THE CONTRIBUTION OF α_2-ADRENOCEPTORS TO SYMPATHETIC NEUROEFFECTOR TRANSMISSION IN THE RABBIT ISOLATED SAPHENOUS AND PLANTARIS VEINS.

A thesis presented for the degree of
Master of Science

by

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Declaration.

With the exception of figures 1-5 (which were done in collaboration with Dr. V.G. Wilson) all of the work contained within this thesis is my own. All of the experiments and data analysis were carried out personally as was the initial experimental design. A list of the publications which have used data derived from this study is given.

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Daly, C.J., McGrath, J.C. & Wilson, V.G. (1987). α-adrenoceptor-mediated contractions in the rabbit saphenous vein - The influence of endothelial cells. *Br. J. Pharmacol.* **92**, 776P.

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MacDonald, A. Daly, C.J., Bulloch, J.M. & McGrath, J.C. (1992). Contributions of α_1-adrenoceptors, α_2-adrenoceptors and P_{2x}-purinoceptors to neurotransmission in several rabbit isolated bloood vessels. *Br. J. Pharmacol.* **105**, 347-354.

Summary

1 The aim of the study was to establish i) the presence or absence of postjunctional α_2-adrenoceptors on the rabbit isolated saphenous and plantaris veins, ii) the contribution of these receptors (if any) to sympathetic neuroeffector transmission in both vessels, iii) the conditions which may influence their contribution to the neuroeffector response.

2 Based on the rank order of α-adrenoceptor subtype specific agonist potency (UK14304 > NA >> phenylephrine) and antagonist potency (rauwolscine = prazosin > corynanthine), the saphenous vein was considered to possess a mixed population of postjunctional α_1- and α_2-adrenoceptors. A possible interaction between these receptors is discussed.

3 Based on the rank order of α-adrenoceptor subtype specific agonist potency (UK14304 > NA >> phenylephrine) and antagonist potency (rauwolscine > corynanthine >> prazosin), the plantaris vein was considered to possess a more homogeneous population of functional postjunctional α_2-adrenoceptors than the saphenous vein.

4 The effects of relatively selective α-adrenoceptor antagonists, on the response to electrical field stimulation (EFS), in the saphenous vein suggested that α_1-adrenoceptors play a major role in the neurovascular response.

5 Responses to EFS in the saphenous vein were unaffected by either propranolol or corticosterone. Cocaine, however, was found to potentiate the height and, more significantly, to prolong the time course of the response to EFS in the saphenous vein.

6 The effects of selective α-antagonists, in the presence of cocaine, indicated a small contribution from postjunctional α_2-adrenoceptors to the response to EFS in the saphenous vein.

7 The apparent lack of effect of rauwolscine and the inhibitory effect of prazosin on responses to EFS in the plantaris vein indicated that, in the absence of cocaine, α_1-adrenoceptors play a major role in neuroeffector transmission in this vessel.

8 In the presence of cocaine, rauwolscine caused a significant inhibition of responses to EFS in the plantaris vein. The effect of prazosin however was unaffected. This indicates that, in the presence of cocaine, postjunctional α_2-adrenoceptors play a significant role in neuroeffector transmission.

9 The non-adrenergic component of the response to EFS in the plantaris vein was found to be highly sensitive to inhibition by α-β,methyleneATP. This suggests that NA & ATP are co-transmitters in the sympathetic nerves of the plantaris vein.

10 As mentioned above (point 5), cocaine increased the duration of the response to EFS in the saphenous vein. This resulted from the emergence of a secondary phase of the response to EFS above 8Hz which was highly sensitive to inhibition by the α_2-adrenoceptor antagonists rauwolscine and CH 38083. The first phase was sensitive to α_1-adrenoceptor antagonists. Thus in the presence of cocaine, α_2-adrenoceptors provide a major contribution to the neurovascular response above 8Hz.

11 Lowering the Po_2 in the saphenous vein caused the appearance of a secondary phase of the response to EFS. In low O_2 prazosin was found to be slightly less effective but the effect of rauwolscine was no greater. This indicated that merely lowering Po_2 was not sufficient to recruit a significant population of postjunctional α_2-adrenoceptors for the response to neuroeffector transmission.

12 In the absence of cocaine and in the presence of selective α-adrenoceptor antagonists, angiotensin II (AII) did not potentiate responses to EFS in the saphenous vein. In the presence of cocaine and prazosin however AII caused a significant potentiation of response at 16Hz and above. Thus AII has a facilitatory effect on postjunctional α_2-adrenoceptors when neuronal uptake is blocked.

13 AII caused potentiation of responses to EFS in the plantaris vein only in the presence of prazosin and regardless of the presence of cocaine. Therefore, AII can be shown to have a selective potentiating effect on postjunctional α_2-adrenoceptors which are involved in the response to EFS in both the saphenous and plantaris veins.

14 The sensitivity to selective and non-selective α-adrenoceptor agonists in the saphenous vein was unaffected by removal of the endothelium. The maximum response to the α_1-adrenoceptor agonists was however increased. This suggests that endogenous nitric oxide (NO) liberated from the endothelium has an inhibitory effect on responses mediated by postjunctional α_1- but not α_2-adrenoceptors.

15 The effect of L-NAME (an inhibitor of NO synthetase) was assessed on the biphasic response to EFS and the response to exogenous NA in the saphenous vein. L-NAME caused a marked potentiation of the response to EFS. However, no selective effect on either phase was found. Endogenous NO had more of an effect on those receptors involved in the response to EFS than those involved in the response to exogenously applied agonists.

16 In conclusion, functional α_2-adrenoceptors are present on both the saphenous and plantaris veins. The addition of cocaine, to block neuronal uptake and thus increase the concentration or duration of NA in the neuroeffector junction, can enhance the contribution of α_2-adrenoceptors to the response to neuroeffector transmission. AII can also enhance the contribution of α_2-adrenoceptors to the neurovascular response. It is not certain whether changes in Po_2 or basal levels of NO can selectively augment α_2-adrenoceptor function although these factors can modulate the α-adrenoceptor mediated response to neuroeffector transmission in the saphenous vein.

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List of abbreviations.

α,β-mATP	α,β-methylene adenosine triphosphate
AII	angiotensin II
Amid.	amidephrine
CH.	CH 38083
Coc.	cocaine
Cort.	corticosterone
Cory.	corynanthine
CRC	concentration response curve
EFS	electrical field stimulation
L-NAME	Nω-nitro-L- arginine methyl ester
NA	noradrenaline
Phen.	phenylephrine
Praz.	prazosin
Prop.	propranolol
Rauw.	rauwolscine
UK	UK 14304
Xyl.	xylazine

Introduction

Demonstration of α_2-adrenoceptor mediated responses in isolated tissues has proven difficult for many years. Prazosin resistant, pressor responses to noradrenaline were first demonstrated in the pithed rat (Bentley *et. al.*, 1977). Further studies, comparing the effects of various α-adrenoceptor subtype selective agonists, confirmed the existence of postjunctional α_2-adrenoceptors on peripheral blood vessels (Docherty *et. al.*, 1979; Drew & Whiting, 1979). Unfortunately, *in-vitro* examination of isolated arteries often failed to identify a functional α_2-adrenoceptor population. Functional α_2-adrenoceptors can, however, be more easily demonstrated on isolated venous tissue. The first isolated vein to be extensively studied was the canine saphenous vein where a heterogeneous population of α-adrenoceptors was clearly demonstrated (De Mey & Vanhoutte, 1981). A few years later the rabbit isolated saphenous vein was shown to possess a similar heterogeneous α-adrenoceptor profile (Alabaster *et. al.*, 1985).

Further studies have shown that α_2-adrenoceptors can be demostrated on a variety of isolated blood vessels including the rabbit saphenous, plantaris and ear veins (Daly *et. al.*, 1988b). The α_2-adrenoceptors of the rabbit saphenous vein appear to interact in some way with the α_1-adrenoceptors (Daly *et. al.*, 1988c). The plantaris vein on the other hand contains an almost homogeneous population of α_2-adrenoceptors. Although α_2-adrenoceptors can be shown to participate in the responses to exogenously applied agonists in saphenous and plantaris veins, their contribution to sympathetic neuroeffector transmission is unclear.

Historical subdivision of adrenoceptors

Adrenergic receptors exist as proteins which are found in the plasma membrane of many different types of cells. These receptor proteins can be activated by the natural catecholamines, noradrenaline and adrenaline, and have thus been termed adrenoceptors (Furchgott, 1972). The first subdivision of the adrenoceptors was based on experiments which showed that a series of phenylethylamines (including adrenaline,

noradrenaline and the synthetic catecholamine, isoprenaline) had different orders of potency depending on the tissue being studied (Ahlquist, 1948). This led Ahlquist to suggest that the adrenoceptors were of two types, α and β. Earlier work by Dale (1913) had shown that adrenaline could have both vasoconstrictor and vasodilator effects, depending on the vascular bed being studied. It is now clear that this is due to the relative proportions of α- and β-adrenoceptors present in a given tissue or vascular bed.

It was not long before the β-adrenoceptors were subdivided into β_1- and β_2- depending on their location and polarity (Lands *et. al.*, 1967). The first subdivision of the α-adrenoceptors was based on their anatomical position. The postjunctional receptors mediated the effector response and were termed α_1- ; the prejunctional receptors inhibited further transmitter release and were termed α_2- (Langer, 1974). The first experimental evidence of an antagonist increasing transmitter outflow was presented seventeen years earlier, however, at the time it was suggested that the antagonist was preventing the receptors from 'utilising' the transmitter (Brown & Gillespie, 1957). This stimulated a great deal of work and led to the suggestion that "..noradrenaline released by nerve stimulation acts on alpha sites of the presynaptic membrane to inhibit its own release" (Kirpekar & Puig, 1971).

It is now generally accepted that within the wall of most blood vessels α_1-adrenoceptors are present on smooth muscle and cause contraction when activated by plasma noradrenaline or by noradrenaline released from sympathetic nerve terminals. The prejunctional (α_2-) adrenoceptors are acted upon by neuronal or plasma noradrenaline to inhibit further transmitter release and are thus termed prejunctional autoreceptors. Current evidence clearly shows, however, that α-adrenoceptors cannot be sub-divided on a purely anatomical basis.

Subdivision of α_1-adrenoceptors

Selective agonists and antagonists were subsequently developed and examined by pharmacologists on a wide variety of tissue types. Based on the action of phenylethanolamines and non-phenylethanolamines on rat anococcygeus and rabbit basilar artery McGrath (1982) first suggested that the α_1-adrenoceptors could be further subdivided into α_{1a}- and α_{1b}. Similarly, the potency of prazosin and yohimbine at α-adrenoceptors varied markedly in various vascular tissues (Drew, 1985). The variable potency of prazosin led to the proposed α_{1H}- and α_{1L}-adrenoceptor sub-division (Flavahan & Vanhoutte, 1986) while the affinity of [^{3}H] WB 4101 in rat brain prompted a subclassification of α_{1A}- and α_{1B}- (Morrow & Creese, 1986). Recent studies have suggested the existance of three adrenoceptor subtypes in vascular smooth muscle (α_{1H}-, α_{1L}- & α_{1N}, Muramatsu *et. al.*, 1990) and rat cerebral cortex (α_{1A}-, α_{1B}- & α_{1L}-, Oshita *et. al.*, 1991).

Subdivision of α_2-adrenoceptors

The existence of postjunctional α_2-adrenoceptors in the vasculature was established following examination of the pressor effects, in pithed rats, of relatively selective α-adrenoceptor agonists in the presence of rauwolscine and prazosin (Docherty *et. al.*, 1979; Docherty & McGrath, 1980; Drew, 1980; Timmermans & Van Zwieten, 1980). Although it was assumed that α_2-adrenoceptors were present in the vasculature, experimental evidence *in-vitro* was not forthcoming. This fact alone is probably responsible for the 'late discovery' of the α_2-subtype. Studies with clonidine on the isolated canine saphenous vein provided the first hard evidence of postjunctional α_2-adrenoceptors *in-vitro* (DeMey & Vanhoutte, 1981). Indeed low concentrations of noradrenaline were found to be relatively resistant to prazosin in this tissue (Flavahan *et. al.*, 1984; Fowler *et. al.*, 1984). In contrast, Alabaster *et. al.*, (1985) found prazosin to be a competitive antagonist at both low and high concentrations of

noradrenaline. Based on reported pA_2 values at pre- and postjunctional α_2-adrenoceptors in different tissues it became clear that α_2-adrenoceptors may not be a homogeneous group (Alabaster & Peters, 1984; Alabaster *et. al.*, 1986).

The development of the 'selective' postjunctional α_2-adrenoceptor antagonist SK&F 104078 provided evidence for intra-species differences (Ruffolo *et. al.*, 1987). Unfortunately the selectivity of this compound has been questioned as it seems unable to distinguish between postjunctional α_1- and α_2-adrenoceptors (Connaughton *et. al.*, 1988; author, unpublished observations). Many attempts have been made at sub-dividing α_2-adrenoceptors on either an anatomical or functional basis. However, the evidence is circumstantial and inconclusive.

Ligand binding studies, which do not rely on functional responsiveness, have provided most of the direct evidence for subdivision of the α_2-adrenoceptors. Based on agonist studies, a two state model for high (α_{2H}-) and low(α_{2L}-) affinity binding at α_2-adrenoceptors was first postulated by Hoffman & Lefkowitz (1980). More recently, Bylund (1988) has used a series of ligands to sub-divide the α_2-adrenoceptors into α_{2A}-, α_{2B}- & α_{2C}-. A later report suggested the existence of an α_{2D}-adrenoceptor subtype (Bylund *et. al.*, 1991).

It is possible to compare antagonist potencies from functional studies with antagonist affinity data derived from ligand binding studies. One such comparison has suggested the existence of subtypes of prejunctional α_2-adrenoceptors (Connaughton & Docherty, 1990). In this study it was concluded that the prejunctional α_2-adrenoceptors of the rat vas deferens are of the α_{2A}-subtype while those of the rat atrium are of the α_{2B}-subtype. Until α_2-subtype selective antagonists are developed we will have to rely on comparative studies of antagonist potency and affinity.

Molecular characterisation of α-adrenoceptors.

The adrenergic receptors are being extensively studied by molecular biologists. The gene sequences / cDNA for three α_1- and three α_2-adrenoceptors subtypes have already been isolated. It would seem therefore that sub-division of the α-adrenoceptors can now be made on a structural basis. Whether the subtypes identified by structural, functional and binding studies will ever be unified remains to be seen but this will probably provide some of the most exciting 'adrenoceptor' research in the coming years.

Mechanism of action of α-adrenoceptors on vascular smooth muscle.

The hydrophilic nature of most agonists prevents them from 'personally' carrying their 'message' into the cell. The receptors are therefore linked through various proteins and enzymes to other chemical pathways inside the cell. These intracellular molecules are thus termed second messengers. The various second messenger cascades ultimately result in an increase in intracellular Ca^{++} which is required if contraction of smooth muscle is to be sustained. The α-adrenoceptors mediate their cellular functions via second messengers which happen to be quite different for the α_1- and α_2-subtypes.

α_1-adrenoceptors.

Stimulation of α_1-adrenoceptors causes the activation of phospholipase C (PLC) which catalyses the hydrolysis of phosphotidylinositol 4, 5-biphosphate (PIP_2) to inositol, 1,4,5,-triphosphate (IP_3) and 1,2-diacylglycerol (1,2-DG). IP_3 can liberate intracellular Ca^{++} stores while 1,2-DG can activate protein kinase C (PKC) which can modulate the activity of receptor- and potential-operated Ca^{++} channels (see Abdel-Latif, 1986 and McGrath *et. al.*, 1989 for review). It should be noted that current

evidence can not correlate increases in IP_3 or 1,2-DG directly with functional responsiveness.

α_2-adrenoceptors.

It is widely accepted that α_2-adrenoceptors are linked to membrane bound adenylate cyclase (AC) through a pertussis toxin sensitive guanine nucleotide regulatory protein (G_i). The G-protein referred to consists of three subunits, α, β & γ. When the receptor is stimulated G_i is activated and this results in the dissociation of the α_i and β/γ complex. Since the β/γ complex is identical to the β/γ complex of G_s (the G-protein for β-adrenoceptors) it can bind α_s and thus become inactive. Alternatively α_i may directly inhibit the catalytic unit of adenylate cyclase or it may compete with α_s for the same site (Gierschik & Jakobs, 1988). The result of inhibiting AC is a reduction of cAMP which is thought to participate in the uptake and storage of intracellular Ca^{++} and activation of cAMP dependent protein kinases which can inhibit Ca^{++} channel function (McGrath *et. al.*, 1989).

Another model has been suggested to take account of the discrepancies in the physiological effects of α_2-adrenoceptor agonists. Limbird (1984) proposed that α_2-adrenoceptor stimulation activates the Na+/H+ exchange system which increases pH and the accessibility of membrane-bound Ca^{++}. This results in activation of phospholipase A_2 and thus arachidonic acid production which can lead to thromboxane A_2 formation which can activate PLC and stimulate IP_3 / 1,2-DG production as described for α_1-adrenoceptors. This rather complicated chain of events may prove very useful for workers trying to explain the possible interactions between α_1- and α_2-adrenoceptors.

α_2-adrenoceptor function

Noradrenaline is present in plasma at concentrations of around 0.1nM. Noradrenaline is also stored in sympathetic adrenergic nerve terminals (or varicosities) and is used as their principal neurotransitter. It follows therefore that adrenoceptors must exist for this hormone as well as adrenaline which is also present in plasma. It cannot be disputed that these receptors exist, what can be (and often is) contested is the existence of particular subtypes of receptors in different regions of the body. α_1-adrenoceptors can be demonstrated in almost all of the tissues upon which both noradrenaline and adrenaline can be shown to act. In pharmacological terms this is generally established by their greater sensitivity to prazosin (an α_1-adrenoceptor antagonist) than to rauwolscine (an α_2-adrenoceptor antagonist) (McGrath, 1982).

From this it follows that identification of a population of α_2-adrenoceptors would require that the response being studied be more sensitive to rauwolscine than to prazosin. While prazosin-resistant, rauwolscine-sensitive responses have been relatively easy to demonstrate *in vivo*, such responses have been difficult to find *in vitro*. Sensitivity to rauwolscine or other α_2-adrenoceptor antagonists can be shown, but the crucialy important prazosin resistance has, until quite recently, been elusive *in-vitro*.

Nevertheless, α_2-adrenoceptors have been show to have a functional relevance in many diverse tissue types.

Central nervous system α_2-adrenoceptors

Presynaptic α_2-adrenoceptors are present on noradrenergic, dopaminergic, cholinergic and serotinergic neurons. α_2-adrenoceptors have also been found in the cerebral cortex of rat (Farnebo & Hamberger, 1971), rabbit (ReichenBacher *et. al.*, 1982) and guinea-pig (Beani *et. al.*, 1978) as well as cat spinal cord (Fleetwood-Walker *et. al.*, 1985). These adrenoceptors are responsible for regulating transmitter release from the host neuron.

The evidence gathered from the studies on cortex is based on the ability of α_2-agonists to inhibit, or α_2-antagonists to enhance, stimulus-evoked [^{3}H]-transmitter release. The spinal cord experiments examined inhibition by NA of a nociceptive response.

Clinically, the α_2-agonist clonidine has been used to treat opiate withdrawal whereas the α_2-antagonist mianserin has been used in the treatment of depression. α_2-agonists are also used as anti-hypertensive agents due to their action at central α_2-adrenoceptor sites.

Peripheral Blood Vessels

With the exception of the capillaries, all other blood vessels contain a tunic of smooth muscle; the tunica media, which in most vessels contains a population of α-adrenoceptors. It is the smooth muscle cells of the media which ultimately determine the degree of vasoconstriction or vasodilation, and thus the luminal diameter of the vessel. The smooth muscle is under the influence of (i) neurotransmitters liberated from sympathetic nerves which innervate the tunica adventitia, in most but not all blood vessels, (ii) endogenous hormones, synthetic drugs and other blood borne factors; (iii)

substances released from the endothelium (e.g. nitric oxide, endothelium derived hyperpolarising factor, endothelin).

In addition, the effects of all of these vasoactive substances can be further modulated by local environmental factors such Po_2, pH, temperature etc.

The possibility of interactions of two or more of these factors adds another level of complexity which must be considered when analysing the results of *in vivo* experiments.

