflect an interaction with postjunctional α_2-adrenoceptors.

A number of other observations however, suggest that this interpretation may be too simplistic. For example, Day & Moore (1976) have demonstrated a non-specific facilitatory action for A II on responses to various contractile agents, including NA, in rabbit isolated aorta. Similarly, Chiba & Tsukada (1986) also observed an increase in responses to KCl, as well as NA, in the canine mesenteric artery in the presence of A II. Since ouabain produced similar effects, these authors concluded that A II modulated responses to vasoactive agents by producing a small depolarization of the cell membrane, thereby reducing the threshold for contraction. Such a non-specific sensitisation of vascular smooth muscle is unlikely to account for the effects of A II in the present study since, when the full receptor complement was present in the saphenous vein, responses to bradykinin and NA were unaffected by A II, as were responses to NA and phenylephrine in the distal saphenous artery.

Two further studies suggest a more complex interaction between A II and postjunctional α-adrenoceptors. This interaction is apparently related to some process responsible for determining the expression of intrinsic activity for synthetic agents, and is irrespective of subtype specificity. For example, Lues & Schumann (1984) have reported that A II converted the action of BHT-920 from a weak antagonist to a partial agonist at postjunctional α_1-adrenoceptors in the rabbit isolated aorta (Lues & Schumann, 1984). These results were repeated in the rabbit mesenteric artery, in which it was also observed that A II conferred α_2-adrenoceptor agonism (prazosin-resistant contractile responses) on two relatively selective α_2-adrenoceptor antagonists, rauwolscine and BDF 6143 (Lues *et al.*, 1984). The introduction of responses, produced by A II, to BHT-920 in these

preparations is essentially similar to that seen with UK-14304 in the distal saphenous artery, although different α-adrenoceptor subtypes are mooted to be involved. Therefore, rather than the α-adrenoceptor subtype being the crucial factor involved, A II may act on some other common feature of responses to these agents, such as their dependence on extracellular Ca^{2+}, to produce its facilitatory effects. However, one of the main tenets of the present study was to examine the influence of various agents on the definitive, endogenous α-adrenoceptor agonist, NA (Furchgott, 1972), and as such A II clearly produces a selective enhancement of responses to this agonist mediated via postjunctional α_2-adrenoceptors.

Effect of A I on postjunctional α_2-adrenoceptor-mediated responses

The physiological precursor of A II, angiotensin I (A I) mimicked the facilitatory action of A II on postjunctional α_2-adrenoceptor-mediated responses. Responses to NA after isolation of postjunctional α_2-adrenoceptors in the lateral saphenous vein were potentiated by A I, while this agent also 'uncovered' a previously unseen component of responses to UK-14304 in the distal saphenous artery. There was however, some difficulty in reversing the potentiating action of A I with angiotensin-converting enzyme (ACE) inhibitors. Cilazaprilat (1μM), a concentration 500-fold greater than its IC_{50} value at inhibiting enzyme-substrate interactions (Natoff & Redshaw, 1987), abolished contractions to A I in the lateral saphenous vein, but this was not associated with a complete reversal of the facilitatory action of this peptide. Similarly, another ACE inhibitor, captopril, at a concentration approximately 150 times greater than its IC_{50} value (1μM) (Natoff & Redshaw, 1987), did not reverse the facilitatory action of A I in the distal saphenous artery. Indeed captopril (1μM) did not even completely abolish contractile responses to A I (0.5μM) in this preparation.

Three possible explanations could be forwarded to explain why the ACE

inhibitors failed to completely reverse the effects of A I. For example, it is possible that A I produces a facilitation of α_2-adrenoceptor-mediated responses alone, although this seems unlikely since A I has no reported biological activity on vascular smooth muscle (See: Peach 1977). Secondly, despite the high concentrations of ACE inhibitors employed, these may have been insufficient to result in complete inhibition of ACE. Thirdly, it is possible that A I was converted to A II by enzymes other than ACE, such as tonins (Boucher *et al.*, 1974).

The fact that ACE inhibitors reduced or abolished the contractile response to A I demonstrates that A II can be generated locally by the action of vascular or endothelial angiotensin converting enzyme in both these preparations. To this extent, therefore, there may be a dissociation between the concentrations of A II found at the level of vascular smooth muscle and those found in the general circulation. The lack of inhibitory effect of either cilazaprilat, captopril or saralasin on responses mediated via postjunctional α_2-adrenoceptors alone, demonstrates that a complete, functional, local renin-angiotensin system is not to be found in either preparation *in vitro*, and is therefore dependent on some other component of the renin-angiotensin system not present in vascular smooth muscle, under the conditions employed. Demonstration of the local conversion of A I to A II, with subsequent facilitatory actions on responses to NA or sympathetic nerve stimulation, has previously been demonstrated in the perfused rat mesentery and kidney vasculatures (Malik & Najletti, 1976, Boke & Malik, 1983).

Effects of Bay K 8644 and nifedipine on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors

There has been considerable debate in recent years concerning the relative importance of different Ca^{2+} pools that may be activated following stimulation of postjunctional α-adrenoceptors. In particular this has focussed on the susceptibility of responses to selective α_1- and α_2-adrenoceptor agonists to 1,4-dihydropyridine derivatives in studies from whole animals. A number of observations have suggested that vasopressor responses evoked by α_2- but not α_1-adrenoceptor agonists are susceptible to the action of these agents, leading to the belief that α_2-adrenoceptor-mediated responses are wholly dependent on the influx of extracellular Ca^{2+} through 1,4-dihydropyridine-sensitive Ca^{2+} channels. In contrast, responses to α_1-adrenoceptor stimulation have been postulated to activate principally the release of Ca^{2+} from intracellular sources with a smaller dependence on the influx of extracellular Ca^{2+} (for review see: van Zwieten & Timmermans, 1987). This hypothesis seems simplistic, however, since responses to some α_1-adrenoceptor agonists are markedly attenuated by calcium channel blockers (CCB's) in the pithed rat (Timmermans *et al.*, 1983a,b).

Several explanations have been forwarded to explain these observations (Timmermans *et al.*, 1983a,b, Nichols & Ruffolo, 1988). However the use of whole animals to study these phenomena is less than ideal for a number of reasons. For example, these studies have generally used bolus injections of agonists, which do not produce 'equilibrium responses'. Responses to α-adrenoceptor agonists are often biphasic, each phase of which can be differentially modulated by a number of factors (O'Brien *et al.*, 1985). In addition, the measurement of mean arterial pressure does not reflect possible differential effects of agonists on cardiac output and total peripheral resistance (see: McGrath *et al.*, 1989). Therefore it was

desirable to examine the effects of 1,4-dihydropyridine derivatives on responses to NA mediated via postjunctional α_1- and α_2-adrenoceptors in isolated vascular preparations.

Responses to NA in rabbit isolated lateral saphenous vein, left renal vein, ear vein and distal saphenous artery have been shown to be at least partly dependent on the influx of extracellular Ca^{2+} for contraction (Daly *et al.*, 1989). No evidence was obtained in these preparations to suggest that 1,4-dihydropyridine derivatives differentially influence responses mediated via either postjunctional α_1- or α_2-adrenoceptors. For example, Bay K 8644, an activator of voltage-dependent Ca^{2+} channels (Schramm *et al.*, 1983), produced an enhancement of responses mediated via α_2-adrenoceptors in the lateral saphenous vein. This was suggested by the reduction in potency of prazosin against responses to NA in the presence of Bay K 8644 and directly demonstrated after isolation of a homogeneous population of postjunctional α_2-adrenoceptors. In addition, this agent also potentiated responses to NA mediated via postjunctional α_1-adrenoceptors in the left renal vein.

Conversely, but in agreement with these observations, nifedipine, an inhibitor of voltage operated Ca^{2+} channels (Fleckenstein, 1977), produced a concentration-dependent reduction in the maximum response to NA after isolation of postjunctional α_2-adrenoceptors in the lateral saphenous vein. This agent did not, however, completely block responses to NA under these conditions. Similarly, nifedipine reduced, but did not abolish, responses to NA after isolation of α_2-adrenoceptors in the rabbit distal saphenous artery. Since responses to NA under these conditions are dependent upon the presence of A II (see later), it was theoretically possible that the reduction in the maximum response could be related to reduction in the activity of this agent. Nifedipine (1μM) however, did not affect the magnitude of the peak contractile response to A II. Therefore, the reduction in responses to NA is likely to reflect an action of nifedipine on the post receptor

events subsequent to α_2-adrenoceptor stimulation. The maximally effective concentration of nifedipine (0.1μM) in both these preparations also reduced the magnitude of α_1-adrenoceptor-mediated responses in the left renal vein. These results therefore demonstrate that at least part of the response to NA in these preparations is mediated via the influx of Ca^{2+} through 1,4-dihydropyridine-sensitive channels, irrespective of the subtype stimulated.

Since responses to NA after isolation of postjunctional α_2-adrenoceptors in the lateral saphenous vein are completely dependent upon the influx of extracellular Ca^{2+} (Daly *et al.*, 1989), the nifedipine-resistant component of responses to NA seen under these conditions must reflect the entry of Ca^{2+} via 1,4-dihydropyridine-resistant, receptor-operated channels. Such channels also appear to be responsible for the influx of extracellular Ca^{2+} following postjunctional α_2-adrenoceptor stimulation in the isolated ear vein, since Bay K 8644 was without effect on responses to NA. In agreement with this hypothesis, nifedipine has been shown to be inactive in this preparation (V.G. Wilson, personal communication).

The few other comparative studies attempted *in vitro* further outline the dangers inherent in generalising the susceptibility of responses mediated via a particular receptor to the blocking action of agents which interfere with one aspect of stimulus-response coupling. For example, while 1,4-dihydropyridines can influence postjunctional α_2-adrenoceptor-mediated responses in a number of vascular preparations such as the rat saphenous vein (Cheung, 1985), feline mesenteric bed (Lippton *et al.*, 1987) or canine saphenous artery (Sulpizio & Hieble, 1987) responses to NA, in the presence of prazosin (α_2-adrenoceptor-mediated), are unaffected by Bay K 8644 in the human hand vein (Arner *et al.*, 1988). In addition, postjunctional α_1-adrenoceptor-mediated responses are facilitated by Bay K 8644 and reduced by nimodipine in human hand veins (Arner

et al., 1988), but in contrast are resistant to the action of Bay K 8644 in the canine saphenous vein (Eskinder & Gross, 1987).

Therefore a sufficient number of exceptions can be demonstrated in isolated vascular preparations, that prevent the direct association between α-adrenoceptor subtype and susceptibility to 1,4-dihydropyridine derivatives, which has been postulated *in vivo*. The differential effect of CCB's in whole animals is more likely to reflect a limitation of measuring only the change in the peak response to the bolus injection of an agonist. For example, CCB's have no differential effect on responses mediated via either postjunctional α_1- or α_2-adrenoceptors, if the area under the curve is used as a measure of the whole response to a given agonist (McGrath & O'Brien, 1987). Likewise, these agents reduce the size of the responses to infusions, which give a more 'equilibrium' type response, of agonists selective for either subtype (McGrath & O'Brien, 1987, Lefevre-Borg *et al.*, 1988).