In view of the problems just outlined, many experiments on blood vessels are carried out *in vitro* where the environmental conditions can be accurately determined by the experimenter.

Arterial α_2-adrenoceptors

As previously mentioned, the presence of arterial α_2-adrenoceptors was confirmed by pressure changes obtained in response to α-adrenoceptor agonsits in pithed rats which were resistant to prazosin (Docherty et al,1979; Drew & Whiting, 1979). The work which followed failed to demonstrate convincingly the presence of α_2-adrenoceptors on isolated arteries *in vitro*.

This could have been due to the relatively large (non-resistance) vessels which were studied at that time or it may have been due to the absence of a co-factor normally present *in-vivo*. In terms of relative size it is interesting that in the rabbit, the thoracic aorta has an almost homogeneous population of post junctional α_1-adrenoceptors (Docherty *et. al.*, 1981). However, the smallest terminal arterioles of the cremaster muscle have an almost homogeneous population of α_2-adrenoceptors as judged by intra vital miscroscopy. (Faber, 1988; McGillivray-Anderson & Faber, 1990; Ohyanagi, 1991). Resistance arteries (~200μm i.d.) from humans but not rats, rabbits or pigs

have also been shown to contain postjuntional α_2-adrenoceptors (Nielsen *et al.*, 1991). Development of the wire myograph (Mulvany & Halpern 1976) and perfusion myograph (Halpern *et. al.*, 1984) has enablled pharmacological study of the small arteries (100-300μm i.d.) most likely to contribute to peripheral resistance. Unfortunately it still remains difficult to demonstrate α-adrenoceptor mediated responses in segments of arteries *in vitro* that are prazosin resistant.

Size (i.e. lumen diameter) is not necessarily the principal factor in demonstrating arterial α_2-adrenoceptors. It may be that the various co-factors present in blood are not routinely present in *in vitro* experiments. It has been shown that angiotensin II (AII) can 'uncover' an otherwise quiescent population of α_2-adrenoceptors in the rabbit isolated distal saphenous artery (Dunn *et al.*, 1989). Responses to clonidine in isolated rat tail arteries can be enhanced by prior activation with 5HT, phenylephrine or vasopressin (Fulsgang *et al.*, 1991). In isolated vascular beds of the rat tail (Templeton *et.al.*, 1989) or rabbit ear (McGrath *et.al.*, 1991) responses to α_2-adrenoceptor agonists can be enhanced if a low concentration of a co-factor, e.g. an α_1-agonist or KCl is included in the perfusate. It is now becoming accepted that arterial α_2-adrenoceptor function requires support from other receptor types or depolarising agents. The recent 'explosion' of work with the NO synthase inhibitors - L-NMMA & L-NAME (Moncada *et al.*, 1991) has suggested that NO released from endothelial and smooth muscle cells plays a part in suppressing adrenergic function and α_2-adrenoceptor mediated responses in rabbit pulmonary arteries (MacLean & McGrath, Personal communication) and in isolated perfused rat lung (Shaw & Pollock, personal communication) as well as in guinea pig pulmonary arterioles (Liu *et.al.*, 1991).

It is now possible to create an *in-vitro* environment which favours α_2-adrenoceptor function in blood vessels. The conditions required, however, make it very difficult to study arterial α_2-adrenoceptors in isolation. Venous tissue, however, poses fewer problems.

Venous α_2-adrenoceptors.

De Mey & Vanhoutte (1981) first demonstrated the existence of a heterogeneous population of postjunctional α-adrenoceptors in the canine saphenous vein. It was later suggested by Alabaster *et. al*, (1985) that the rabbit saphenous vein contained a more homogeneous population of postjunctional α_2-adrenoceptors. Using the same tissue Levitt & Hieble (1985) observed prazosin-sensitive NA responses and therefore suggested that α_1-adrenoceptors contributed to the response. More recent work has suggested a functional interaction between α_1- & α_2-adrenoceptors in response to exogenously applied NA in the rabbit saphenous vein (Daly *et al.*, 1988c) as well as to NA released from sympathetic nerve terminals (Daly & Wilson, 1991; MacDonald *et al.*, 1992). Although responses to α_2-agonists such as UK 14304, BHT-920 & BHT 933 can be obtained in many veins these responses are often sensitive to prazosin. This again highlights the difficulty in obtaining a homogeneous population of postjunctional α_2-adrenoceptors.

It has been suggested that venous α_2-adrenoceptors have a thermoregulatory role and are primarily located on the more cutaneous veins. (Flavahan & Vanhoutte,1986; McAdams & Waterfall, 1989). This is supported by the resistance to prazosin of NA-evoked responses in the rabbit plantaris vein and rabbit ear vein (Daly *et al.*, 1988b). The rabbit ear vein appears to contain, almost exclusively, a population of postjunctional α_2-adrenoceptors (Daly *et al.*, 1988a).

Other 'systemic' veins have also been shown to possess post junctional α_2-adrenoceptors. These include: Dog pulmonary vein (Shebuski *et al.*, 1987); Cat hepatic venous circulation (Segstro & Greenway, 1986); Human femoral vein (Glusa & Markwadt, 1983a).

Clearly α_2-adrenoceptors have the potential to play an important role in the regulation of blood flow. Presently it appears that postjunctionally they may be more important on the venous side. However, the difficulty in locating and studying their function on isolated arteries must not be allowed to diminish their functional importance.

Importance of the venous system.

It is surprising, in view of their important role in the circulatory system, that the veins have not been as widely studied as the arterial side of the circulation. In their review on 'veins and venous tone', Folkow & Mellander (1964) describe a "monumental negligence" on the part of vascular researchers and physiologists with respect to the study of veins.

The veins contain around 70% of the total blood volume and their major function is, therefore, one of capacitance although they do play a minor part in resistance. The rate of exchange and the direction of filtration across the capillary walls is dependent on the ratio of precapillary to postcapillary resistance. It follows therefore that, for this function at least, the small veins are as important as the small arteries. The venous return plays a crucial role in relation to cardiac output. Since it is a relatively low pressure system the blood in the veins must be actively mobilised towards the heart, particularly from the lower limbs. The veins are not just passive tubes, they are under local, reflex and central nervous control.

Venous morphology.

The veins are relatively thin walled by comparison with arteries. Like arteries, they have a concentric ring structure which consists of three functionally distinct layers.

The outermost sheath is called the 'tunica adventitia' and comprises mostly macrophage and fibroblast cells. The adventitia surrounds the tunica media, which is made up of smooth muscle cells which are generally arranged in a helical fashion. The media also contains collagen and elastin fibres. The inner most layer of cells, the endothelium, line the inner surface of the the internal elastic laminae and are in contact with the blood. The smooth muscle cells which are responsible for determining the calibre of the vessel, are under the influence of a variety of physiological processes. The endothelial cells are known to release a variety of substances, both contractile (e.g. endothelin) and relaxatory (e.g. endothelium derived relaxing factor, nitric oxide (which may be EDRF) and endothelium derived hyperpolarising factor). These endothelial derived factors can exert a powerful influence on the tonicity of the overlying smooth muscle. Other blood borne factors such as circulating catecholamines (eg. noradrenaline and adrenaline) and hormones (eg. angiotensin II) can stimulate smooth muscle contraction. Postganglionic sympathetic neurones, whose varicosities are present in the adventitia and outer layers of smooth muscle, can liberate neurotransmitters, which can cause a powerful vasoconstriction, and are important for the mobilisation of blood. These three modulating influences (i.e. neurotransmitters, endothelium derived factors and blood borne substances) create a complex system which serves to regulate the activity of the medial smooth muscle.

Adrenergic nerves.

Most veins are innervated with adrenergic fibers whose principal neurotransmitter is noradrenaline (NA). The transmitter is stored within vesicles at, or around, release sites on the nerve varicosities. The absence of Schwann cells in the region of the varicosities makes it possible for the transmitter to be released into the extra-terminal space. The transmitter can then diffuse into the tunica media and act on smooth muscle cells to cause activation. In arteries the distance between the varicosities and the nearest

smooth muscle cells, called the synaptic celft, can be as little as 80nm (Burnstock *et. al.*, 1970).

Cutaneous veins and splanchnic veins such as the saphenous and mesenteric have been shown to constrict vigorously to adrenergic nerve stimulation while the 'deeper' femoral vein gives a relatively poor response (Vanhoutte, 1974). This highlights the importance of neurogenic control of vessels which are not massaged by skeletal muscle in order to propel blood through the lumen. In the rabbit saphenous and plantaris veins, therefore, sympathetic control of smooth muscle tone must be of prime importance.

Activation of sympathetic nerves, to cause transmitter release leading to venoconstriction, can be obtained with field stimulation (Holman & McLean, 1967). This enables the *in-vitro* study of sympathetic nerve stimulation in isolated segments of blood vessels. Interaction of the physiological factors mentioned above with the sympathetic neuroeffector response can therefore also be studied *in-vitro*.

Origin of transmitter noradrenaline.

Noradrenaline (NA) is synthesised from the amino acid tyrosine. Hydroxylation of tyrosine by tyrosine hydroxylase yields 3,4-dihydroxyphenylalanine (dopa). Decarboxylation of dopa by decarboxylase yields dopamine. Hydroxylation of dopamine by dopamine-β-hydroxylase finally produces NA (Blaschko, 1939).

Once synthesised, NA is stored in vesicles (von Euler & Hillarp, 1956) ready for release by exocytosis. Depolarisation of the nerve terminal caused by the arrival of an action potential activates voltage-operated Ca^{++} channels in the terminal. The influx of Ca^{++} leads to an increase in intraterminal Ca^{++} concentration and this triggers the exocytotic process.

Three types of vesicle have been identified; small dense vesicles (SDV, which contain NA and ATP), small clear vesicles (SCV, contents unknown) and large dense vesicles (LDV, which contain NA, ATP & neuropeptides). The release of vesicles in differing amounts and ratios is frequency dependent. Low frequency stimulation preferentially releases SDVs. High frequency stimulation releases LDVs (Stjarne, 1989). The release of transmitter may be either quantal or graded.

Disposal of released NA.

There are a variety of routes for the elimination of NA from the synaptic cleft. The major route is largely determined by the geometry of the particular cleft. If the cleft is very narrow then most of the released NA will be removed locally via the neuronal pump (uptake$_1$) or by uptake into nonneuronal sites (uptake$_2$). NA removed by the nerve varicosities via uptake$_1$ may be used for further release, although most of it will be deaminated by monoamine oxidase (MAO). The deaminated metabolite then diffuses out of the nerve terminal where it can be methylated by catechol-O-methyl transferase (COMT). NA removed by the uptake$_2$ mechanism suffers a similar fate to that removed by uptake$_1$. If the synaptic cleft is very wide, there is more scope for NA to avoid the uptake mechanisms and escape into the blood stream. NA in the blood will eventually be cleared by the lungs.

Junctional hypothesis.

The responses of isolated blood vessels to stimulation of the sympathetic nerves are much faster than those obtained to exogenously applied NA. α-adrenoceptors must therefore be present near to the site of release. Most nerve mediated responses in isolated blood vessels are sensitive to prazosin. This has led to the widely held belief that α_1-adrenoceptors are present in the synaptic cleft (Docherty & McGrath, 1980; Langer *et. al.*, 1980; Wilffert *et.al.*, 1982). Since responses to nerve stimulation are not generally inhibited by addition of α_2-adrenoceptor antagonists, it has been assumed that α_2-adrenoceptors are mainly extrajunctional.

Evidence is now available to support the view that α_2-adrenoceptors may be located in the synaptic cleft of some tissues. The saphenous vein of the dog (Flavahan *et. al.*, 1984) and human (Docherty & Hyland, 1985) have been shown to possess 'innervated' α_2-adrenoceptors. Other tissues in which this has been shown are; canine mesenteric vein (Kou *et. al.*, 1984), rat tail artery (Medgett, 1985) and human digital arteries (Stevens & Moulds, 1985).

It has been suggested by some workers that, in guinea pig arterioles, α-adrenoceptors are only present in extrajunctional regions and that the synaptic cleft is populated by 'specialised receptors' (Hirst & Nield, 1981). When balanced against the weight of the evidence in favour of intrajunctional α-adrenoceptors, however, this seems unlikely.

Co-transmission.

Early experiments identified the presence of both ATP and NA in SDVs and LDVs of sympathetic nerves (Stjarne, 1964). While the very existence of ATP in the vesicles suggested a role as a co-transmitter, the ratio of ATP to NA was very low. In

the nerve trunk the NA : ATP ratio was found to be 4 : 1 (Stjarne, 1964) and in the terminal regions it was 50 : 1 (Klein, 1982). The low proportion of ATP was therefore considered to make co-transmission unlikely (Fredholm *et. al.*, 1982).

With the development of a selective P_2-purinoceptor antagonist (ANAPP3) and a P_{2x}-purinoceptor desensitising agent (α-β-methylene ATP) it has been possible to study the pharmacology of non-adrenergic (i.e. α-adrenoceptor antagonist resistant) responses to nerve stimulation. The work which followed the development of the P_{2x}-selective compounds has provided compelling evidence for co-transmission in a variety of preparations including the pithed rat (Bulloch & McGrath, 1988), guinea pig vas deferens (Sneddon *et. al.*, 1983), mouse vas deferens (Stjarne & Astrand, 1984), rabbit mesenteric artery (Ishikawa, 1985), rabbit saphenous artery (Burnstock & Warland, 1987), rabbit ileocolic artery (Bulloch & Starke, 1990) and more recently the rabbit dorsal cutaneous arteries (Daly *et.al.*, 1993).

Presynaptic mechanisms.

As previously mentioned, α_2-adrenoceptors can be shown to exist on sympathetic nerve varicosities and to inhibit further release of transmitter NA. It is now apparent that these are not the only presynaptic receptors which can regulate transmitter release. Beta-adrenoceptors are also present and can enhance further transmitter release. The possibility exists therefore that α- and β-adrenoceptors can function together to finely regulate the release of sympathetic transmitter. Langer (1977) proposed that at low concentrations of NA (i.e. low frequency stimulation) the presynaptic β-adrenoceptors are activated and cause further transmitter release. At higher concentrations of NA (i.e. high frequency stimulation) the α-adrenoceptors are activated and can inhibit transmitter release.

Several presynaptic receptors have now been described for noradrenergic nerves; muscarinic receptors (inhibitory, -); dopamine receptors (-); opiate receptors (-); prostaglandin E1 & E2 receptors (-); adenosine receptors (-); angiotensin II receptors (+); nicotinic receptors (+) (Langer, 1977). These receptors have not been described for all types of noradrenergic nerves and are listed here only to emphasise the importance of the presynaptic site as a regulatory mechanism.

Physiological influences and conditions required for α_2-adrenoceptor expression.

The composition of the extracellular fluid is essential to the functionality of vascular smooth muscle. Consequently, changes in the chemical composition of the extracellular fluid can modify vascular reactivity (Bohr, 1964). The composition of venous blood is more variable than that of arterial blood, since the venous blood reflects the degree of metabolism within the tissues. It follows that venous smooth muscle must function under more variable conditions (particularly with respect to oxygen content) than arterial smooth muscle.

As stated earlier, a difficulty in demonstrating postjunctional α_2-adrenoceptors can be the relatively un-physiological conditions, caused by the absence of co-factors, that many *in-vitro* experiments are performed in. Sensitivity to selective α_2-agonists has been demonstrated *in-vivo* , as has prazosin resistance. The response to α_2-adrenoceptor agonists can be enhanced if the rats are ventilated with a gas mixture containing low oxygen and high carbon dioxide (Grant *et. al.*, 1985). These authors concluded that the α_1-adrenoceptor is more dependent on blood oxygen content while the α_2-adrenoceptor is more dependent on carbon dioxode / pH. Since the condition which exists in venous blood relative to arterial blood is low O_2 / high CO_2, it is perhaps not surprising that both subtypes of α-adrenoceptors can be easily demonstrated in isolated veins.

It is now apparent, from the accumulating evidence, that postjunctional adrenoceptor subtypes can interact with each other in a manner which suggests a link at the second messenger level (McGrath *et. al.*, 1989). There is also a particularly interesting interaction between angiotensin II and postjunctional α_2-adrenoceptors (Schumann & Lues, 1983; Dunn *et. al.*, 1989; McGrath *et. al.*, 1989). Angiotensin II may therefore be one of the important endogenous co-factors which are generally absent from *in-vitro* experiments.

The vascular endothelium liberates inhibitory factors which supress the responses to α_1-adrenoceptor agonists in the isolated saphenous vein (Daly, *et. al.*, 1987). The effects of these inhibitory factors have not yet been assessed on sympathetic neuroeffector transmission on this vessel but it is not unlikely that they will be effective.

Aims of the project.

The purpose of this project was a) to establish the presence of α_2-adrenoceptors on the rabbit isolated saphenous and plantaris vein, b) to assess, in these vessels, the contribution of α_2-adrenoceptors to neuroeffector transmission and c) to investigate some of the physiological factors which may modulate the neuroeffector response mediated by α_2-adrenoceptors.

Methods

Male New Zealand White rabbits (2-3Kg) were killed by stunning followed by exsanguination. The lateral saphenous vein or plantaris vein was carefully removed with as little connective tissue as possible and placed in cold physiological salt solution (PSS) before being divided into ring segments of 3-4mm in length. The rings were then suspended between two wire supports, similar to that described by Hooker *et. al.*, (1976). The upper supports were connected to transducers for isometric recording. The lower supports were attached to small vessel electrodes upon which are mounted parallel platinum plates to facilitate electrical field stimulation (EFS). In the experiments which did not require EFS the lower supports were attached to glass tissue holders. The complete assemblies were then placed in 30ml glass organ baths containing physiological salt solution (PSS) maintained at 37°C and saturated with 95% O_2 : 5% CO_2. All the preparations were then placed under an initial tension of 1.5g. The preparations generally relaxed to a resting tension of around 0.75 - 1.0g. Any preparations which fell below 0.75g were re-tensioned to that level.

Experimental apparatus.

The small vessel electrodes were purchased from HSE electronics. The glass tissue holders were specially manufactured by the Chemistry Department of Glasgow University. The transducers were of type FTO3c (isometric), manufactured by Grass. The transducers were connected through, and balanced by, Fylde Electronics FE BBS bridge conditioners. The final signal was amplified and displayed on a Linseis L2065 chart recorder. Electrical square wave pulses were delivered by a 'Square 1' multi channel stimulator, supplied by Square 1 instruments.

Experimental protocol for the construction of concentration response curves.

After a period of equilibration (45 minutes), a sighting concentration of 3μM noradrenaline was given and each tissue was allowed to contract to a plateau at the point of equilibrium. After washing, a full 60 minutes was left before a concentration response curve (CRC) to the chosen agonist was constructed. All the agonists were tested over the range 1nM-0.1mM. Concentrations were added to the bath cumulatively in 3 times steps, taking into account the concentration already present. This gives final increments of 0.5 log. units. The transient nature of the response to certain concentrations of agonist dictated that the next, higher, concentration be given as close as possible to the peak response. This method was adopted for all of the CRCs. Since cumulative addition of agonists will increase the volume of the bath, individual drug additions were given in small amounts not exceeding 70μl. Antagonists were allowed 40 minutes to equilibrate before construction of the next CRC.

Electrical field stimulation.

After a period of equilibration (45 minutes), a sighting concentration of 3μM noradrenaline was given and each tissue was allowed to contract to a plateau. After washing and a further 30 minutes, frequency response curves (FRCs) were constructed. Stimulation parameters used were 4, 8, 16, 32 and 64 Hz, 1 second train duration, 0.1ms pulse width, 35 volts. A low-frequency, long-duration (4Hz/10sec.) train of pulses was also tested. Stimuli were delivered at 5 minute intervals. The initial FRC is referred to as FRC-1 or control FRC. At the end of FRC-1 a period of 30 minutes was left before the construction of FRC-2. This allows ample time (incubation period) for an antagonist or other drug to be added to one or more of the organ baths. The same time (30 min.) was

left between subsequent FRCs to allow further incubations. No more than four FRCs were constructed in any single preparation.

In some experiments cocaine (10μM) and propranolol (1μM) were present in the bathing medium (PSS) throughout, while in others they were added as the protocol demanded.

Drugs & solutions.