Postjunctional α-adrenoceptors in rabbit isolated distal saphenous artery

The rank order of potencies for agonists in this preparation, NA > phenylephrine > UK-14304 > Sgd 101/75 strongly indicates a predominance of postjunctional α_1-adrenoceptors in mediating contractile responses, and is similar to that observed in various other vascular preparations from the rabbit which contain homogeneous populations of α_1-adrenoceptors (see: Daly *et al.*, 1988c). In addition, prazosin produced a concentration-dependent rightward displacement of the CCRC to NA, with a pA_2 value of 8.57 and the slope of the Schild plot did not significantly differ from unity, indicating competitive antagonism. This pA_2 value for prazosin is consistent with reported pA_2 values against α_1-adrenoceptor-mediated responses in other vascular preparations from the rabbit such as the thoracic aorta (8.7) (Docherty *et al.*, 1981), ear artery (8.6) (Hieble *et al.*, 1982) or rabbit pulmonary artery (8.8) (Kaposci *et al.*, 1987).

Conversely, rauwolscine (1μM), a concentration approximately 100 times greater than that equivalent to its pA_2 value at postjunctional α_2-adrenoceptors in the rabbit ear vein (Daly *et al.*, 1988b) and its dissociation constant in the lateral saphenous vein after isolation of postjunctional α_2-adrenoceptors (present study), produced only a small rightward displacement of the CCRC to NA in this preparation. Therefore, the estimated -log K_B value for rauwolscine (6.32) in the rabbit distal saphenous artery is slightly higher, but not dissimilar to its reported pA_2 value at postjunctional α_1-adrenoceptors in other preparations, such as the thoracic aorta (6.1) and ear artery (5.5) (Daly *et al.*, 1988c). Further evidence that under normal experimental conditions this preparation contains solely postjunctional α_1-adrenoceptors is provided by the observation that prazosin (0.1μM) was a more potent antagonist against responses to phenylephrine, Sgd 101/75 and UK-14304

than was rauwolscine (1μM).

Influence of A II on α-adrenoceptor-mediated responses

As mentioned previously, A II, without altering resting baseline tension, produced a marked increase in the sensitivity of responses to UK-14304 in the rabbit isolated distal saphenous artery. Thus, previously absent responses were 'uncovered' at low concentrations of UK-14304. These responses were unaffected by prazosin at a concentration which is approximately 50 times greater than for its pA_2 value at α_1-adrenoceptors in this preparation. The CCRC to UK-14304 in the presence of A II however, was shifted to the right at higher concentrations of UK-14304, indicating the continued expression of responses mediated via postjunctional α_1-adrenoceptors. In contrast, in the presence of A II, rauwolscine (1μM) antagonised the responses to UK-14304 more effectively than prazosin (0.1μM), in effect preventing the increase in sensitivity to UK-14304 produced by A II. Therefore in the presence of A II responses to an α-adrenoceptor agonist, UK-14304, were more suceptible to rauwolscine than they were to prazosin, and are likely to have been mediated by postjunctional α_2-adrenoceptors.

In contrast to the marked effects of A II on responses to UK-14304, this agent was without effect on responses to NA (non-selective) and the selective α_1-adrenoceptor agonist phenylephrine. It did however produce a small increase in the sensitivity of the preparation to the partial agonist Sgd 101/75, providing the first evidence in the present study that A II can produce a facilitation of responses mediated via postjunctional α_1-adrenoceptors.

Before classifying the uncovered responses to UK-14304 in the presence of A II as being mediated via postjunctional α_2-adrenoceptors, it was desirable to

demonstrate activity for NA at these receptors. In accord with this hypothesis, A II made responses to NA more resistant to the antagonistic action of prazosin, particularly on the lower portion of the CCRC. This is similar to the effect of A II on prazosin antagonism seen in the lateral saphenous vein and the introduction of a prazosin-resistant component of responses strongly indicates the presence of both postjunctional α_1- and α_2-adrenoceptors in the distal saphenous artery. These results also parallel observations in the rat tail artery, in which the lower third of the CCRC to NA is slightly 'resistant' to prazosin over a limited prazosin concentration range (Rajanayagam & Medgett, 1987) and in which postjunctional α_2-adrenoceptor-mediated responses have subsequently been demonstrated (Templeton *et al.*, 1989). The lack of effect of A II on the antagonism produced by prazosin on responses to Sgd 101/75 and phenylephrine demonstrates that responses produced by these agonists in the absence and presence of A II were mediated via postjunctional α_1-adrenoceptors.

Attempted isolation of postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery

Phenoxybenzamine (0.3μM), an irreversible α-adrenoceptor antagonist (Furchgott, 1972), virtually abolished responses to NA in the rabbit isolated distal saphenous artery. The basis for receptor isolation, therefore, was to provide conditions whereby any postjunctional α_2-adrenoceptors could be selectively protected by the inclusion of 1μM rauwolscine in an analogous manner to that used in the lateral saphenous vein. This concentration of α_2-adrenoceptor antagonist was considered to be selective since it was without effect on responses to phenylephrine in the distal saphenous artery.

In contrast to the saphenous vein, the inclusion of rauwolscine (1μM) prior to and during exposure to phenoxybenzamine, did not result in part of the response

to NA being spared from phenoxybenzamine. This does not indicate the absence of postjunctional α_2-adrenoceptors however, since in the presence of A II concentration-dependent responses were observed to the α-adrenoceptor agonists UK-14304, NA and phenylephrine. The rank order of potencies for these agonists, UK-14304 > NA > phenylephrine differs from the potency order for these agents under normal circumstances, NA > phenylephrine >UK-14304, and is similar to the potency order for agonists found in the rabbit ear vein, which contains predominantly postjunctional α_2-adrenoceptors (Daly *et al.*, 1988b).

Additionally, responses to NA after the receptor protection protocol in the presence of A II were resistant to 0.1μM prazosin, a concentration approximately 150-times greater than for its pA_2 value on postjunctional α_1-adrenoceptors in this preparation. They were however, susceptible to rauwolscine (1μM) which produced an approximately 100-fold rightward displacement of the CCRC to NA, with an estimated $-\log K_B$ value of 8.01. This value corresponds well with that obtained against responses to NA in the lateral saphenous vein after isolation of postjunctional α_2-adrenoceptors and against responses to NA in the rabbit ear vein (Daly *et al.*, 1988b). Further evidence favouring the existence of a homogeneous population of postjunctional α_2-adrenoceptors under these conditions, is provided from the observation that the combination of prazosin (0.1μM) and rauwolscine (1μM) was no more effective at inhibiting responses to NA than rauwolscine (1μM) alone. Essentially these results are similar to those obtained under normal experimental conditions, where A II was a prerequisite for the demonstration of prazosin-resistant responses to both UK-14304 and NA.

Interaction between postjunctional α_1- and α_2-adrenoceptors

A number of observations in the rabbit isolated lateral saphenous vein and distal saphenous artery suggest that postjunctional α_1- and α_2-adrenoceptors do not co-exist in a simple manner. For example, despite the fact that postjunctional α_2-adrenoceptors can be clearly demonstrated in both preparations, under appropriate conditions, no component of the response to NA is resistant to prazosin when both α-adrenoceptors are present. In addition, having clearly demonstrated a facilitatory role for A II on responses mediated via postjunctional α_2-adrenoceptors, we may then have expected this agent to enhance responses to NA, a non-selective agonist, when the full receptor complement was present in each preparation. Such an action was observed in neither.

These apparently paradoxical observations could be reconciled if the two postjunctional α-adrenoceptors reside on the same cells and interact at the level of a common post-receptor site in the events leading to contraction. Thus, responses mediated by postjunctional α_2-adrenoceptors in the distal saphenous artery may be dependent upon simultaneous stimulation of the α_1-adrenoceptor population. With the latter predominating, this would explain the relatively high potency of prazosin, with no component of responses to NA (or other agonists) being resistant to the α_1-adrenoceptor antagonist, and the relatively low potency of rauwolscine. Since both α-adrenoceptors are present under normal experimental conditions, the positive influence required for the expression of α_2-adrenoceptor-mediated responses would be provided by stimulation of postjunctional α_1-adrenoceptors. Assuming a similar mechanism to be involved, the requirement for A II would therefore be superfluous, explaining the lack of a facilitatory action for this agent against responses to NA when the full receptor complement was active.

If however, α_1-adrenoceptors are inhibited, by either prazosin or phenoxybenzamine, this positive influence for A II would subsequently be a prerequisite for the expression of α_2-adrenoceptor-mediated responses. This therefore explains the induction of prazosin-resistance produced by A II, and the requirement for the presence of this agent to produce responses to NA mediated via postjunctional α_2-adrenoceptors, after irreversible inactivation of postjunctional α_1-adrenoceptors with phenoxybenzamine. An interaction between the α-adrenoceptors in this preparation was directly demonstrated by inducing a small degree of tone in the distal saphenous artery with a selective concentration of phenylephrine (0.01μM). Stimulation of α_1-adrenoceptors resulted in the introduction of a previously 'unseen' component of responses to UK-14304, at very low concentrations, analogous to that introduced by A II. Attempting to examine the effects of α-adrenoceptor antagonists on these responses would have negated the experiment. However it is reasonable to assume that the uncovered responses to UK-14304 were mediated via postjunctional α_2-adrenoceptors. The concept of interacting postjunctional α-adrenoceptors in the distal saphenous artery is represented diagramatically in Figure 71.

A similar interaction between postjunctional α-adrenoceptors is also implicated in the lateral saphenous vein, although the dependency of α_2-adrenoceptor-mediated responses on α_1-adrenoceptor stimulation is somewhat less. This is apparent since NA can clearly sustain a response alone after isolation of postjunctional α_2-adrenoceptors, contrasting with the distal saphenous artery, where responses are wholly dependent on the presence of a stimulatory agent. The relatively high pA_2 value obtained for rauwolscine in this preparation indicates that α_2-adrenoceptors are the predominant subtype, but their susceptibility to prazosin indicates some dependence upon a small degree of stimulation of the α_1-adrenoceptor population. Despite a competitive action of prazosin at postjunctional α_1-adrenoceptors, this would not necessarily result in a parallel shift in the agonist