PSS was of the following composition (mM) NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$. $7H_20$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2 and glucose 11.1. Na_2 EDTA 23μM was included to prevent oxidative degradation of NA.

The following drugs were used: noradrenaline bitartrate (Sigma); UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); phenylephrine HCl (Sigma); prazosin HCl (Pfizer), rauwolscine HCl (Roth); corynanthine HCl (Roth); CH-38083 (7,8-methylenedioxi-14-α-hydroalloberbane HCl, Chinoin); α,β-methyleneadenosine 5-triphosphate lithium salt (Sigma); L-NAME (Nω-nitro-L-arginine methyl ester, Sigma); amidephrine HCl (Mead Johnstone); xylazine HCl (Bayer); cocaine HCl (Macarthys); propranolol HCl (Sigma); corticosterone (Sigma). All drugs were dissolved in distilled water.

Anatomical locations.

Saphenous vein.

The lateral saphenous vein was regarded as being that portion beginning at the knee and continuing, towards the femoral, as far as the first visible bifurcation. A section of no more than 20mm starting from just above the knee was taken.

Plantaris vein.

The plantaris vein precedes the 'distal' saphenous vein which begins at the paws and extends towards the ankle where the saphenous vein begins. A 10-15mm section was removed from each foot.

Expression of CRC data

Responses to the agonists tested were expressed as a percentage of the maximum response to noradrenaline. The order of agonist potency in both veins was determined by comparing the pD_2 (-log EC_{50}) values for each agonist. This value corresponds to the negative log. concentration of the agonist required to produce half (or 50%) of the maximum response attainable with that agonist and is obtained by graphical interpolation in the linear portion of the concentration response curve.

Antagonist potency was determined as follows; EC_{50} values were obtained for a) the agonist in the presence of the antagonist and b) the agonist in the absence of the

antagonist. The agonist concentration ratio (ACR) was determined by dividing a) by b). ACRs were determined over an antagonist concentration range of 100-600 fold. If antagonism is competetive then a plot of antagonist concentration against ACR minus 1 should yield a straight line with a slope of 1. The point where this line crosses the abscissa is equal to the pA_2 of the antagonist in that particular test system (Arunlakshana & Schild, 1959). A software package (Stats 7) was used to determine the slope of the line, the confidence limits and the estimated pA_2 value. An antagonist which yields a line with confidence limits that overlap unity was considered to be competative.

Expression of FRC data

The absolute sizes of responses to nerve stimulation varied considerably between tissues for example, although uptake1 blockade consistently increased individual responses, the range of absolute values for 'blocked' and 'unblocked' tissues overlap. For this reason all the results of this study have been expressed as a percentage of the maximum contractile response obtained in the control frequency response curve (FRC-1). On all figures columns represent mean values and vertical lines represent the S.E.M.

Statistical analysis.

Student's paired or unpaired t-tests were used to test for significant differences. Results were considered to be significant where $p<0.05$ (*), 0.01(**) or 0.001(***). In cases where 3 FRCs were compared, FRC 2 was tested against FRC 1 and FRC 3 was tested against FRC 2. For the analysis of biphasic responses, phase 1 and phase 2 of the response prior to treatment were measured and each designated as 100%. Each individual phase of the response after treatment was expressed (graphically) as a

percentage of the relevant control phase. Statistical tests, however, were made on the raw data.

Criteria for the choice of antagonist concentrations in EFS experiments.

Rauwolscine.

Potentiation, by rauwolscine, of the first phase of the response to nerve stimulation in the isolated saphenous vein was maximal at a concentration of 1μM (Daly & Wilson, 1991). Increasing the concentration to 10μM inhibited the responses at all frequencies (unpublished observations). This inhibition was due to blockade of α_1-adrenoceptors indicating a loss of "selectivity". The potentiation of responses observed with 1μM rauwolscine was due to activity at prejunctional α_2-adrenoceptors. In a previous study (Daly *et al* ., 1988b) responses to low concentrations of noradrenaline were found to be resistant to rauwolscine at concentrations up to 2.5μM. This resistant component was sensitive to prazosin confirming rauwolscines' relative selectivity at a concentration 2.5 times greater than that used in the present study. In the rabbit plantaris vein prazosin was virtualy without effect versus responses to noradrenaline. Both prazosin and rauwolscine (2.5μM) in combination however effected a much greater rightward shift of the CRC to noradrenaline. In summary, this evidence confirms that 2.5μM is the highest concentration of rauwolscine which can be presumed to be selective for α_2-adrenoceptors compared with α_1-adrenoceptors.

Prazosin.

In the rabbit ear vein; a preparation with an almost homogeneous population of post-junctional α_2-adrenoceptors (Daly *et al.*, 1988a), prazosin (1μM) was virtually without

effect. In the rabbit plantaris vein it was not possible to obtain a pA_2 value for prazosin because of its lack of effect. This confirms that prazosin 0.1μM can be presumed to be selective for α_1-adrenoceptors compared with α_2-adrenoceptors

Corynanthine.

The estimated pA_2 value for corynanthine against NA in the isolated rabbit saphenous vein was 6.36. Although the pA_2 is lower than may be expected for an action at α_1-adrenoceptors, the slope of the schild plot indicated competative antagonism. It was assumed that a concentration of 1μM would be relatively selective for α_1-adrenoceptors.

CH 38083.

When tested against NA in the isolated saphenous vein, CH 38083 (10μM) produced a biphasic rightward shift of the CRC. A resistant component, like that seen in the presence of rauwolscine, was also seen in the presence of CH 38083. This resistant component was sensitive to prazosin (Daly *et.al.*, 1988c). For this reason it was presumed that 1μM of CH 38083 would be relatively selective for α_2-adrenoceptors.

α,β-methylene ATP.

After addition of 3μM α,β-methylene ATP, subsequent addition of the drug produced no response indicating that this concentration was sufficient to desensitize P_{2x}-purinoceptors. This was found to be the case in both of the vessels used for this study.

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Cocaine.

In the presence of cocaine (10μM) the CRC for NA in the isolated saphenous vein is shifted to the left. This concentration has been reported to be at the upper limit of that required to block uptake$_1$ without having any anaesthetic effect (McGrath *et. al.*, 1989).

Results

α-adrenoceptor profile of the rabbit isolated saphenous vein.

Effects of cocaine, propranolol & normetanephrine on responses mediated by exogenously applied noradrenaline.

Following the blockade of neuronal uptake ($uptake_1$) of noradrenaline (NA) with cocaine (10μM), the concentration response curve (CRC) for NA was significantly shifted to the left by 0.5±0.08 of a log unit (n=6). After pre-treatment with 3μM phenoxybenzamine, NA induced a relaxation of KCl (40mM)-induced tone which propranolol (1μM) inhibited. Normetanephrine (30μM) produced a slight leftward shift of the NA CRC (0.1±0.04). This shift was statistically significant but sufficiently small to indicate that extraneuronal uptake ($uptake_2$) is not a major route in NA removal.

All CRCs were therefore performed in the presence of cocaine (10μM) and propranolol (1μM) to block $uptake_1$ and β-adrenoceptors respectively.

α-adrenoceptor agonist potency.

Based on the magnitude of responses, relative to NA, obtained to the selective agonists, phenylephrine was found to be a full agonist. The selective α_2-adrenoceptor agonist UK-14304 produced only 80% of the maximum response obtained with NA and is therefore apparently not a full agonist if acting at the same receptors as NA (Figure 1).

The order of potency based on the calculated pD2 values was;

UK-14304 (7.85±0.1) > NA (7.53±0.1) >> phenylephrine (5.83±0.06).

This suggests the presence of a population of postjunctional α_2-adrenoceptors. The potency of phenylephrine is sufficiently high to suggest the presence of α_1-adrenoceptors.

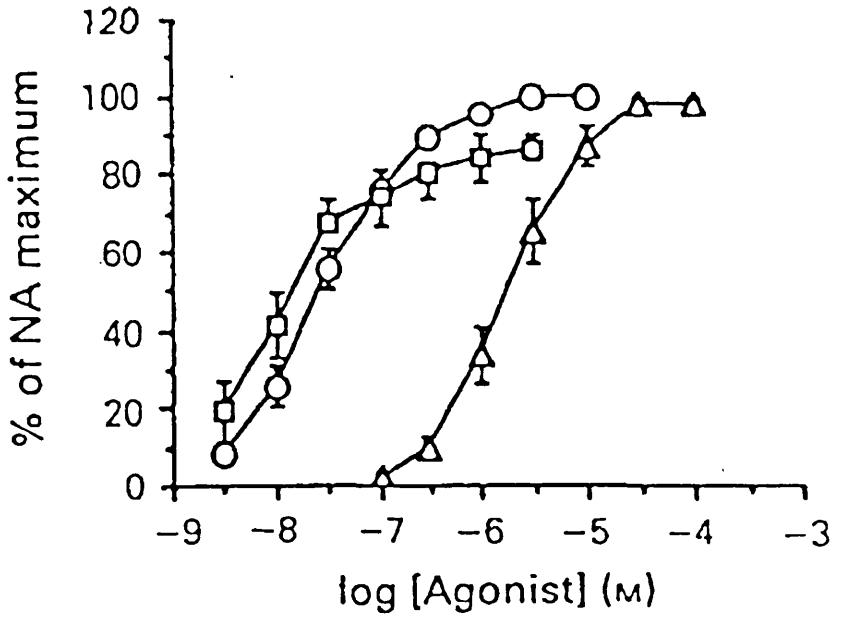

Figure 1. A comparison of the effect of various agonists at α-adrenoceptors in the rabbit isolated saphenous vein. The effect of (-)-noradrenaline (NA) (○), phenylephrine (△) and UK-14304 (□). All responses are expressed as a percentage of the maximum response to (-)-NA and are the mean of 4-9 observations. The vertical lines indicate the s.e.m.

α-adrenoceptor antagonist potency.

The relatively selective α_1-adrenoceptor antagonist corynanthine produced dose dependent, rightward shifts of the NA CRC (Figure 2a). The Schild plot for corynanthine against NA produced a line of slope 0.89 (1.06-0.71). Since the confidence limits overlap unity, corynanthine's antagonism could be considered competetive. Close inspection of the graph however does not reveal classical parallel shifts. The estimated pA_2 for corynanthine (0.5-50μM) was 6.36 (6.55-6.16) which is lower than might be expected at α_1-adrenoceptors (see discussion).

The more potent α_1-adrenoceptor antagonist prazosin, also produced dose dependent rightward shifts of the NA CRC (Figure 2b). The maximum response to NA in the presence of 0.1μM and 1μM prazosin was reduced. The Schild analysis produced a line of slope 0.58 (0.68-0.51). The confidence limits did not overlap unity, therefore prazosin is a non-competetive antagonist against NA in this tissue. The estimated pA_2 for prazosin (0.005μM-3μM) was 8.44 (8.72-8.18).

Rauwolscine produced dose dependent rightward shifts of the upper part of the NA CRC (Figure 2c). Responses to low concentrations of NA appeared to be resistant to rauwolscine. These rauwolscine resistant responses were found to be prazosin-sensitive (Figure 3) and are therefore mediated by α_1-adrenoceptors. Schild analysis was performed on the agonist concentration ratios at 75% of the maximum response. The slope of the line was 0.85 (0.74-0.96). The estimated pA_2 for rauwolscine (0.05-2.5μM) was 8.56 (8.89-8.22).

The rank order of potency, based on the pA2 values, was;

rauwolscine = prazosin >> corynanthine.

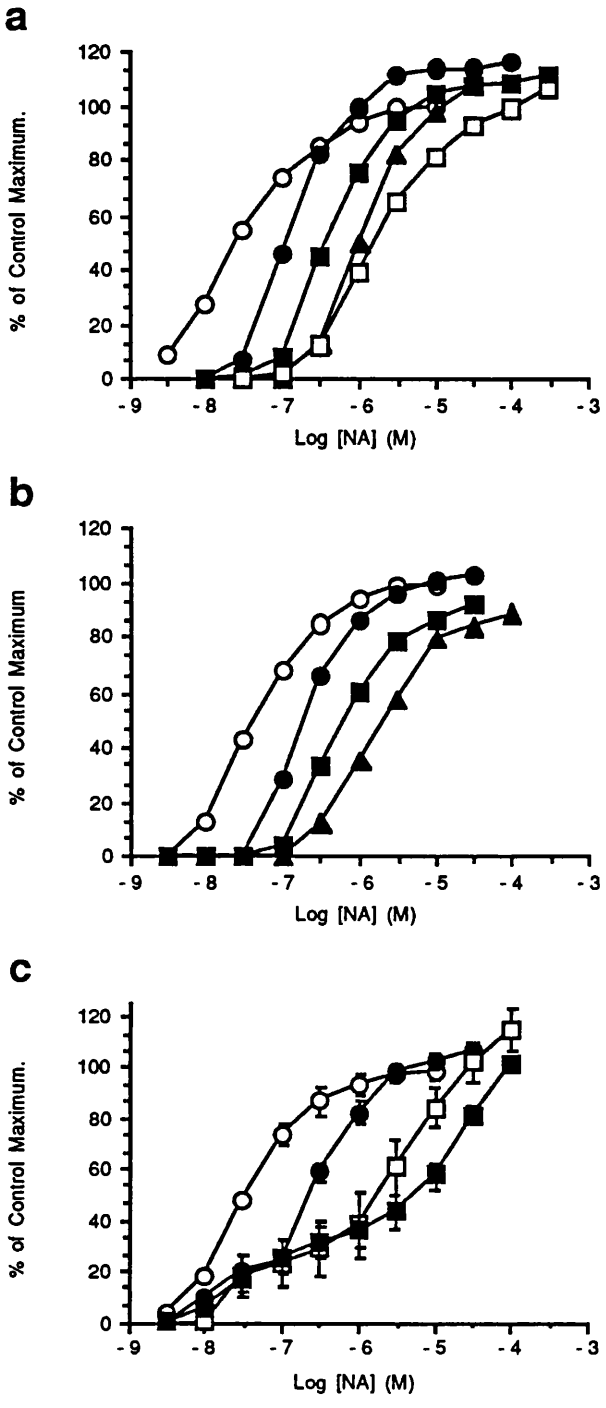

Figure 2. The effect of various antagonists at α-adrenoceptors on the isolated saphenous vein. The effect of a) corynanthine 0.5μM (●), 2.5μM (■), 10μM (▲) and 50μM (□). b) prazosin 0.03μM (●), 0.3μM (■) and 3μM (▲). c) rauwolscine 0.05μM (●), 0.5 μM (□) and 2.5μM (■). All responses are expressed as a percentage of the maximum response to NA and are the mean ± s.e.m. (n=4)

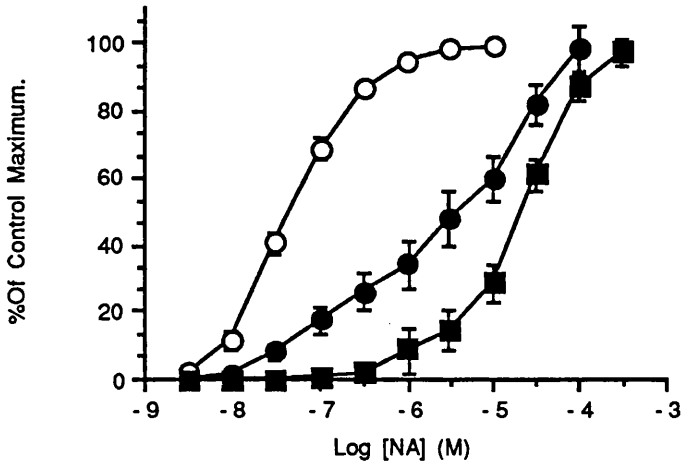

Figure 3. The combined effect of prazosin (0.1μM) and rauwolscine (2.5μM) on responses to NA in the isolated saphenous vein. Control (○) rauwolscine alone (●) and rauwolscine + prazosin (■). All responses are expressed as a percentage of the maximum response to NA and are the mean ± s.e.m. (n=4).

The relative potencies of rauwolscine and corynanthine support the presumption that the saphenous vein contains an effective population of α_2-adrenoceptors.

α-adrenoceptor profile of the rabbit isolated plantaris vein.

All experiments were performed in the presence of cocaine (10μM) and propranolol (1μM).

α-adrenoceptor agonist potency.

Figure 4 shows the concentration response curves (CRCs) for UK-14304, NA and phenylephrine. UK-14304 produced only 60% of the maximum contraction to NA but exhibited greater potency (~3 fold) than NA. Based on the pD_2 values, the observed order of potency was ;

UK-14304 (7.58±0.09) > NA (6.98±0.06) >> phenylephrine (5.81±0.06).

This order of potency suggests the presence of a population of postjunctional α_2-adrenoceptors. Phenylephrine is sufficiently potent to suggest the presence of α_1-adrenoceptors.

α-adrenoceptor antagonist potency.

Corynanthine (0.5-50μM) produced dose-dependent, parallel rightward displacements of the NA CRC (Figure 5a). The 95% confidence limits of the slope of the Schild plot overlapped unity, therefore the antagonism can be considered competitive. The slope of the Schild plot was 0.94 (1.18-0.71) and the estimated pA_2 was 6.32 (6.66-5.97) which is on the border line of expected activity at α_1- and α_2-adrenoceptors (see discussion).

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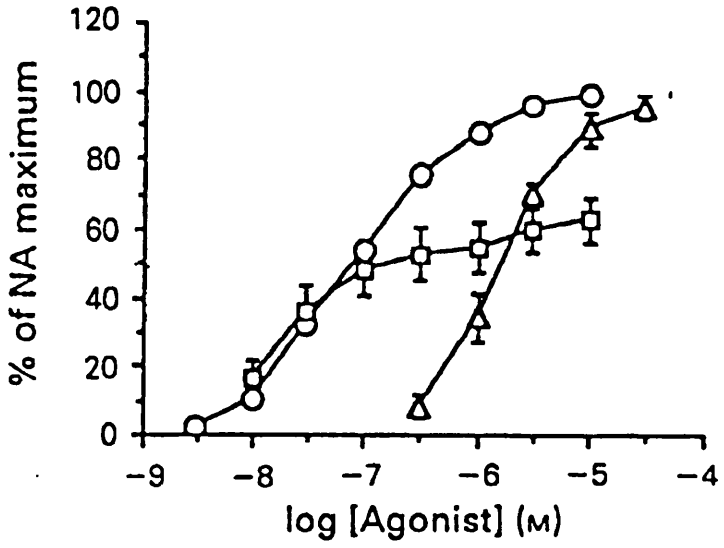

Figure 4. A comparison of the effects of various α-adrenoceptor agonists in the rabbit isolated plantaris vein. The effect of (-)-noradrenaline (NA) (○), phenylephrine (△) and UK-14304 (□). All responses are expressed as a percentage of the maximum response to (-)-NA and are the mean of 4-9 observations. The vertical lines indicate the s.e.m.