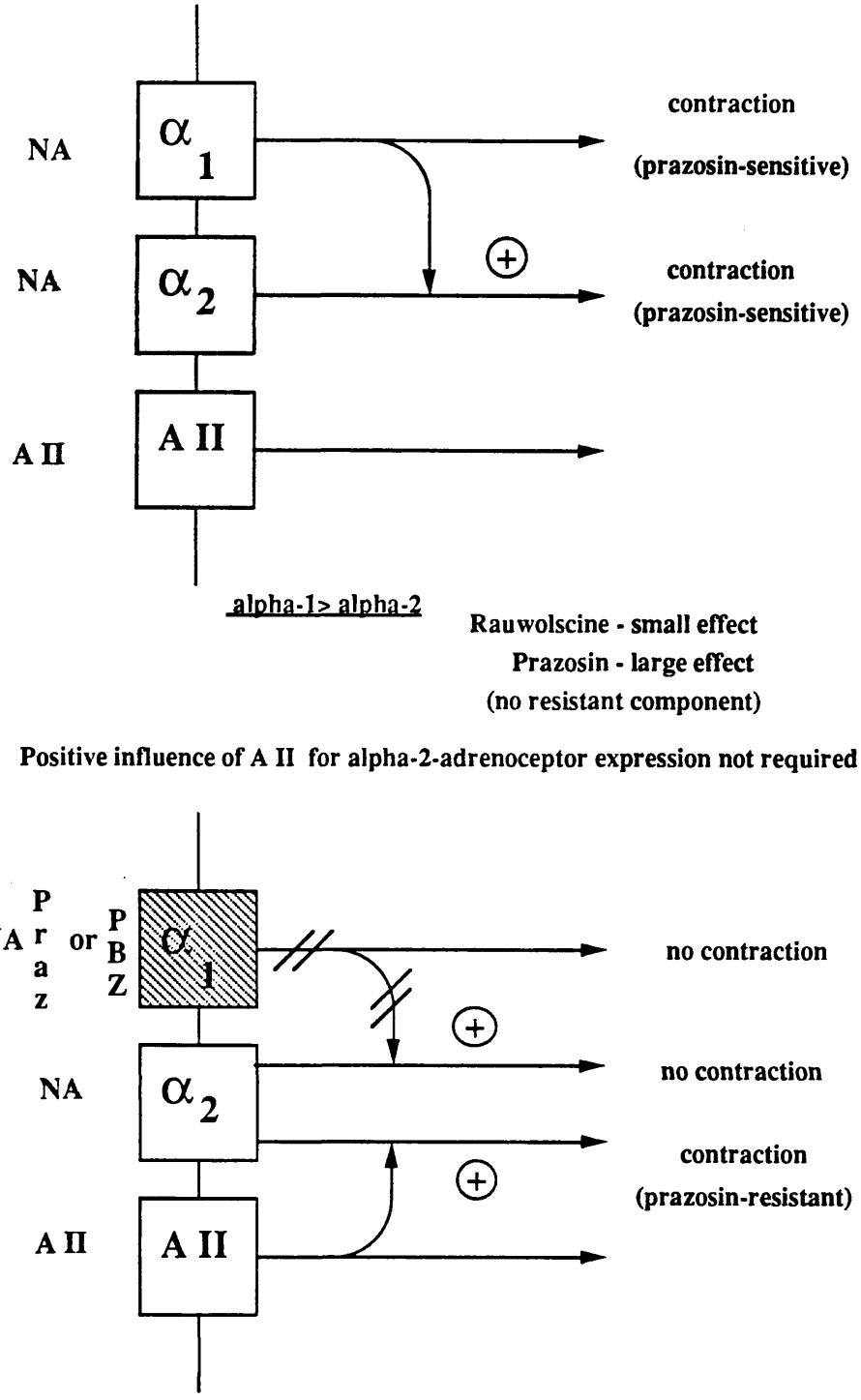


Figure No. 71

Diagrammatic representation of the postulated interaction between postjunctional α-adrenoceptor subtypes in the rabbit isolated distal saphenous artery. Note that under normal circumstances, α_1-adrenoceptors provide the positive input required for the expression of α_2-adrenoceptors, therefore all responses are sensitive to prazosin. A II, is only required if α_1-adrenoceptors are blocked with either prazosin or phenoxybenzamine.

CCRC, because of the unequal influence of prazosin on the two sets of receptor-mediated events i.e. blockade of α_1-adrenoceptor mediated contractions and facilitation and lack of blockade of α_2-adrenoceptor-mediated contractions. Again under these circumstances, the facilitatory action of A II on postjunctional α_2-adrenoceptor-mediated responses is apparent only if α_1-adrenoceptors are blocked by prazosin or phenoxybenzamine.

In contrast to both the distal saphenous artery and lateral saphenous vein responses to NA in the rabbit isolated ear vein are resistant to prazosin, but susceptible to rauwolscine (Daly *et al.*, 1988b). Therefore, the positive co-operation of postjunctional α_1-adrenoceptors is not required for the expression of α_2-adrenoceptor-mediated responses in this preparation. These three preparations therefore, provide a spectrum for the ability of α_2-adrenoceptors to sustain a response alone. It is interesting to note that the facilitatory action of A II is greatest where α_2-adrenoceptor-mediated responses are least manifest (distal saphenous artery), and least where they are easily demonstrated (ear vein), and that this parallels the degree of interaction between postjunctional α-adrenoceptor subtypes.

An analogous situation, to the interaction between α-adrenoceptor subtypes in the lateral saphenous vein, appears to exist for β_1- and β_2-adrenoceptors that mediate relaxation to NA in guinea-pig isolated tracheal strips (Carswell & Nahorski, 1983). Here, the existence of the β_1-subtype was clearly evident from the presence of a resistant component of responses to NA in the presence of the selective β_2-adrenoceptor antagonist ICI 118,551. However, no component of the NA CCRC was resistant to the selective β_1-adrenoceptor antagonist atenolol and the slope of the Schild plot was less than unity. The authors suggested that this was indicative of a functional interaction between two subtypes of adrenoceptors that mediate the same functional response. Similarly, an even more subtle interaction

between a functionally active population of α_1-adrenoceptors and a 'quiescent' population of α_2-adrenoceptors on the cat nictitating membrane has been demonstrated (Shepperson, 1984). Contractile responses to selective α_1-adrenoceptor agonists were potentiated by prior exposure to a selective α_2-adrenoceptor agonist, even though these agonists failed to produce a functional response. This effect could be blocked by selective α_2-adrenoceptor antagonists.