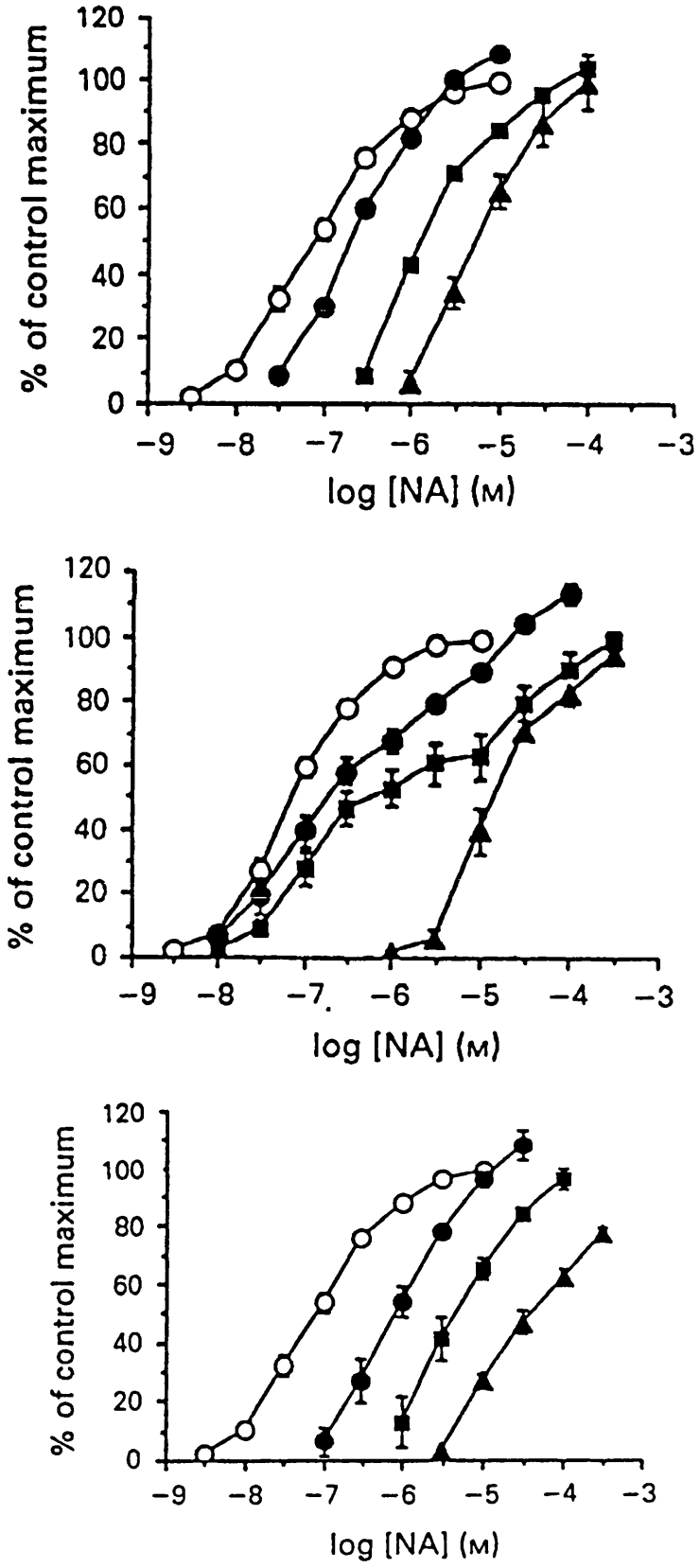

Figure 5. A comparison of the effects of various α-adrenoceptor antagonists, on contractions elicited by (-)-NA (○), in the rabbit isolated plantaris vein. (a) The effect of 0.5μM (●), 10μM (■) and 50 μM (▲) corynanthine. (b) The effect of 0.1μM (●), 1μM (■) prazosin and a combination of prazosin (0.1μM) and rauwolscine (2.5μM) (▲). (c) The effect of 0.5μM (●), 10μM (■) and 50μM (▲) rauwolscine. All responses are expressed as a percentage of the maximum response in the control concentration response curve and are the mean of 4-9 observations. The vertical lines indicate the s.e.m.

Prazosin (0.01-1μM) caused a non-parallel rightward displacement of the NA CRC (Figure 5b). The lower part of the curve was resistant to prazosin but sensitive to the further addition of rauwolscine (2.5μM) (Figure 5b). The non-parallel displacement by prazosin made Schild analysis impossible and so a pA_2 value could not be determined.

Rauwolscine (0.5-50μM) caused a parallel, dose dependent, rightward shift of the NA CRC (Figure 5c). The highest concentration of rauwolscine (50μM) caused a slight depression in the maximum response. The slope of the Schild plot was 0.8 (1.03-0.56) and the 95% confidence limits overlapped unity. The estimated pA_2 value was 7.56 (8.31-6.8) which, although rather high, is indicative of an action at α_2-adrenoceptors (see discussion).

The rank order of potency, based on the pA_2 values, was;

rauwolscine > corynanthine >> prazosin.

This indicates an effective population of α_2-adrenoceptors.

α-adrenoceptor subtypes involved in the response to electrical field stimulation in the isolated saphenous vein.

Electrical field stimulation (EFS) at 4, 8, 16, 32 & 64Hz for 1 second duration produced monophasic responses which returned quickly to baseline (Figure 6). The response to 4Hz for 10 seconds continued to rise for the duration of the stimulus and started to decay immediately at the end of the train. The maximum response was generally obtained at either 32 or 64Hz and was found to be approximately 38% of the maximum response to NA. Much greater stimuli would have been required to discover the parameters needed to achieve 100% of the NA maximum response. Such experiment were attempted unsuccessfully due to equipment failure (stimulus overload) at voltages greater than 50v.

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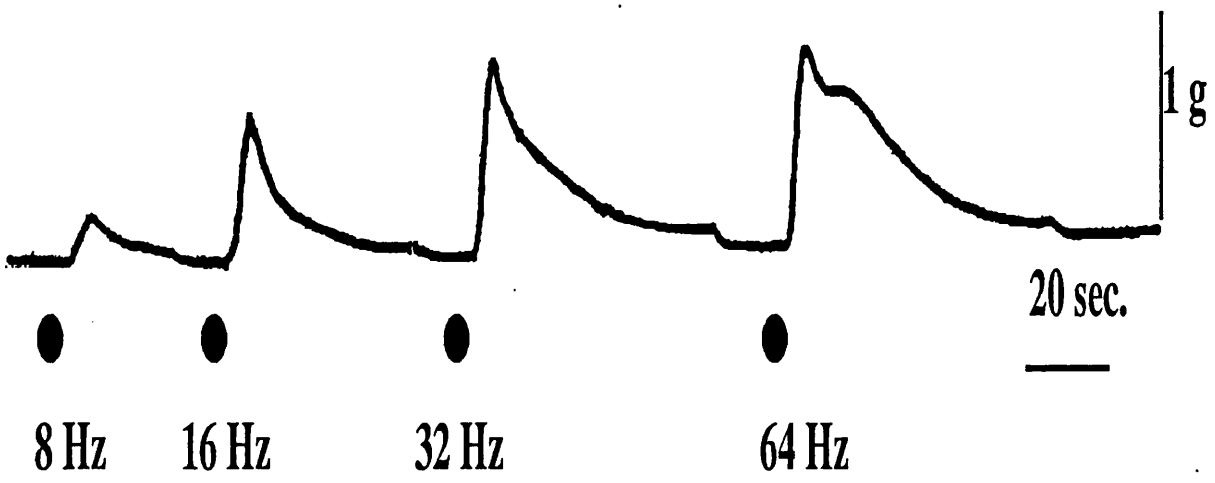

Figure 6. Representative tracing of the effect of electrical field stimulation on the rabbit isolated saphenous vein. Responses were frequency dependent and the parameters of stimulation were; 0.1ms pulse width, 1 second duration, 35 volts. Trains of pulses were delivered at 5 minute intervals

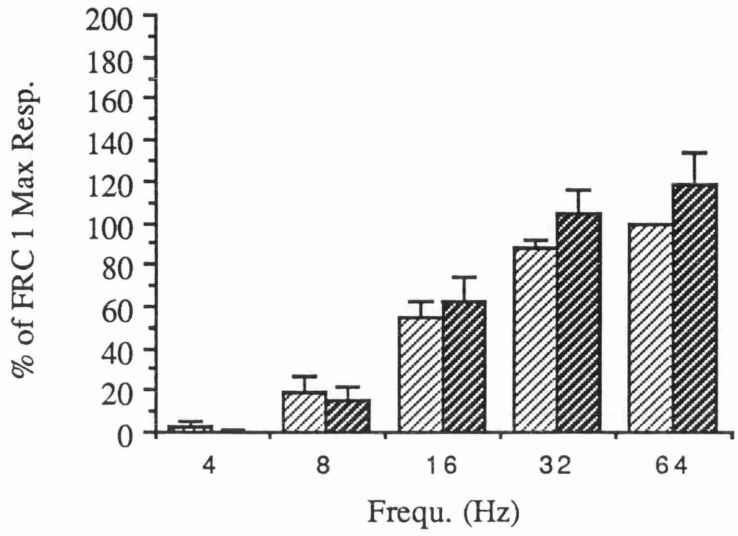

Figure 7. The effect of time on responses to EFS in the isolated saphenous vein. The light columns represent the control responses. The dark columns represent responses to a second FRC. The interval between FRC 1 and FRC 2 was 30 minutes Values represent the mean ± s.e.m. (n=5).

The responses tended to increase with time. However this was not statistically significant and therefore the results of the antagonist studies were not corrected to account for this (Figure 7).

Tetrodotoxin (0.1μM) completely abolished the motor response at frequencies up to and including 64Hz. Taken together with the fact that the pulse width was maintained at 0.1ms, this confirms that responses at these frequencies are entirely neurogenic. Under certain conditions blockade of α-adrenoceptors can almost abolish the nerve induced response, which further supports the view that there is little, if any, direct muscle stimulation.

Rauwolscine (2.5μM) tended to enhance the responses at 4Hz and 8Hz but inhibited the responses at higher frequencies (Figure 8a). These effects were definite in individual experiments but not statistically significant and may reflect the countervailing effects of pre- and postjunctional blockade. Prejunctional α_2-adrenoceptors are known to be present in the rabbit saphenous vein (Levitt & Hieble, 1986).

Prazosin (0.1μM) inhibited the responses at all frequencies tested. Inhibition of the response at 4 Hz & 8Hz was not statistically significant (Figure 8b).

The combination of prazosin and rauwolscine produced a greater inhibition, than prazosin alone, in only the low frequency / long duration response (4Hz / 10 seconds). The response to 1 second trains of stimuli were inhibited significantly only at 32Hz (Figure 8c).

Since prazosin produced inhibition but rauwolscine did not, there is no evidence that the population of postjunctional α_2-adrenoceptors is involved in the response to short bursts of noradrenergic stimuli. The response remaining after prazosin and rauwolscine together may suggest the presence of a co-transmitter, possibly ATP.

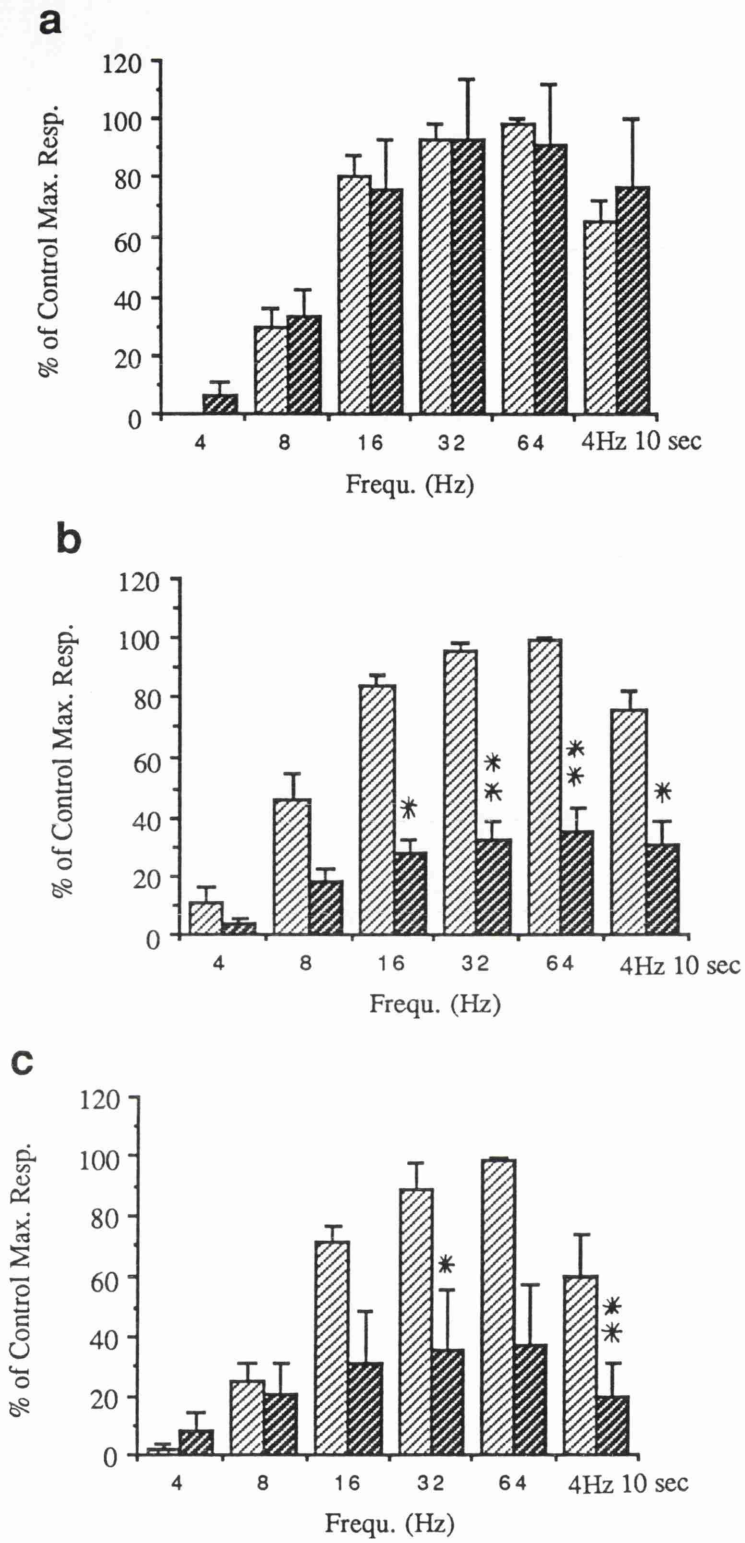

Figure 8. The effects of α-adrenoceptor inhibition on responses to EFS in the isolated saphenous vein. The light columns indicate the control responses, the dark columns indicate responses in the presence of the antagonist. a) effect of rauwolscine (1μM). b) effect of prazosin (0.1μM). c) effect of prazosin (0.1μM) plus rauwolscine (1μM). Responses are expressed as the mean ± s.e.m. (n=4), $p<0.01$ (**), $p<0.05$ (*).

Effects of cocaine, propranolol & corticosterone in the isolated saphenous vein.

The agonist studies which identified the population of postjunctional α_2-adrenoceptors on the isolated rabbit saphenous vein relied on the presence of cocaine and propranolol in the bathing solution (PSS). The effects of cocaine, propranolol and corticosterone on responses to EFS were therefore examined. In these experiments corticosterone was used in place of the less selective compound, normetanephrine (see discussion) The effects of the adrenoceptor antagonists were also studied in the presence of cocaine and propranolol.

Corticosterone (30μM) and propranolol (1μM) did not significantly alter the responses to EFS (Figure 9b, 9c).

In contrast, cocaine (10μM) could increase the overall height of the response at any frequency. Over 4 experiments, however, the increase was significant at 8Hz only (Figure 9a). Cocaine (10μM) also increased the duration of the responses, particularly at frequencies above 8Hz (Figure 10). The increase in response duration was due to a secondary phase which appeared as a shoulder on the response at 16, 32 & 64Hz. The height of the shoulder varied and in some instances exceeded the height of the initial 'fast' component. This produced a great variability in the overall height of the responses and accounts for the low level of statistical significance of the cocaine potentiation. The biphasic response caused by the presence of cocaine is examined in detail in a later section.

Cocaine always produced a potentiation of responses as measured by their peak height in individual tissues (Figure 11a). However, a comparison of different tissues in the presence and absence of cocaine (Figure 11b) demonstrates that it is possible for a tissue with an intact uptake$_1$ mechanism to generate greater peak tension, in response to EFS, than a tissue where uptake$_1$ is blocked. Therefore, uptake$_1$ blockade does not result in extraordinarily large responses.

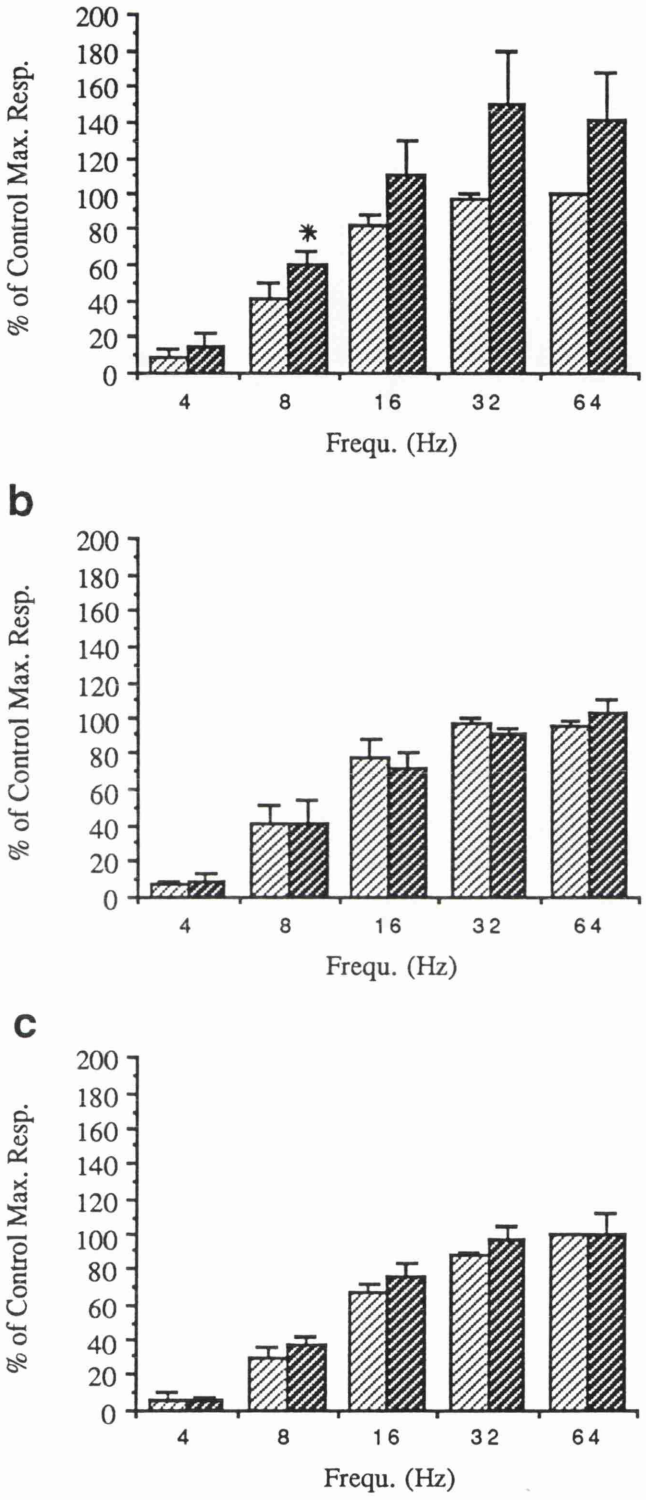

Figure 9. The effects of a) cocaine (10μM); b) corticosterone (30μM) and c) propranolol (1μM) in the isolated saphenous vein. Light columns represent control values, dark columns represent values in the presence of the drug. Values are given as mean ± s.e.m. (n=4).

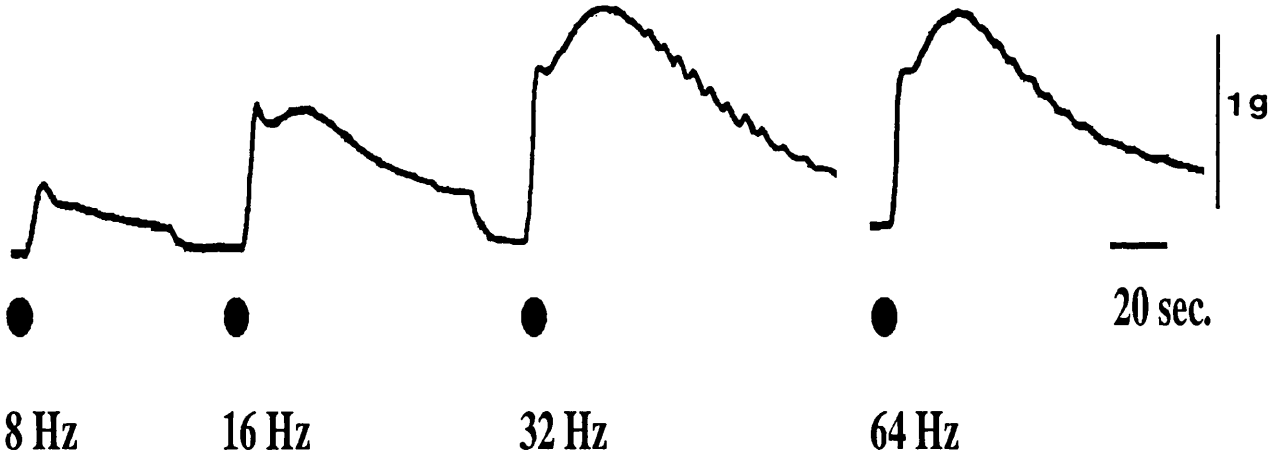

Figure 10. Representative tracing of the effect of electrical field stimulation on the rabbit isolated saphenous vein, in the presence of cocaine (10μM). Responses were frequency dependent and the parameters of stimulation were; 0.1ms pulse width, 1 second duration, 35 volts. Trains of pulses were delivered at 5 minute intervals.