As mentioned in the introduction, since the original demonstration of postjunctional α_2-adrenoceptors in the pithed rat (Drew & Whiting, 1979, Docherty *et al.*, 1979, Docherty & McGrath, 1980), identification of this subtype on isolated vascular preparations, both responding to NA and resistant to prazosin or a suitable α_1-adrenoceptor antagonist, has proved very difficult. While a number of explanations have been forwarded to explain the elusive nature of this receptor subtype (see: McGrath *et al.*, 1989), it is clear from the present study that one such factor that may conspire to render contractions mediated by postjunctional α_2-adrenoceptors 'prazosin-sensitive', is the dependency of α_2-adrenoceptor-mediated contractions on stimulation of α_1-adrenoceptors. Since other contractile agents can substitute for the positive influence of α_1-adrenoceptors, prazosin-resistant responses could be easily demonstrated in whole animals due to the presence of circulating vasoactive substances. In this respect, A II would assume great importance, particularly in models such as the pithed rat where the renin-angiotensin system is highly activated (Grant & McGrath, 1988b).

Such an interaction between postjunctional α-adrenoceptors has widespread implications for the characterisation of the α-adrenoceptor populations in blood vessels currently available for study *in vitro*. This is perhaps best illustrated by two further examples, which suggest that the clear demonstration of α_2-adrenoceptor-mediated responses has been obscured by their indirect sensitivity to prazosin.

Firstly, responses to NA in the isolated vascular bed of the rat tail are prazosin-sensitive, rauwolscine-resistant (α_1-mediated) under normal experimental conditions, but in contrast, a sizeable component of the response to NA becomes prazosin-resistant, rauwolscine-sensitive (α_2-mediated) in the presence of vasopressin induced tone (Templeton *et al.*, 1989). Similarly, inducing tone with phenylephrine also introduced rauwolscine-sensitive responses to UK-14304 in this preparation (Templeton, 1988). Secondly, responses to NA in the rabbit femoral artery were shown to be prazosin-sensitive, rauwolscine-resistant, and therefore by definition α_1-adrenoceptor-mediated (Purdy & Weber, 1988). In the present study however, A II introduced a component of responses to UK-14304 in the femoral artery in an analogous manner to that seen in the distal saphenous artery. It would seem reasonable therefore, to predict that further analysis would demonstrate unequivocably the presence of postjunctional α_2-adrenoceptors in the femoral artery.

Such an interaction between postjunctional α_1- and α_2-adrenoceptors may also partially explain the wide disparity in the relative potencies of α_1- and α_2-adrenoceptor antagonists found between vascular preparations (see: Drew, 1985, McGrath *et al.*, 1989). If, for example, the expression of postjunctional α_2-adrenoceptors is completely dependent upon α_1-adrenoceptor stimulation, then we may expect that prazosin, in all situations, should potently antagonise responses in a competitive manner (as is the case in the distal saphenous artery). The relative potency of α_2-adrenoceptor antagonists will however, depend upon the relative predominance of this subtype. Therefore, with an increasing contribution of postjunctional α_2-adrenoceptors to the total response, the pA_2 values for α_2-adrenoceptor antagonists will increase accordingly. It is interesting to note therefore, that in the human femoral artery and canine portal vein, in both of which there is some evidence suggesting the presence of postjunctional α_2-adrenoceptors (Glusa & Markwardt, 1983, Furuta, 1988), that prazosin is only 11-35-fold more

potent than yohimbine (De Mey & Vanhoutte, 1981, Shoji *et al.*, 1983), in contrast to the rabbit pulmonary artery (Starke, 1981), rat aorta or portal vein (Digges & Summers, 1983) where the differential is greater than 200-fold.

This situation becomes even more complex if postjunctional α_2-adrenoceptors in a particular preparation, have some ability to sustain responses alone, but are facilitated by stimulation of the α_1-adrenoceptor subtype. Under these circumstances, the nature of the antagonism produced by α-adrenoceptor antagonists would tend to be associated with non-parallel shifts of the agonist CCRC, due to their unequal influence on the two sets of receptor-mediated events, and may also be associated with the appearance of a resistant component of the response. The rat tail artery (Rajanayagam & Medgett, 1987) and the lateral saphenous vein (present study, Daly *et al.*, 1988a) represent two functional examples of such an interaction.

The facilitatory influence of vasoactive agents on postjunctional α_2-adrenoceptors is not limited to A II and α_1-adrenoceptors, since the thromboxane mimetic U46619 also produced a similar potentiation of responses to UK-14304 in the rabbit distal saphenous artery. Similarly, Furuta (1988) has shown that the selective α_2-adrenoceptor agonist, B-HT 920, produced yohimbine-sensitive, prazosin-resistant contractile responses of the canine portal vein in the presence of a wide range of vascular stimulants. A number of striking differences exist between this preparation and the distal saphenous artery however. For example, Bay K 8644 and KCl also uncovered responses to B-HT 920 in the canine portal vein, leading the author to conclude that the uncovering of α_2-adrenoceptor-mediated responses was associated with an increase in intracellular Ca^{2+} levels. These agents although potentiating responses to UK-14304 in the rabbit distal saphenous artery, did not produce a marked increase in the threshold concentration of UK-14304 for

contraction, which was characteristic of the introduction of postjunctional α_2-adrenoceptor-mediated responses. This suggests that the potentiation produced by Bay K 8644 and KCl in this preparation, represents an enhancement of UK-14304 responses mediated via postjunctional α_1-adrenoceptors. Therefore it is unlikely that an increase in the intracellular Ca^{2+} levels alone is an adequate explanation for the effects of various vasoactive agents on α_2-adrenoceptor-mediated responses in the distal saphenous artery.

Since postjunctional α_2-adrenoceptors can be uncovered in the distal saphenous artery by a number of agents which stimulate specific receptors, it would seem reasonable to assume that α_2-adrenoceptor expression is dependent upon (or facilitated by) some common process activated by these agents. It is therefore interesting to note that α_1-adrenoceptors, A II receptors and thromboxane receptors are linked to activation of the phosphatidylinositol pathway in vascular smooth muscle (Abdel-Latif, 1986). Due to the inability to clearly demonstrate functionally active postjunctional α_2-adrenoceptors in isolated vascular preparations, little is known about the biochemical consequencies of their activation. However in a variety of non-vascular tissues, stimulation of α_2-adrenoceptors is closely linked with the production of cyclic AMP (Isom & Limbird, 1988). It is therefore possible, that the interaction between vasoactive agents and postjunctional α_2-adrenoceptors in vascular smooth muscle, may be the result of an interaction between two differing second messenger systems, mediating the same functional response.

In relation to the nature of the potentiation produced by vasoactive agents on responses to UK-14304, it is interesting to observe that the combination of agonists which mediate the same functional response, via different second messenger systems, interact in a synergistic manner, while those which utilise the same second

messenger pathway are merely additive in non-vascular tissues (Sugden *et al.*, 1984, Cocks *et al.*, 1984). While such a synergistic interaction between different second messenger systems would be a suitable explanation for the effects of A II, phenylephrine and U46619 on responses to UK-14304, it should be noted that 5-HT receptor stimulation is also associated with increasing the turnover of the phosphatidylinositol pathway in vascular smooth muscle (Abdel-Latif, 1986). This agent did not uncover a component of responses to UK-14304. This observation is difficult to explain in terms of the above simplistic hypothesis. However it is possible that the vascular receptors activated by 5-HT do not reside on the same smooth muscle cells as postjunctional α_2-adrenoceptors. This would therefore preclude any intracellular, biochemical interaction subsequent to stimulation of these receptors.

Sympathetic neurovascular transmission in the rabbit isolated distal saphenous artery

Sympathetic neurovascular transmission in the rabbit isolated distal saphenous artery is the resultant of a complex interaction between three postjunctional receptor systems, α_1-adrenoceptors, purinoceptors and α_2-adrenoceptors. In order to demonstrate contractile responses to each of these three receptor systems, responding to a nerve released transmitter, some manipulation of the experimental conditions was required.

α_1-adrenoceptors

Postjunctional α_1-adrenoceptors clearly have a predominant role in mediating the end-organ contractile responses to sympathetic nerve stimulation in the rabbit isolated distal saphenous artery. Prazosin (0.1μM), a concentration selective for α_1-adrenoceptors in this preparation (see previous section), markedly attenuated or abolished nerve-mediated responses when applied alone, or after the combination of rauwolscine (1μM) and α,β-methylene ATP (3μM).

Purinoceptors

The demonstration of a purinergic component of responses to sympathetic nerve stimulation was apparent only after complete adrenergic blockade. The P_2-purinoceptor desensitising agent α,β-methylene ATP (3μM) (Kasakov & Burnstock, 1983) abolished the residual response remaining after the combination of prazosin (0.1μM) and rauwolscine (1μM). This inhibitory effect of α,β-methylene ATP is consistent with the blockade of purinergic transmission which has been described in a number of other vascular preparations (eg. Muramatsu,

1986, Ramme *et al.*, 1987). In the absence of any other antagonist however, α,β-methylene ATP (3μM) produced an enhancement of responses to sympathetic nerve stimulation. Similar results have been obtained in both the rabbit isolated lateral saphenous vein and ileocoelic artery (unpublished observations). It appears likely therefore, that determination of an inhibitory effect of α,β-methylene ATP under these circumstances is offset by a more dominant facilitatory influence of this agent on adrenergic transmission. The mechanism for the facilitatory action of α,β-methylene ATP is unknown, although two possible explanations could be forwarded. Firstly, it could be the result of a postjunctional enhancement of adrenergic responses or alternatively it may be due to the inhibition of a prejunctional purinoceptor-mediated negative feedback mechanism. The former suggestion seems more likely however, since responses to exogenous NA in the ileocoelic artery are potentiated by α,β-methylene ATP (J. Bulloch, personal communication).

It should be noted that in a previous study, Burnstock & Warland (1987) demonstrated a marked inhibitory effect for α,β-methylene ATP on nerve-mediated responses in the rabbit saphenous artery. It is now apparent that these authors used the proximal saphenous artery rather than the distal saphenous artery used in the present study (G. Burnstock, personal communication). These results are particularly interesting because they clearly indicate that marked differences can occur in the sympathetic transmission process between sequential segments of a vascular preparation .

α_2-adrenoceptors

Rauwolscine (1μM) can be considered selective for α_2-adrenoceptors since it was without effect on responses to phenylephrine or Sgd 101/75 in this

preparation. Under normal conditions rauwolscine (1μM) produced a potentiation of responses to sympathetic nerve stimulation in the distal saphenous artery. This effect is in accord with its inhibitory influence on α_2-adrenoceptor-mediated autoinhibition (for reviews see: Westfall, 1977, Starke, 1987, Stjarne, 1989). Furthermore, rauwolscine produced an enhancement of responses in the presence of prazosin (0.1μM). Since this response was subsequently abolished by α,β-methylene ATP (3μM) it seems reasonable to conclude that this effect represents an increase in the output of the purinergic transmitter by a similar blockade of prejunctional feedback.

This prejunctional α_2-adrenoceptor mediated negative feedback system can obviously complicate the demonstration of 'innervated' postjunctional α_2-adrenoceptors, due to the mutually opposing actions of antagonists, particularly if α_2-adrenoceptors are not the predominant subtype. This is further complicated in the distal saphenous artery (and maybe in other preparations) by the dependency of postjunctional α_2-adrenoceptors upon a degree of stimulation of the α_1-subtype. The combination of these two factors may explain the difficulty associated in demonstrating postjunctional α_2-adrenoceptors responding to NA released from nerves in isolated vascular preparations. Indeed, 'innervated' postjunctional α_2-adrenoceptors have been demonstrated most convincingly in the saphenous veins from various species (Flavahan *et al.*, 1984, Cheung, 1985, Docherty & Hyland, 1985), in each of which α_2-adrenoceptors are the predominant postjunctional subtype and their expression is not completely dependent upon a positive influence from α_1-adrenoceptor stimulation (Flavahan & Vanhoutte, 1987).