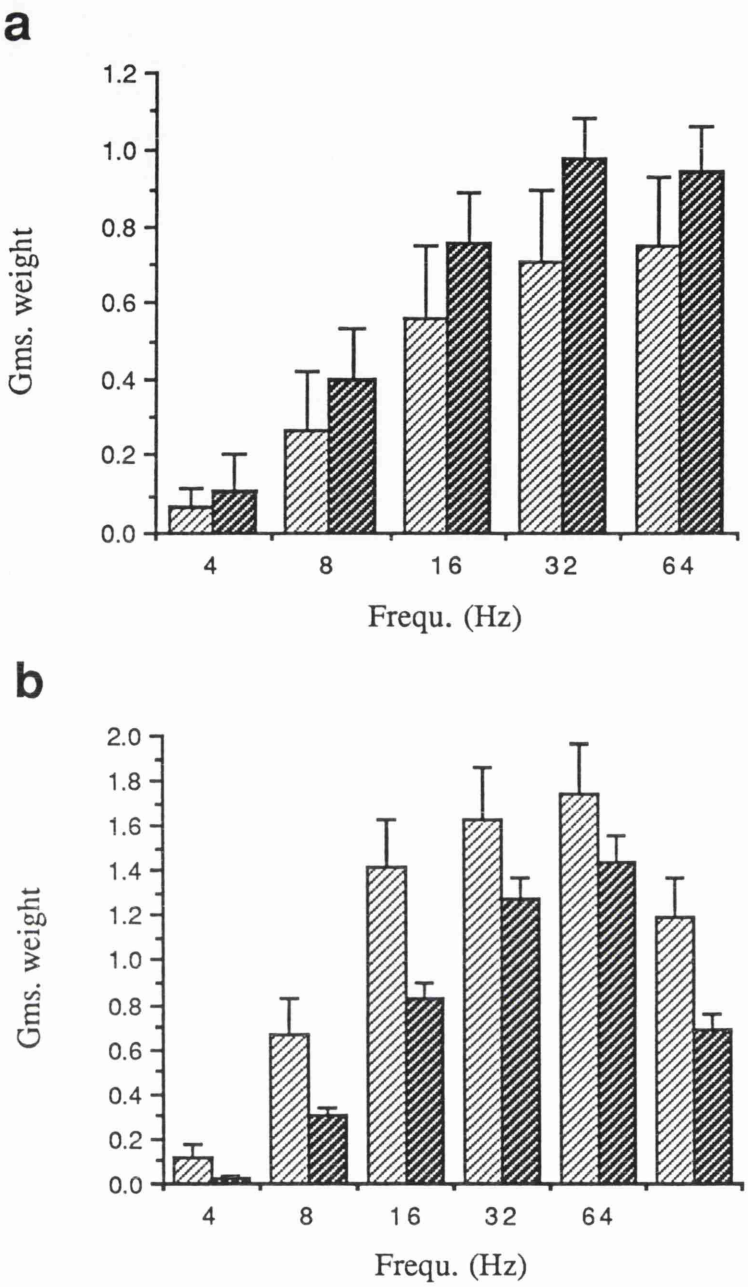

Figure 11. The effect of cocaine (10μM) on responses to electrical field stimulation in the isolated saphenous vein. The data in a) represents the effect of cocaine on the same tissue while the data in b) represents a comparison of different tissues with and without cocaine. Responses are expressed in grammes. Light columns represent control values (cocaine absent), dark columns represent values obtained in the presence of cocaine. Columns and bars indicate mean values ± s.e.m. for a) n=4 and b) controls n=12, cocaine treated n=16.

Functional antagonism was therefore assessed using data expressed as a percentage of maximum responsiveness rather than size of response, which is too variable.

Effects of α-adrenoceptor antagonists on response to E.F.S. in the presence of cocaine and propranolol in the isolated saphenous vein.

In the presence of cocaine and propranolol, responses to E.F.S. were not significantly altered with time (Figure 12).

Rauwolscine (1μM) failed to inhibit the response at any frequency and, in fact, enhanced the response at all except the 64Hz frequencies (Figure 13a). The response of 4Hz / 10 secs was significantly potentiated suggesting an increase in prejunctional α_2-adrenoceptor activity in the presence of uptake$_1$ blockade.

Prazosin (0.1μM) produced a greater inhibition of responses in the presence of cocaine and propranolol (Figure 13b) than in their absence (Figure 8b). The inhibition was significant at all frequencies above 4Hz / 1 sec. A comparison (ANOVA) between the reductions in both the presence and absence of cocaine and propranolol however failed to show statistical significance.

The combination of prazosin and rauwolscine produced an even greater inhibition in the presence of cocaine and propranolol (Figure 13c) than in their absence (Figure 8c). The responses at 4Hz and 8Hz were abolished while those above 8 Hz were markedly reduced. A comparison of the reductions in the presence and absence of cocaine and propranolol was found to be statistically significant at 8Hz & 16Hz (ANOVA; $p<0.05$)

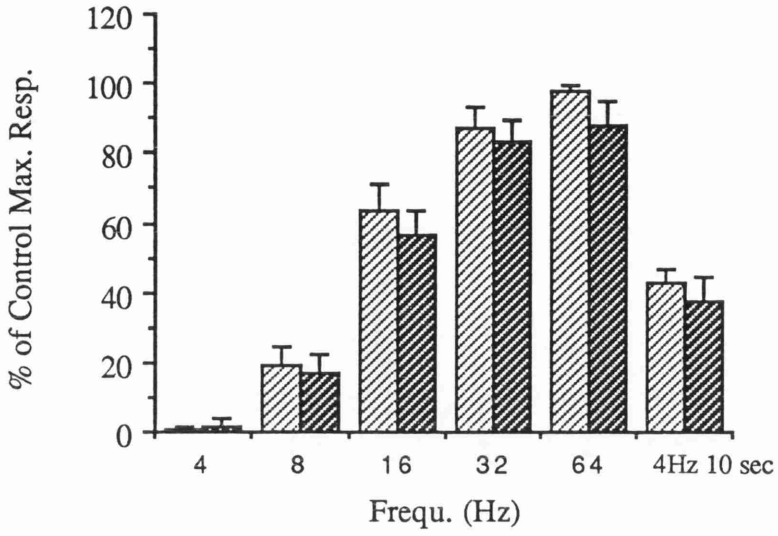

Figure 12. The effect of time on responses to electrical field stimulation in the presence of cocaine in the isolated saphenous vein. Light columns represent the control FRC, dark bars represent the *second* control FRC. Time between FRC 1 & 2 was 30 minutes Values are given as mean ± s.e.m (n=4).

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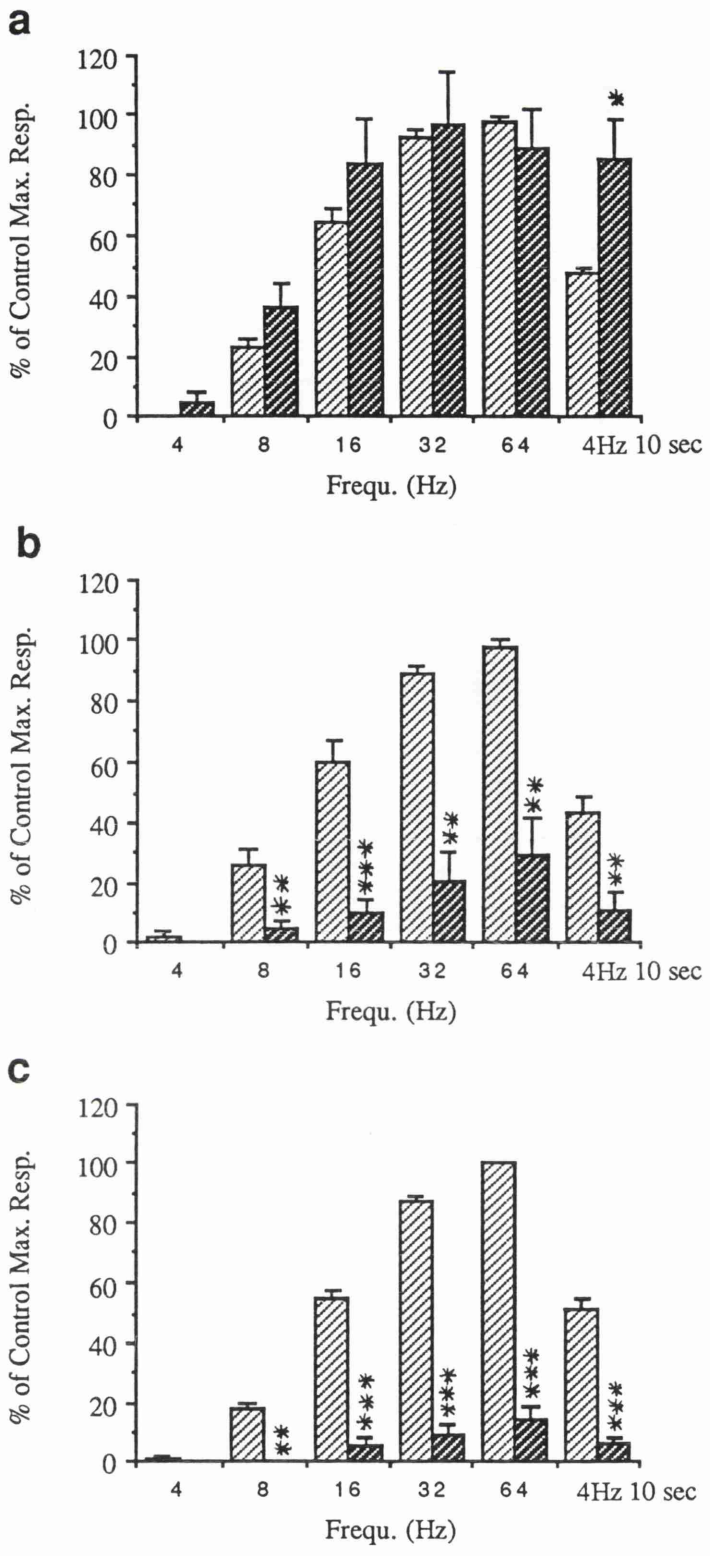

Figure 13. The effects of α-adrenoceptor inhibition on responses to EFS in the isolated saphenous vein and in the presence of cocaine (10μM). The light columns indicate the control responses, the dark columns indicate responses in the presence of the antagonist. a) effect of rauwolscine (1μM). b) effect of prazosin (0.1μM). c) effect of prazosin (0.1μM) and rauwolscine (1μM). Responses are expressed as the mean ± s.e.m. (n=7), $p<0.001$ (***), $p<0.01$ (**), $p<0.05$ (*).

α-adrenoceptor sub-types involved in the response to E.F.S. in the rabbit isolated plantaris vein.

Electrical field stimulation (4-64 Hz) for 1 second's duration caused twitches which were monophasic and returned to baseline within 1 minute. The response was maximal at between 32 and 64 Hz and generated approximately 1g of tension which was approximately 26% of the maximum response to NA (Figure 14).

At all frequencies, responses tended to increase with time. However this increase was not significant (Figure 15) at any, bar the 4Hz/1sec, response.

Rauwolscine (1μM) failed to affect the response significantly at any frequency (Figure 16a).

Prazosin (0.1μM) significantly inhibited the responses at 16, 32 and 64 Hz (Figure 16b).

The combination of prazosin and rauwolscine caused a significant inhibition at only 32 and 64 Hz (Figure 16c).

Effect of cocaine on responses to E.F.S. in the isolated plantaris vein.

The inclusion of cocaine (10μM), caused the appearance of a 'shoulder' or secondary phase which tended to prolong the time course of the response (Figure 17). The relative size of this secondary component compared with the first phase was generally less than in the saphenous vein.

In the presence of cocaine (10μM) the responses over time were slightly depressed. This was only significant however, at 64 Hz and at 4Hz for 10 seconds (figure 18). This may be the result of activation of prejunctional α_2-adrenoceptors by the increased concentration of junctional NA.

Addition of cocaine always increased the size of the response. Comparison of unpaired experiments showed that tissues in the presence of cocaine did not always exhibit greater responses than different tissues in the absence of cocaine. For this reason responses have been expressed as a percentage of the maximum response, as they were for the saphenous vein experiments.

Effects of α-adrenoceptor antagonism after uptake$_1$ blockade in the isolated plantaris vein.

In the presence of cocaine, rauwolscine (1μM) produced a powerful inhibition of responses at all of the frequencies tested (Figure 19a). A comparison of the reductions in the presence and absence of cocaine was found to be statistically significant (ANOVA; $p<0.05$) (Compare Figure 16a & 19a)

Prazosin also caused a powerful inhibition of the responses at all frequencies (Figure 19b).

The combination of rauwolscine and prazosin almost completely abolished the response to nerve stimulation.

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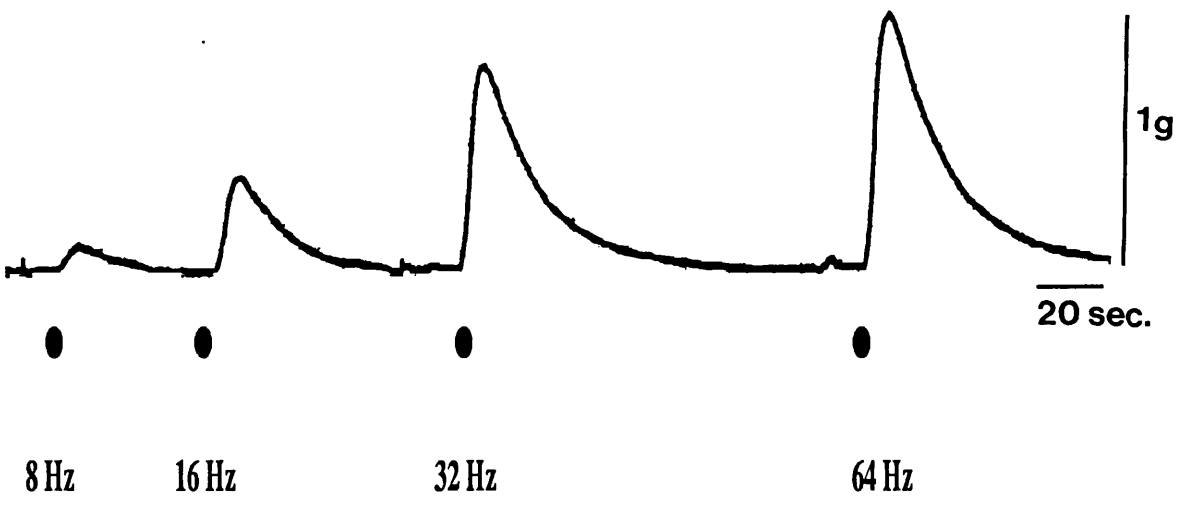

Figure 14. Representive tracing of the effect of electrical field stimulation on the rabbit isolated plantaris vein. Responses were frequency dependent. The parameters of stimulation were; 0.1ms pulse width, 1 second train duration, 35 volts. Trains of pulses were delivered at 5 minute intervals.

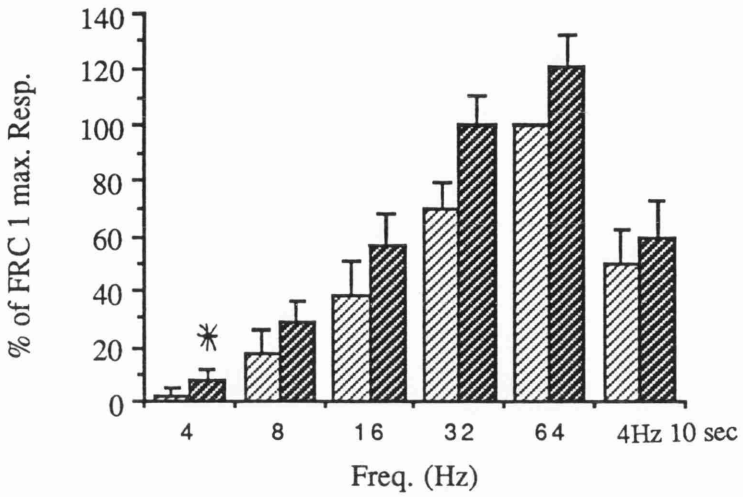

Figure 15. The effect of time on responses to electrical field stimulation in the rabbit isolated plantaris vein. The light columns represent the control responses (FRC 1). The dark columns represent the *second* responses (FRC 2). The interval between FRC1 and FRC 2 was 30 minutes. Values are given as mean ± s.e.m. (n=4), $p<0.1$ (*).

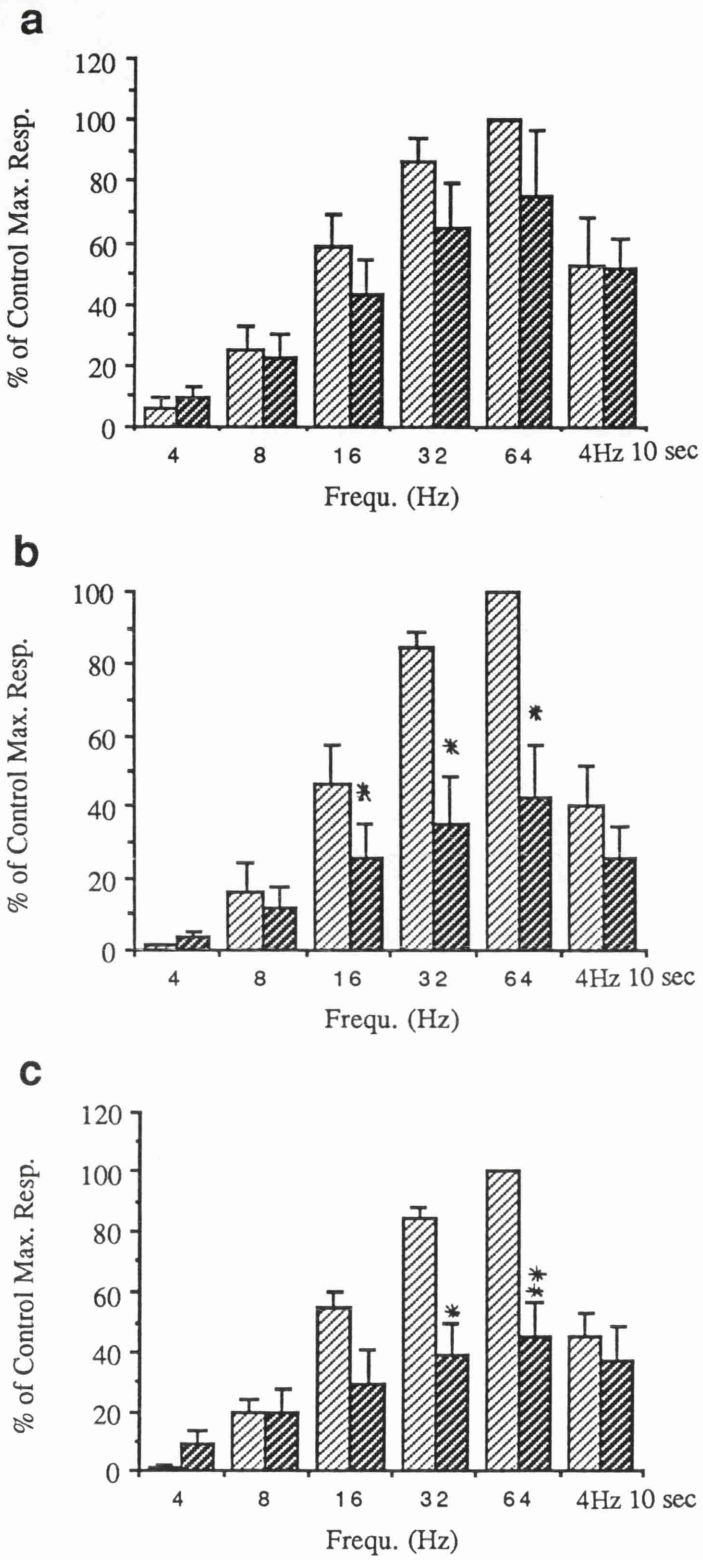

Figure 16. The effect of α-adrenoceptor blockade on responses to EFS in the isolated plantaris vein. The light columns represent the control responses, the dark columns represent responses in the presence of the antagonist. a) effect of rauwolscine (1μM). b) effect of prazosin (0.1μM). c) effect of prazosin (0.1μM) and rauwolscine (1μM). Responses are expressed as mean ± s.e.m. (n=4), $p<0.1$ (*), $p<0.01$ (**), $p<0.001$ (***).

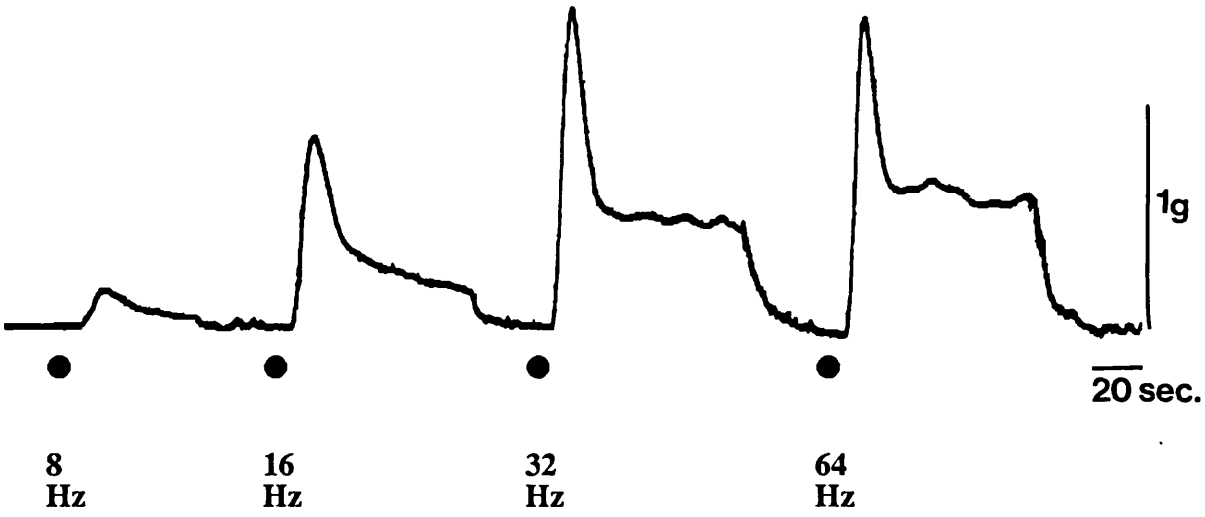

Figure 17. Representative tracing of the effect of electrical field stimulation on the rabbit isolated plantaris vein, in the presence of cocaine (10μM). Responses were frequency dependent and the parameters of stimulation were; 0.1ms pulse width, 1 second train duration, 35 volts. The interval between trains of pulses was 5 minutes.

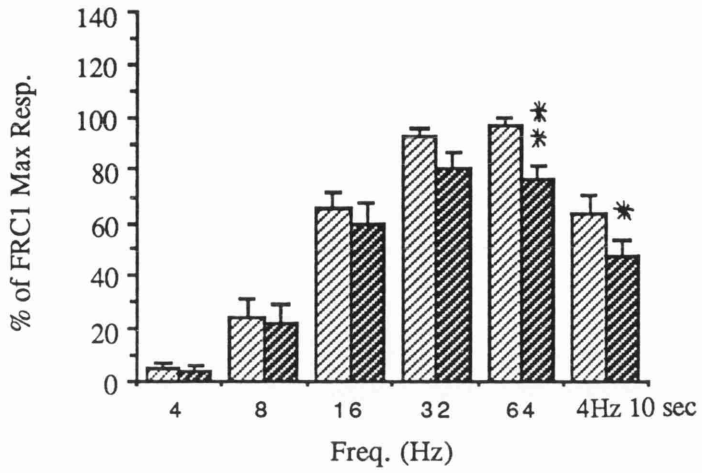

Figure 18. The effect of time on responses to EFS in the isolated plantaris vein, in the presence of cocaine (10μM). The light columns represent the control responses (FRC 1). The dark columns represent the *second* responses (FRC 2). Interval between FRC 1 & 2 was 30 minutes. Values are given as mean ± s.e.m. (n=6), $p<0.1$ (*), $p<0.01$ (**).

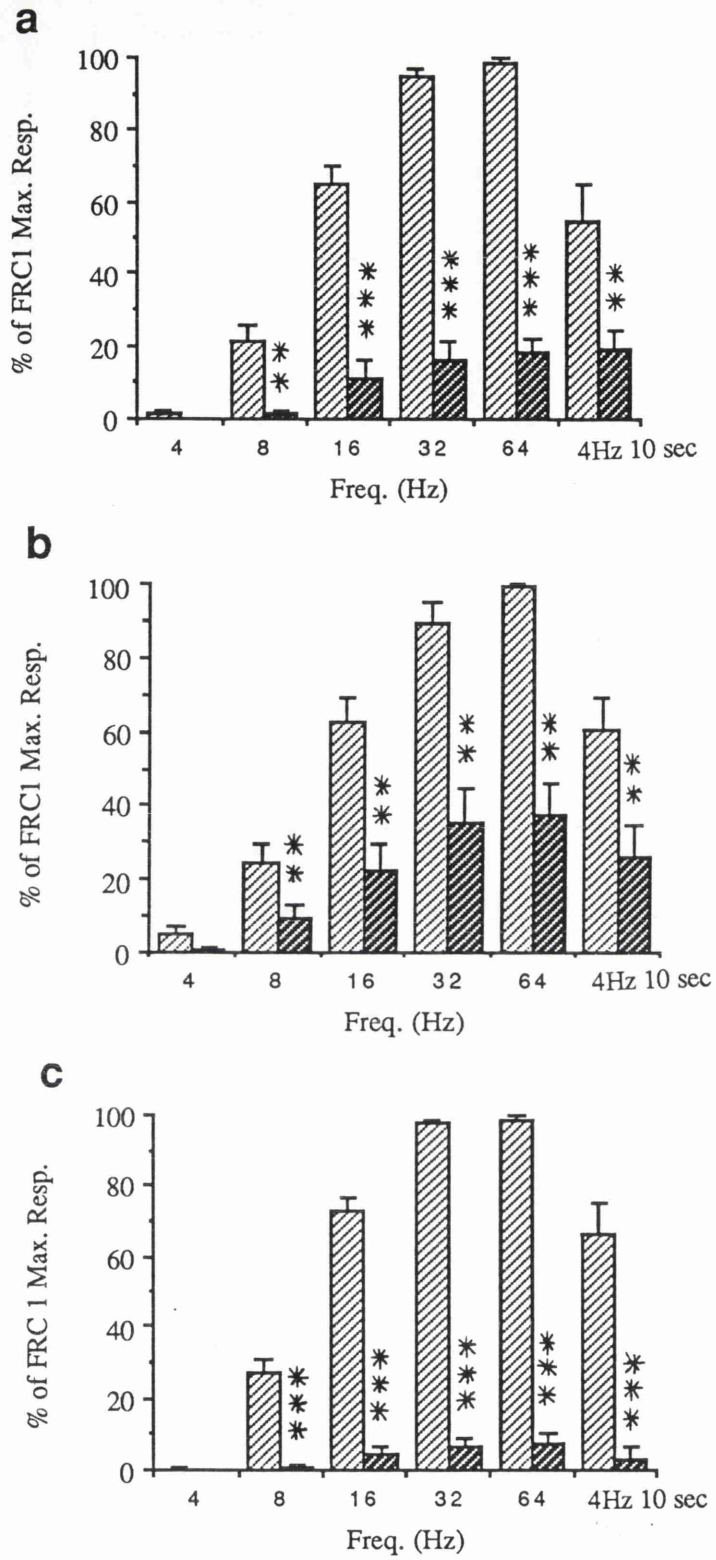

Figure 19. The effect of α-adrenoceptor blockade on responses to EFS in the isolated plantaris vein. The light columns represent the control responses, the dark columns represent responses in the presence of the antagonist. a) effect of rauwolscine (1μM). b) effect of prazosin (0.1μM). c) effect of prazosin (0.1μM) plus rauwolscine (1μM). Responses are expressed as mean ± s.e.m. (n=4), $p<0.1$ (*), $p<0.01$ (**), $p<0.001$ (***).

The Effect of α-β-mATP on Responses to Electrical Field Stimulation in the Isolated Plantaris Vein.

The previous experiments showed that α-blockade was most effective in the presence of cocaine. The following experiments were designed to establish the degree of co-transmission in the plantaris vein.

α-β-mATP (3μM) caused a transient response which was 80.84±9.59% of the response to 3μM NA. Subsequent additions of α-β-mATP caused no further response and so the P_{2x}-purinoceptors were assumed to be desensitised.

During exposure to α-β-mATP (3μM) the frequency response curve was slightly potentiated (Figure 20). However since the time controls (in the absence of cocaine) were also slightly potentiated this result is probably not significant. It should be noted that α-β-mATP alone had no inhibitory effects at any of the frequencies tested.

In paired experiments prazosin (0.1μM) and rauwolscine (1μM), in combination, caused a marked inhibition of the neuroeffector response (Figure 21). The inhibition was altered to the same degree by cocaine as was found in the unpaired experiments of the previous section.

In the presence of α-blockade addition of α-β-mATP caused a further, significant, inhibition of the response to E.F.S. In the presence of cocaine, α-β-mATP was unable to abolish the non-adrenergic component of the response. In the absence of cocaine α-β-mATP was slightly more effective and was able to totally abolish the non-adrenergic response at 4Hz and 8Hz.

These results indicate the involvement of ATP as a co-transmitter with NA in the rabbit plantaris vein.

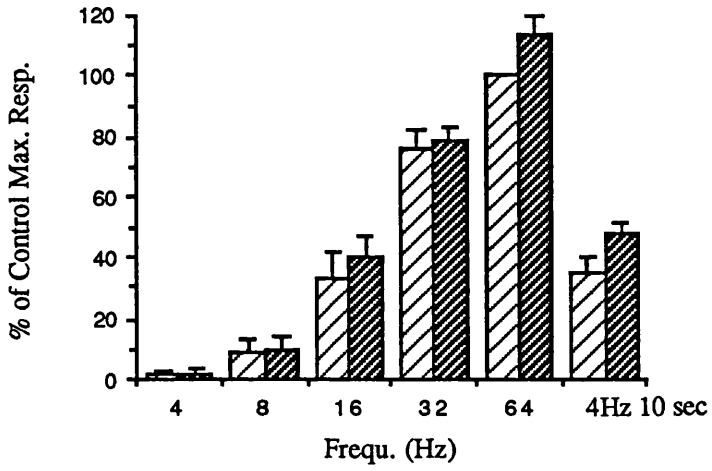

Figure 20. The effect of α-β-methylene ATP (3μM) on responses to EFS in the isolated plantaris vein. Light columns represent control responses, dark columns represent responses in the presence of α-β-methylene ATP. Values are given as mean ± s.e.m. (n=4).

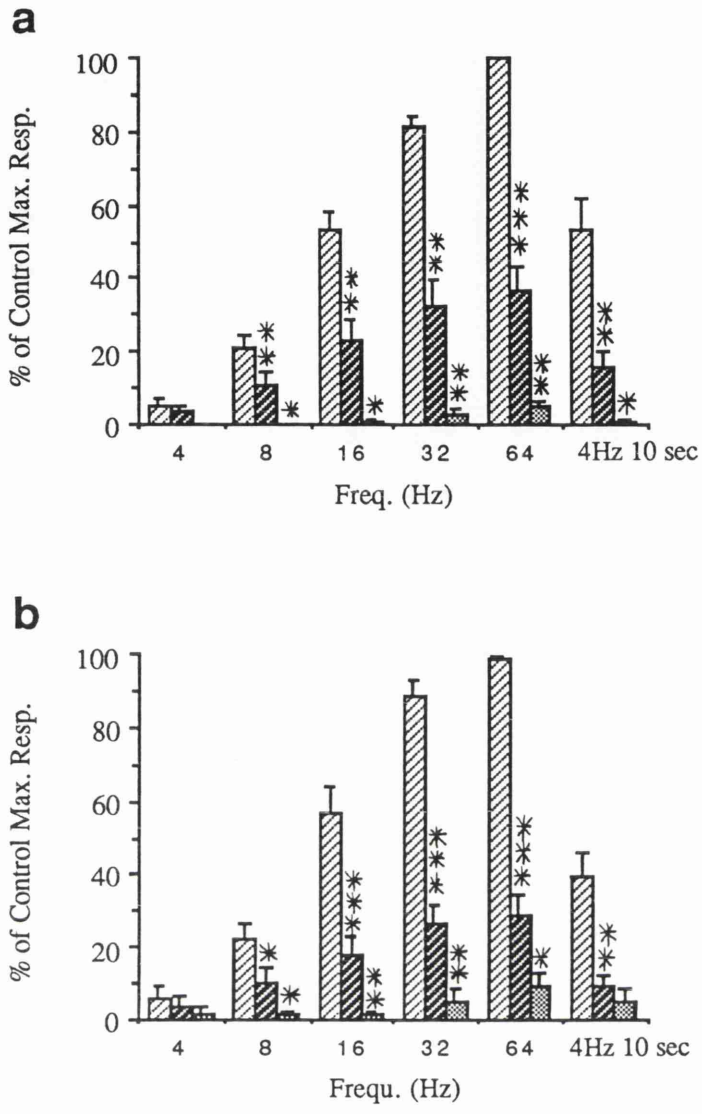

Figure 21. The effect of prazosin (0.1μM) and rauwolscine (1μM) together (dark hatched columns) and in combination with α-β-methylene ATP (3μM, dark stippled columns) on responses to EFS (control, light hatched columns) in the isolated plantaris vein. a) in the absence of cocaine. b) in the presence of cocaine (10μM). Values are given as mean ± s.e.m. (n=8). $p<0.1$ (*), $p<0.05$ (**), $p<0.001$ (***).

Investigation of the Bi-Phasic Responses Obtained After Uptake$_1$ Blockade in the Rabbit Isolated Saphenous Vein.

As mentioned earlier, addition of cocaine, to block neuronal uptake and increase the concentration of junctional NA, increases the duration of the neuro-effector response in saphenous vein. The increased duration is due to the emergence of a secondary 'slow' phase of the response to E.F.S. above 8Hz (Figure 22a). The initial 'fast' component peaks at around 6 seconds while the secondary 'slow' phase peaks at around 18 seconds (Figure 22a). The following experiments were designed to identify the population of post-junctional adrenoceptors responsible for this uncovered component.

Figure 22a shows the effect of cocaine uncovering a slow secondary component to 32Hz stimulation. Rauwolscine (1μM) enhanced the first phase and inhibited the secondary phase (Figure 22b, Figure 25c). Prazosin (0.1μM) on the other hand, caused a non-selective inhibition of both phases (Figure 22c, Figure 25b). The effect of rauwolscine raised the possibility that the secondary component was mediated by α_2-adrenoceptors. Experiments were thus performed using the structurally dissimilar α_2-adrenoceptor antagonist CH-38083 (Vizi *et. al.*,1986).

Figure 23a shows the response to 16, 32 and 64 Hz in the absence of cocaine. After uptake$_1$ blockade a secondary phase emerged (Figure 23b). The secondary phase was completely blocked by CH-38083 (Figure 23c, Figure 25b).

The nature of the response to 16, 32 and 64Hz in the presence of cocaine makes it necessary to examine closely the effects of selective antagonists on the individual phases. Control phases were measured at 6 and 18 seconds for each frequency and these were considered to be 100% of the first and second phases respectively. Responses in the presence of the antagonists were expressed as a percentage of these controls.

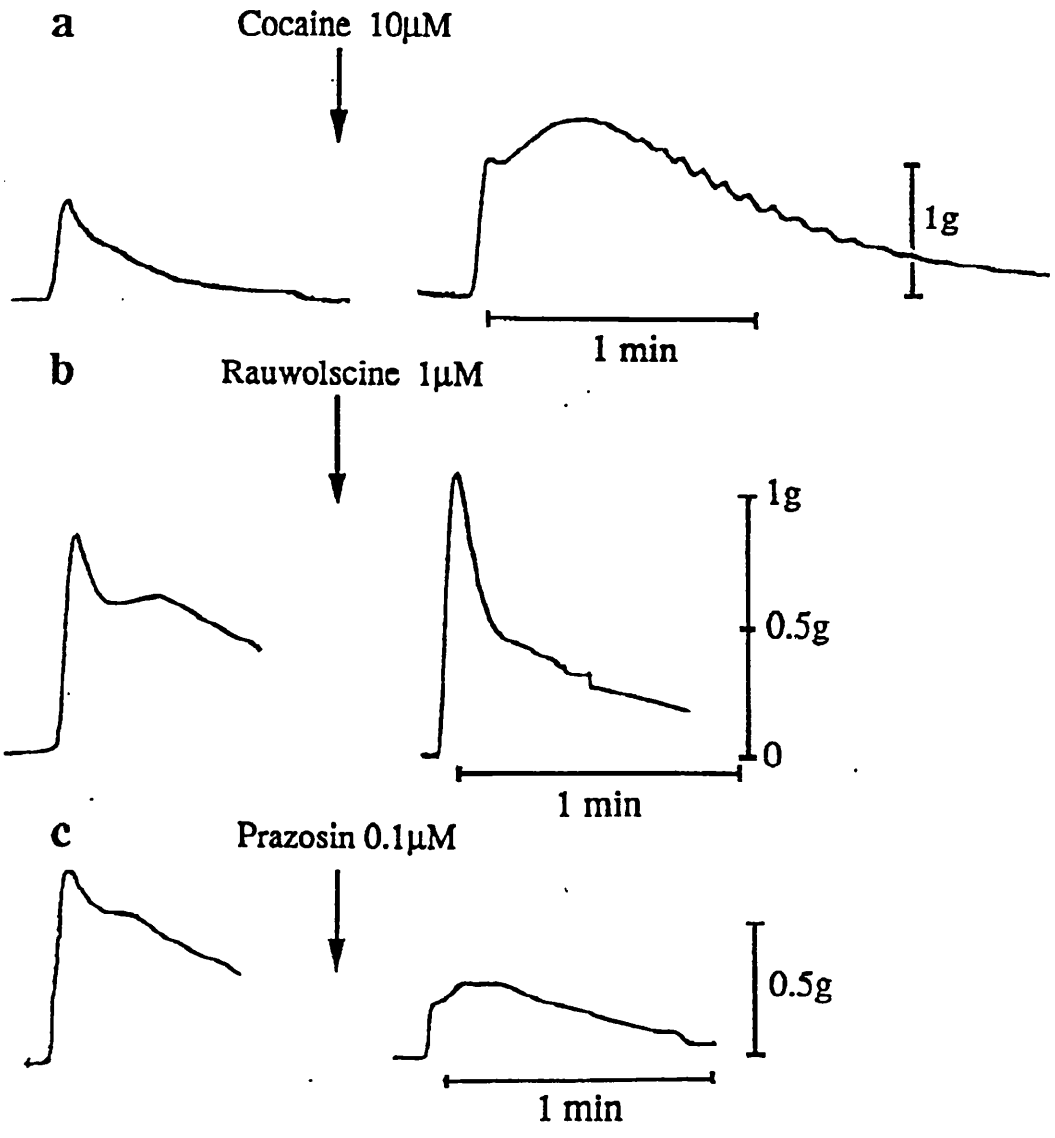

Figure 22. Representative tracings showing the effect of a) cocaine on the response to 32 Hz stimulation in the isolated saphenous vein; b) rauwolscine on the biphasic response obtained in the presence of cocaine to 32 Hz stimulation and c) prazosin on the biphasic response obtained in the presence of cocaine to 32 Hz stimulation. Parameters used were 0.1ms pulse width, 1 second train duration, 35 volts.