Contractile responses to sympathetic nerve stimulation, mediated by postjunctional α_2-adrenoceptors, could be demonstrated under the appropriate conditions in the distal saphenous artery. When this preparation was exposed to

A II (0.05μM), the small residual response remaining after the combination of prazosin (0.1μM) and α,β-methylene ATP (3μM) was markedly potentiated. This response was subsequently inhibited by rauwolscine (1μM) providing the direct demonstration that postjunctional α_2-adrenoceptors are located postjunctionally, sufficiently close to the neurovascular junction that they can respond to NA released from nerves. This situation is essentially analogous to that seen with exogenous NA, where postjunctional α_2-adrenoceptor-mediated responses are only demonstrable after inhibition of α_1-adrenoceptors in the presence of A II.

A number of other studies also suggest the existence of postjunctional interactions between α-adrenoceptors involved in mediating responses to sympathetic nerve stimulation. For example in the canine saphenous vein, responses to low frequencies of nerve stimulation are abolished by both α_1- and α_2-adrenoceptor antagonists (Flavahan *et al.*, 1984). Since the selectivity of the concentrations of antagonist employed remained unquestioned, these observations strongly suggested that responses to nerve stimulation reflected a supra-additive interaction between the α-adrenoceptor subtypes. Similar observations have been made in the hind limb of the dog and in human digital arteries (Gardiner & Peters, 1982, Stevens & Moulds, 1985). Therefore, in analogous manner to the interaction between α-adrenoceptors responding to exogenous NA, α_2-adrenoceptor-mediated responses to nerve stimulation may be rendered sensitive to prazosin and may, in part, account for their elusive nature in isolated vascular preparations.

Effect of A II on responses to sympathetic nerve stimulation

As mentioned in the introduction, A II has been demonstrated to potentiate responses to sympathetic nerve stimulation in a wide variety of smooth muscle preparations. This effect has largely been ascribed to a prejunctional facilitatory mechanism, since equivalent-sized responses to NA in most preparations were

prazosin (0.1μM) and α,β-methylene ATP (3μM) was markedly potentiated. This response was subsequently inhibited by rauwolscine (1μM) providing the direct demonstration that postjunctional α_2-adrenoceptors are located postjunctionally, sufficiently close to the neurovascular junction that they can respond to NA released from nerves. This situation is essentially analogous to that seen with exogenous NA, where postjunctional α_2-adrenoceptor-mediated responses are only demonstrable after inhibition of α_1-adrenoceptors in the presence of A II.

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potentiated to a smaller degree than those to nerve stimulation (for reviews see: Zimmermann, 1979, Westfall, 1977). Without measuring the output of radioactively labelled neurotransmitters it is difficult to determine the site of action, whether pre- or postjunctional, of an agent which produces facilitation of nerve-mediated responses. However, a number of experimental observations suggest that A II can act at both levels to potentiate sympathetic nerve-mediated responses in the rabbit isolated distal saphenous artery.

The uncovering by A II of prazosin-resistant, rauwolscine-sensitive nerve-mediated responses is analogous to the effects of this agent on responses to exogenous NA. This, therefore, indicates a postjunctional interaction between A II and one of the components of sympathetic nerve mediated transmission in the distal saphenous artery. In addition, however, A II probably has a prejunctional facilitatory action. This seems likely since this peptide potentiated responses to sympathetic nerve stimulation under all circumstances, the exception being in the presence of rauwolscine and α,β-methylene ATP, when nerve responses were already markedly increased. Further experiments would be required however, to determine the site of action of A II.

Abstract

The results presented in this thesis provide evidence that postjunctional α_2-adrenoceptors can be demonstrated in an isolated arterial and venous preparation from the rabbit, under appropriate conditions. Under normal circumstances, responses to NA in both the lateral saphenous vein and distal saphenous artery, are susceptible to low concentrations of selective α_1-adrenoceptor antagonists. This could be taken as an indication either for atypical α-adrenoceptors (saphenous vein) or for a homogeneous population of postjunctional α_1-adrenoceptors (saphenous artery). However, a closer examination of each preparation suggests that the effectiveness of agents such as prazosin or YM 12617 in these preparations, results from an interaction between postjunctional α_1- and α_2-adrenoceptors. Therefore, the expression of postjunctional α_2-adrenoceptor-mediated responses are partially or wholly dependent upon a degree of stimulation of postjunctional α_1-adrenoceptors. Such an interaction may mask the presence of postjunctional α_2-adrenoceptors in other isolated vascular preparations. These results have implications for classifying receptors by the susceptibility of agonists to prazosin (or equivalent). Such an interaction also complicates the demonstration of postjunctional α_2-adrenoceptors responding to nerve-released NA in the saphenous artery. The ease in demonstrating prazosin-resistant responses *in vivo* could be explained by the presence of vasoactive substances, with A II of particular importance, which can substitute for α_1-adrenoceptors to provide the necessary positive influence for α_2-adrenoceptor expression. Having isolated homogeneous populations of both α_1- and α_2-adrenoceptors, comparative studies on the effects of agents such as calcium channel blockers/facilitators on responses to the endogenous ligand NA could be attempted. This approach overcomes the limitations of studies *in vivo*. No differential effects for 1,4-dihydropyridines were observed on responses to NA mediated via either subtype.

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