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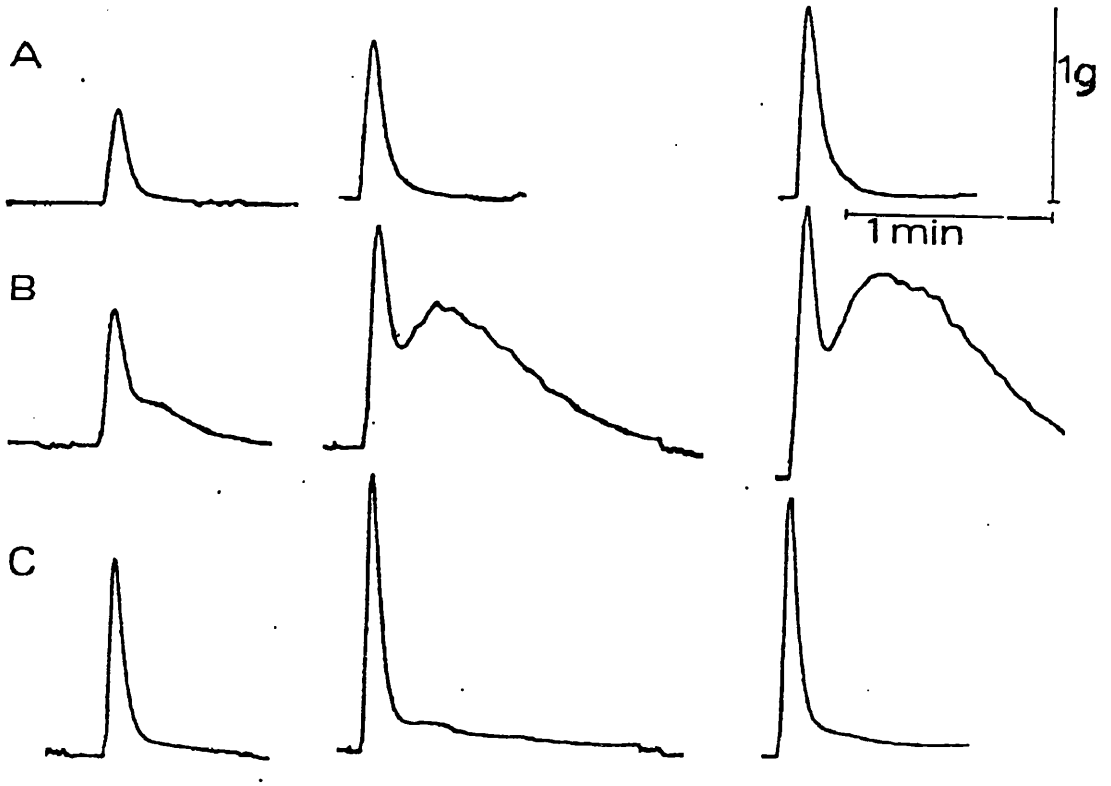

Figure 23. Representative tracings showing the effect of 16, 32 & 64 Hz stimulation (from left to right) in the same preparation of isolated saphenous vein. Top panel (A) shows control responses. Middle panel (B), after addition of cocaine (10μM) to uncover the secondary phase. Bottom panel (C) , in the presence of cocaine, after addition of the α_2-adrenocepor antagonist CH 38083 (1μM)

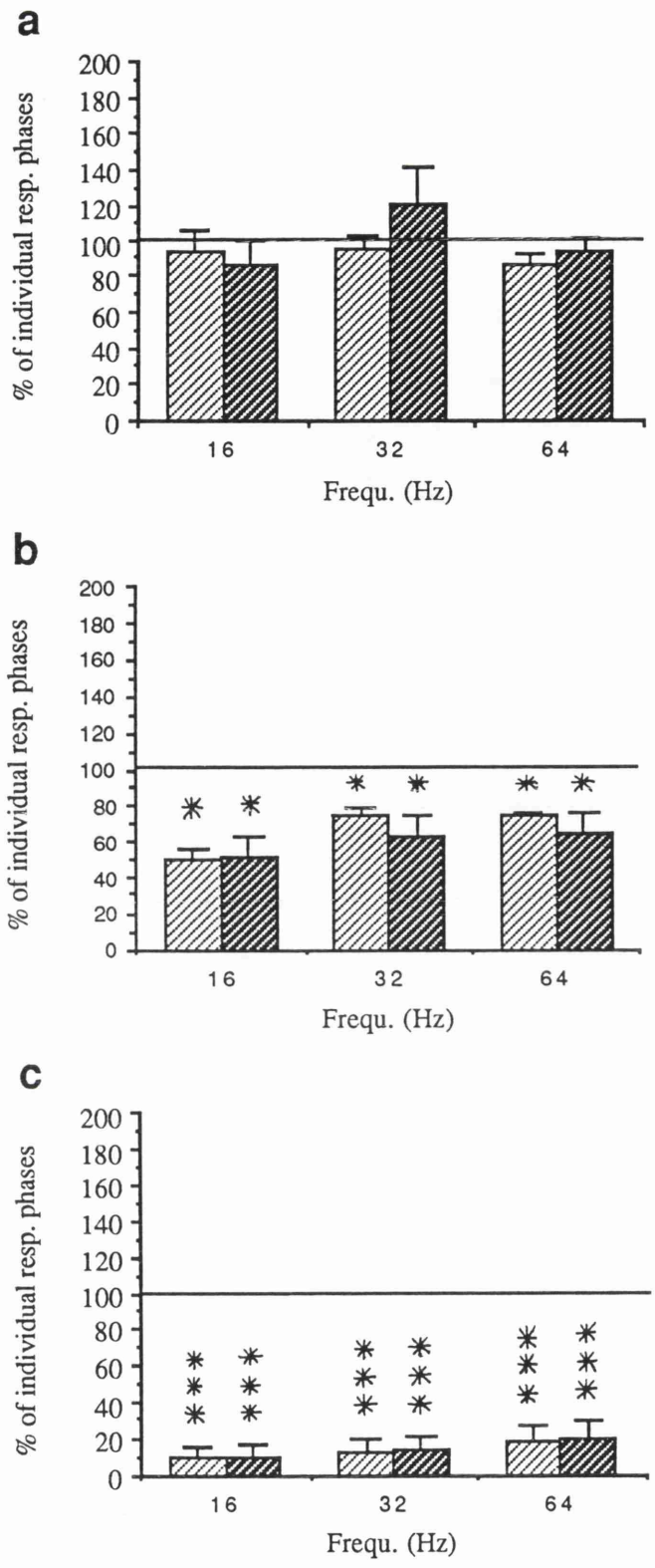

Figure 24. The effect of time and α_1-adrenoceptor blockade on the biphasic responses obtained in the isolaed saphenous vein, in the presence of cocaine (10μM). Light columns represent the first phase of contraction (measured at 6 seconds) the dark columns represent the second phase of contraction (measured at 18 seconds). Responses are expressed as a percentage of the control phases after a) time, b) corynanthine (1μM) and c) prazosin (0.1μM). Values are given as mean ± s.e.m. (a, n=4; b, n=3; c, n=6). For statistical analysis, comparisons (of raw data) were made of individual phases using a paired t-test; $p<0.1$ (*), $p<0.01$ (**), $p<0.001$ (***).

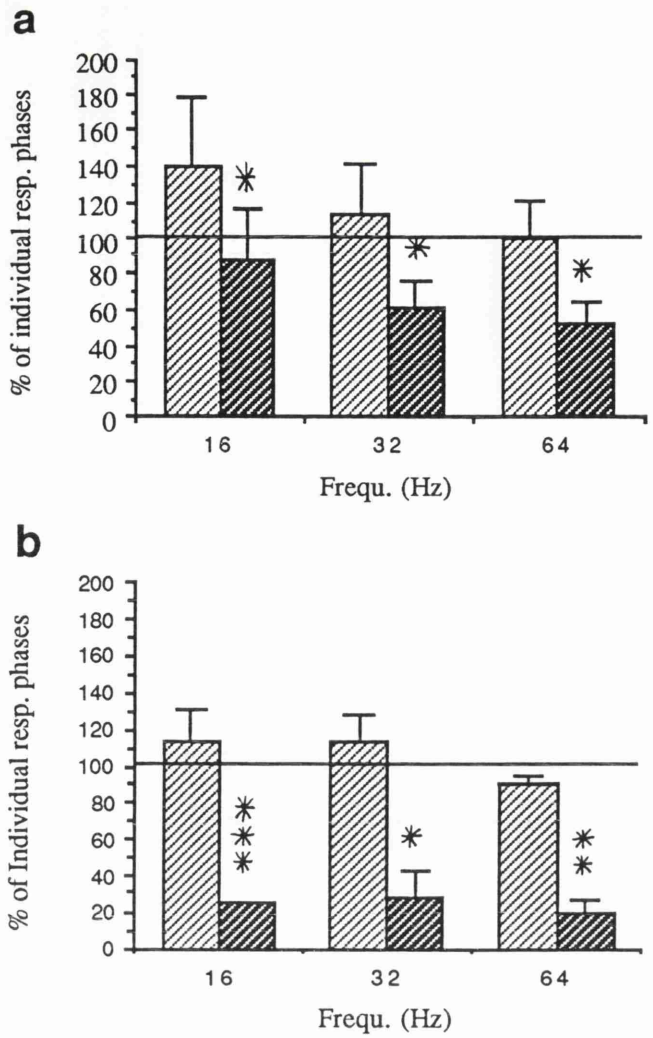

Figure 25. The effect of α_2-adrenoceptor blockade on the biphasic responses obtained in the isolated saphenous vein, in the presence of cocaine (10μM). Light columns represent the first phase of contraction (measured at 6 seconds) the dark columns represent the second phase of contraction (measured at 18 seconds). Responses are expressed as a percentage of the control phases after a) rauwolscine (1μM), b) CH 38083 (1μM). Values are given as mean ± s.e.m. (a, n=6; b, n=4). For statistical analysis comparisons (of raw data) were made of individual phases using a paired t-test; $p<0.1$ (*), $p<0.01$ (**), $p<0.001$ (***)

Bi-phasic responses were highly reproducible and were not significantly altered with time (Figure 24a).

The relatively weak α_1-adrenoceptor antagonist, corynanthine (1μM), inhibited both phases of the response equally at all frequencies (Figure 24b).

The more potent α_1-adrenoceptor antagonist, prazosin (0.1μM) effected a powerful inhibition of the whole response at all frequencies (Figure 24c).

Rauwolscine (1μM) potentiated the first phase of responses at 16 and 32 Hz which, although not significant, is probably due to its pre-junctional α_2- activity. Rauwolscine significantly inhibited the secondary phase of the response at all frequencies (Figure 25a).

CH-38083 had a very slight, non-significant, effect on the initial phase of the response but had a marked effect on the secondary phase of the response at all frequencies (Figure 25b).

These results suggest that, in the isolated saphenous vein, the increased concentration of NA in the synaptic cleft can stimulate a population of postjunctional α_2-adrenoceptors whose exact location remains unclear (see discussion).

Factors which influence the α-adrenoceptor contribution to the neuroeffector response.

The previous section presented data which showed that if the neuronal uptake mechanism is blocked, the increased amount of transmitter can recruit α_2-adrenoceptors. It is possible therefore, that other physiological factors may influence the participation of α_2-adrenoceptors in the response to nerve stimulation.

The following section presents data from experiments which were designed to assess the effects of oxygen (O_2), Angiotensin II (AII) and the endothelium on the participation of postjunctional α_2-adrenoceptors.

The effect of O_2 on the adrenoceptor contribution to neuroeffector transmission in the isolated saphenous vein.

In the absence of cocaine, the response to 32Hz stimulation is transient and has a slight shoulder (Figure 26 L.H. responses). When the oxygenation was decreased to 16% the response developed a secondary response which was similar to that obtained in the presence of cocaine (Figure 26 centre responses). Increasing the oxygenation back to 95% eliminated the secondary phase of the responses (Figure 26 R.H. responses).

After uptake$_1$ blockade, with cocaine (10μM), and in PSS gassed with 95% O_2 / 5% CO_2, prazosin caused a significant inhibition of the biphasic responses to 16, 32 & 64 Hz (Figure 24c, 27a). When the PSS was subsequently gassed with 16% O_2 / 5% CO_2 the responses, in the presence of prazosin, were largely restored (Figure 27a). In the reverse experiment, where the tissues were treated with prazosin (0.1μM) in 16% O_2 / 5% CO_2 the observed α_1-adrenoceptor blockade was reduced (Figure 27b). The prazosin resistant responses were unaffected by changing the gassing to 95% O_2 / 5% CO_2 (Figure 27b). By comparing the effect of prazosin in the high O_2 environment with the effect in the low O_2 environment, it was found that prazosin was more effective in the high O_2 environment (Figure 27a&b, light columns).

The effect of rauwolscine (1μM) was similar in the 95% O_2 and 16% O_2 environment (Figure 28).

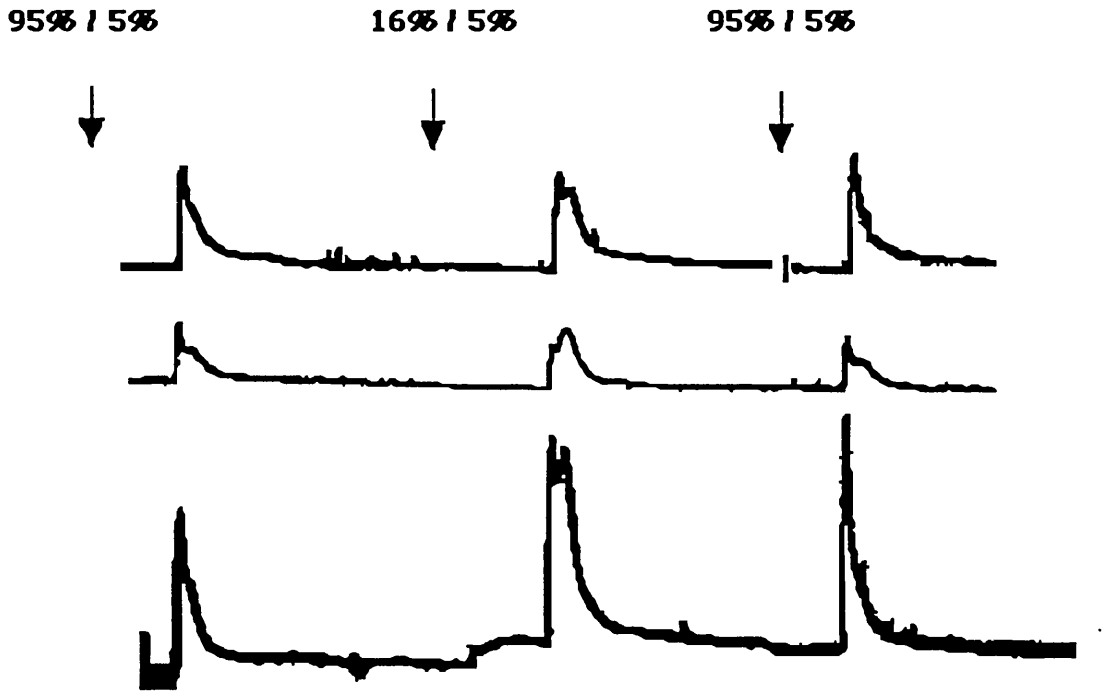

Figure 26. The effect of low oxygenation on the biphasic responses obtained to 32 Hz (0.1ms p.w., 1 sec., 50 volts) in the isolated saphenous vein. Top, middle and bottom panels show the tracings of three different tissue segments. The left hand tracings show the control responses (slow chart speed) in 95% 0_2 / 5% CO_2. Center tracings show responses in 16 % O_2 / 5% CO_2. Right hand responses show responses after returning to 95% 0_2 / 5% CO_2.

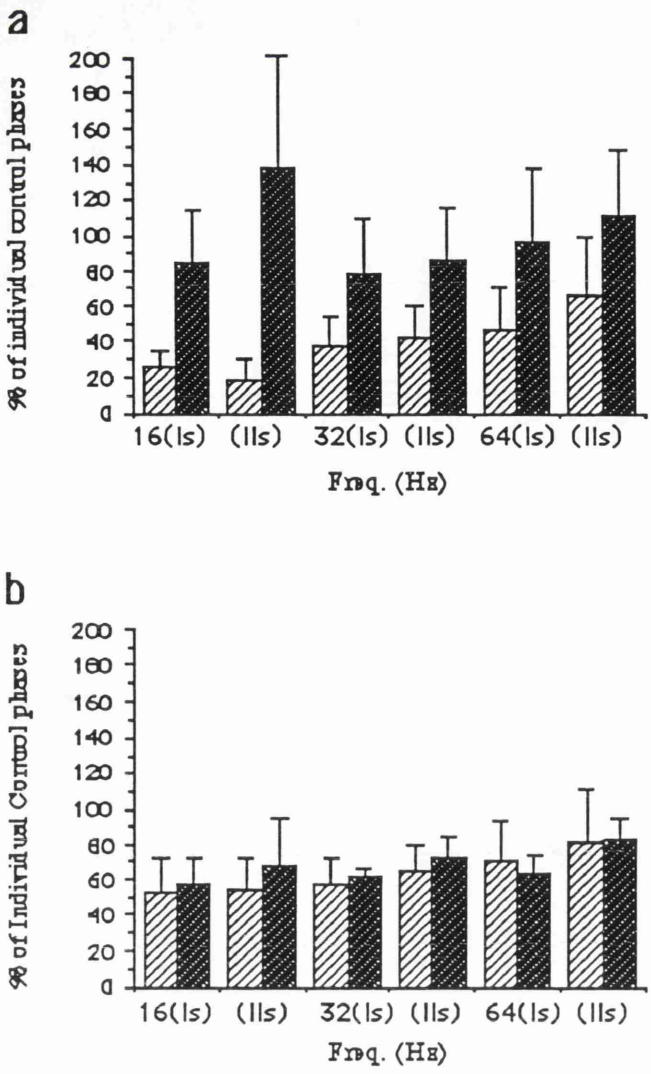

Figure 27. The effect of prazosin (0.1μM) on the biphasic responses of the isolated saphenous vein in both low and high O_2. Labels on the x-axis denote 16 Hz, first phase (Is) and 16 Hz, second phase (IIs) etc. The light columns represent the initial effect of prazosin in a) 95% O_2 / 5% CO_2 and b) 16% O_2 / 5% CO_2. The dark columns represent responses in the presence of prazosin and after the O_2 has been switched to a) 16% O_2 / 5% CO_2 and b) 95% O_2 / 5% CO_2. Values are expressed as a percentage of the control phases (in the absence of prazosin) and represent the mean ± s.e.m. (n=4).

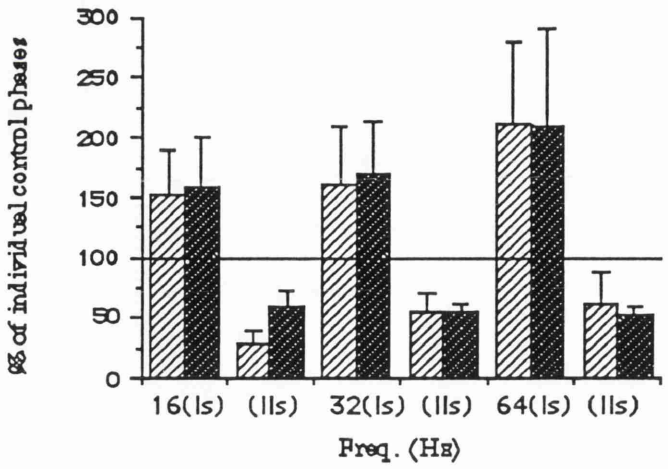

Figure 28. The effect of rauwolscine (1μM) on the biphasic responses of the isolated saphenous vein in both low and high O_2. Labels on the x-axis indicate 16 Hz, first phase (Is); 16 Hz, second phase (IIs) etc. The light columns represent the effect of rauwolscine in 16% O_2 / 5% CO_2. The dark columns represent responses in the presence of rauwolscine and after the gassing has been switched to 95% O_2 / 5% CO_2. Values are expressed as a percentage of the control phases (in the absence of rauwolscine) and represent the mean ± s.e.m. (n=4).

The low frequency, monophasic responses were slightly increased on switching from 95% O_2 to 16% O_2. The 4Hz response (measured isometrically) increased from 30mg weight to 80mg weight, while the 8Hz response increased from 150mg weight to 250mg weight (data not shown).

Many technical difficulties limited the scope of this line of research (see discussion) and these probably contributed to the contradictory nature of these data. Nevertheless there appears to be an α-adrenoceptor mediated component of the nerve induced response which is more resistant to prazosin in the more physiological conditions of 16% O_2 / 5% CO_2.

Effect of AII in the saphenous vein and in the absence of uptake$_1$-blockade and β-blockade.

AII (0.03μM) caused a transient monophasic response which returned to baseline within 2 minutes.

After treatment with rauwolscine (1μM), which itself caused no significant effect on responses, AII caused a slight decrease in responses which was variable and non-significant (Figure 29a).

In the presence of prazosin (0.1μM) AII caused a potentiation of the responses at all frequencies, except the 4Hz / 1 sec. response. However, the potentiation was variable and non-significant (Figure 29b).

After α-blockade with a combination of prazosin and rauwolscine, AII caused a potentiation of the responses at the higher frequencies. However, the potentiation was variable and non-significant (Figure 29c).

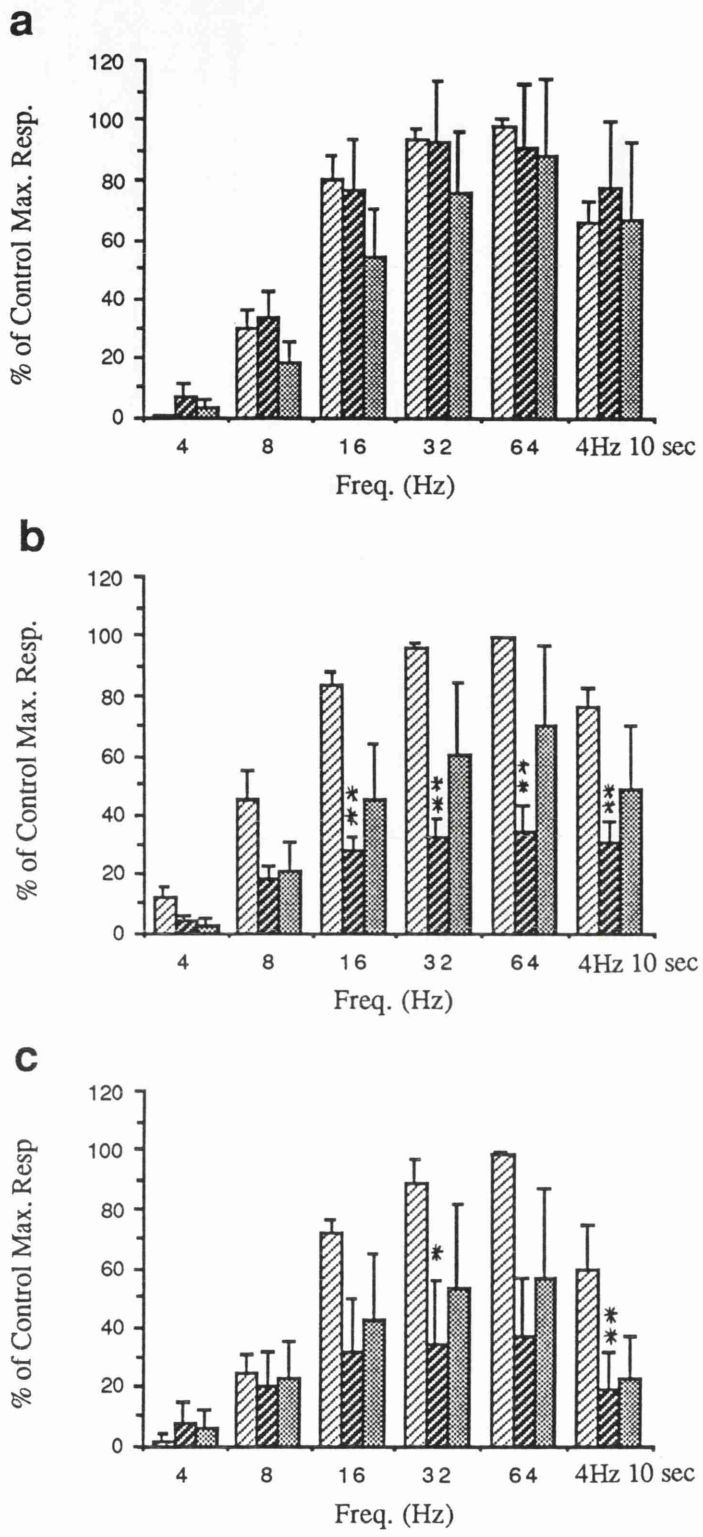

Figure 29. The effect of angiotensin II (0.03μM) on responses to EFS in the isolated saphenous vein, in the absence of cocaine. Light hatched bars represent control responses to EFS. Dark hatched bars represent responses to EFS after a) rauwolscine (1μM), b) prazosin (0.1μM) and c) prazosin and rauwolscine together. Stippled bars represent responses to EFS after addition of AII and in the presence of the α-blockers. Values are given as mean ± s.e.m (n=4). Statistical significance was tested using paired t-test; column 2 vs column 1; column 3 vs column 2; $p<0.05$ (*); $p<0.01$(**).

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Effect of AII in the saphenous vein and in the presence of uptake$_1$-blockade and β-blockade.

AII (0.03μM) caused a transient monophasic response which returned to baseline within 2 minutes.

Rauwolscine caused no significant change in responses and these were unaffected by further addition of AII (Figure 30a).

Prazosin caused an inhibition of responses at all frequencies. This inhibition was partially reversed by addition of AII. The AII induced potentiation was significant at 16, 32 & 64 Hz (Figure 30b).

AII was more effective after prazosin alone than after α-blockade with a combination of prazosin and rauwolscine since the only significant effect was at 16 Hz (Figure 30b&c).

While the results were variable, they do point to AII potentiating responses only after blockade of α_1-adrenoceptors. This suggests that AII may have a selective facilitatory effect on responses mediated by postjunctional α_2-adrenoceptors.

The saphenous vein may not be the ideal tissue to examine this because of the possible interaction between the postjunctional α_1- and α_2-adrenoceptors. A similar set of experiments was therefore performed on the isolated plantaris vein which is apparently devoid of such an interaction but possesses a population of postjunctional α_2-adrenoceptors.

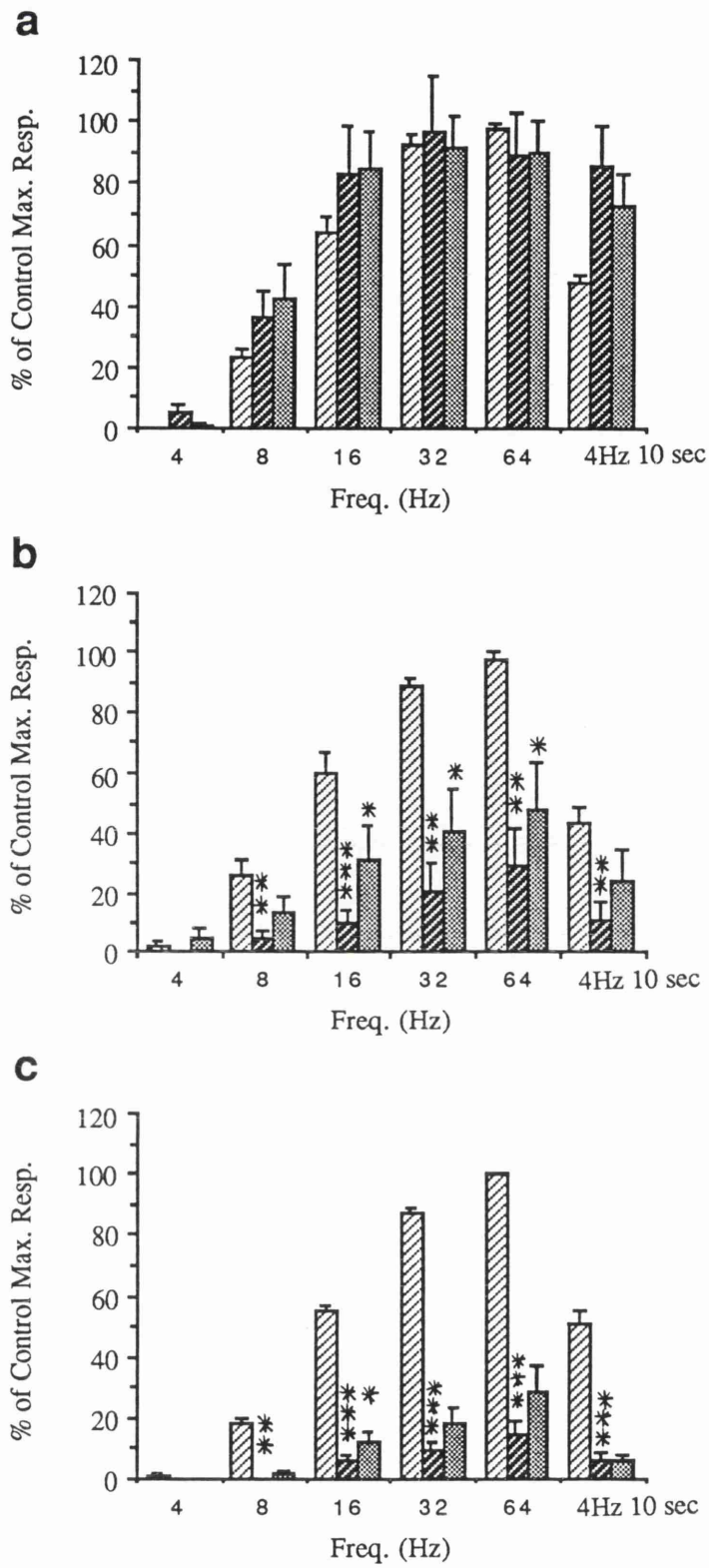

Figure 30. The effect of angiotensin II (0.03μM) on responses to EFS in the isolated saphenous vein, in the presence of cocaine (10μM). Light hatched bars represent control responses to EFS. Dark hatched bars represent responses to EFS after a) rauwolscine (1μM), b) prazosin (0.1μM) and c) prazosin and rauwolscine together. Stippled bars represent responses to EFS after addition of AII and in the presence of the α-blockers. Values are given as mean ± s.e.m (n=4). Statistical significance was tested using a paired t-test; column 2 vs column 1; column 3 vs column 2; $p<0.05$ (*); $p<0.01$(**); $p<0.001$(***).

Effect of AII on responses to E.F.S. in the isolated plantaris vein in the presence and absence of cocaine and propranolol.

AII (0.03μM) caused a transient monophasic response which returned to baseline within 2 minutes.

In the absence of cocaine and propranolol, AII caused a significant potentiation of the responses elicited by E.F.S. at 4, 16, 32 & 64 Hz (Figure 31a). In the presence of cocaine and propranolol, AII still caused a potentiation of responses in some experiments. However, this effect was not as marked and was not statistically significant (Figure 31b).

After blockade of α_1-adrenoceptors with prazosin (0.1μM) and in the presence of cocaine, AII caused a significant potentiation of responses at all frequencies except 4Hz / 1 sec. (Figure 32b). In the absence of cocaine and propranolol, AII caused a potentiation resulting in responses which greatly exceeded the size of the control responses (Figure 32a).

Blockade of α_2-adrenoceptors was more effective with than without cocaine and propranolol (Figure 33a&b). Addition of AII caused a potentiation of all responses, with the exception of the 4Hz responses, in the tissues treated with cocaine and propranolol. The potentiations were not great as those following α_1-adrenoceptor blockade and only the lower frequency (4, 8 & 16 Hz) responses in the absence of cocaine were potentiated beyond control levels (Figure 33a).

Combined α-blockade was less effective in tissues with an intact uptake$_1$ mechanism (Figure 34a). AII caused potentiation of responses at all frequencies both in the presence and absence of cocaine and propranolol although in the presence of cocaine the potentiation was less reliable. AII was unable to potentiate responses above control levels in this experiment (Figure 34a&b).

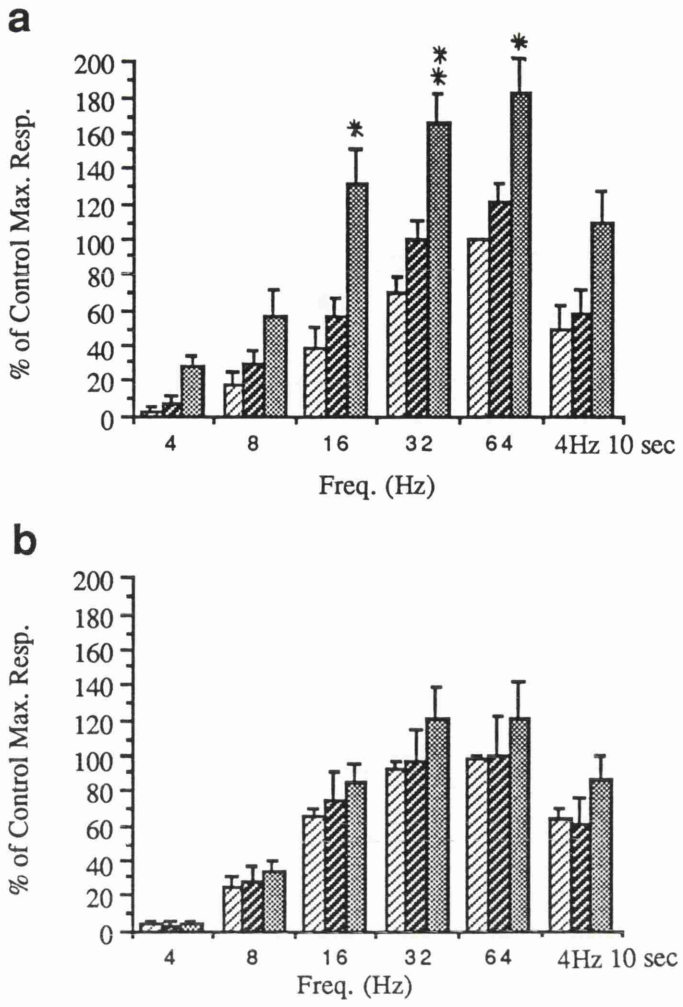

Figure 31. The effect of angiotensin II (0.03μM) on responses to EFS in the isolated plantaris vein in either the absence (a) or presence (b) of cocaine (10μM). Light hatched bars represent the responses to the first frequency response curve (FRC 1). Dark hatched bars represent the responses to FRC 2. Dark stippled bars represent the responses to FRC 3 in the presence of AII. FRCs were taken at 30 minute intervals. Values are given as mean ± s.e.m. (n=5, a; n=7 b). Statistical significance was tested using a paired t-test; column 2 vs column 1; column 3 vs column 2; $p<0.05$ (*); $p<0.01$(**); $p<0.001$(***).

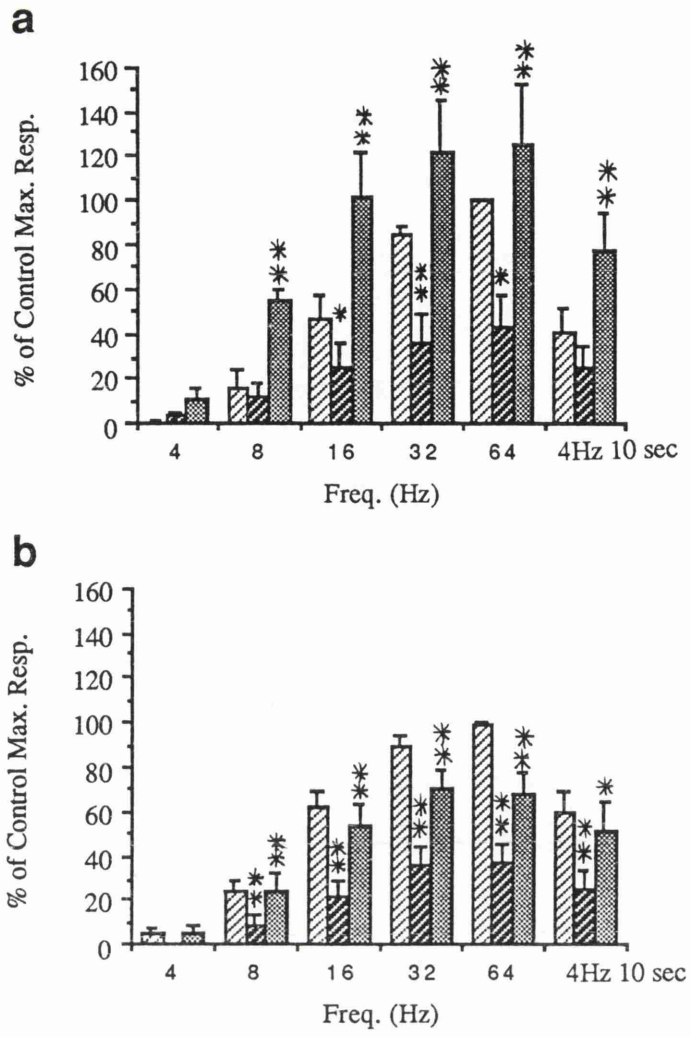

Figure 32. The effect of angiotensin II (0.03μM) on responses to EFS in the isolated plantaris vein, after α_1-blockade, in either the absence (a) or presence (b) of cocaine (10μM). Light hatched bars represent the responses to the first frequency response curve (FRC 1). Dark hatched bars represent the responses to FRC 2, in the presence of prazosin (0.1μM). Dark stippled bars represent the responses to FRC 3 in the presence of AII and prazosin. Values are given as mean ± s.e.m. (n=5, a; n=6 b). Statistical significance was tested using a paired t-test; column 2 vs column 1; column 3 vs column 2; $p<0.05$ (*); $p<0.01$(**); $p<0.001$(***).

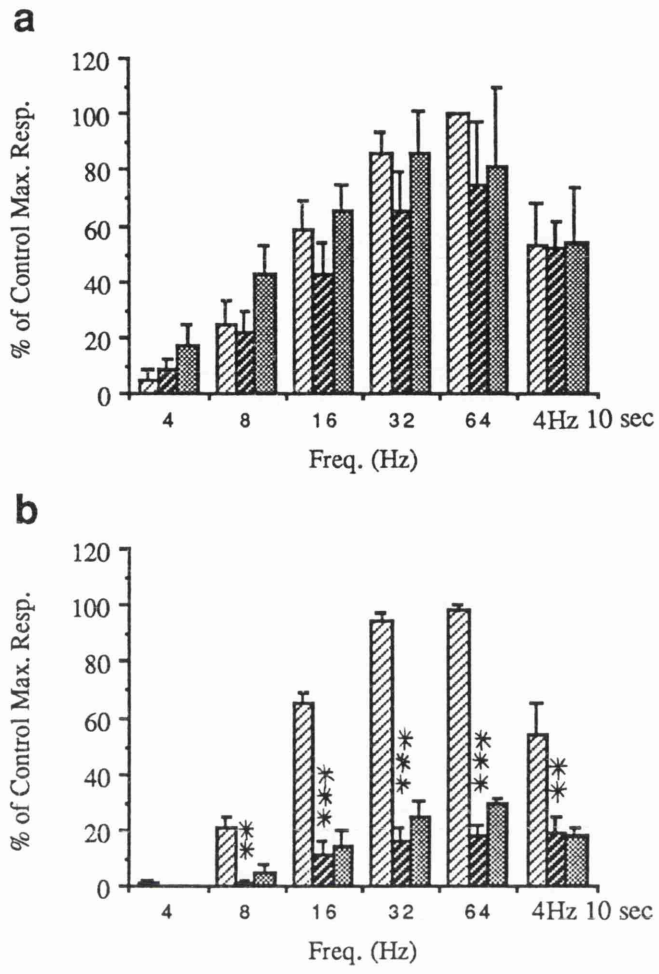

Figure 33. The effect of angiotensin II (0.03μM) on responses to EFS in the isolated plantaris vein, after α_2-blockade, in either the absence (a) or presence (b) of cocaine (10μM). Light hatched bars represent the responses to the first frequency response curve (FRC 1). Dark hatched bars represent the responses to FRC 2, in the presence of rauwolscine (1μM). Dark stippled bars represent the responses to FRC 3 in the presence of AII and rauwolscine. Values are given as mean ± s.e.m. (n=5, a; n=6 b). Statistical significance was tested using a paired t-test; column 2 vs column 1; column 3 vs column 2; $p<0.05$ (*); $p<0.01$(**); $p<0.001$(***).

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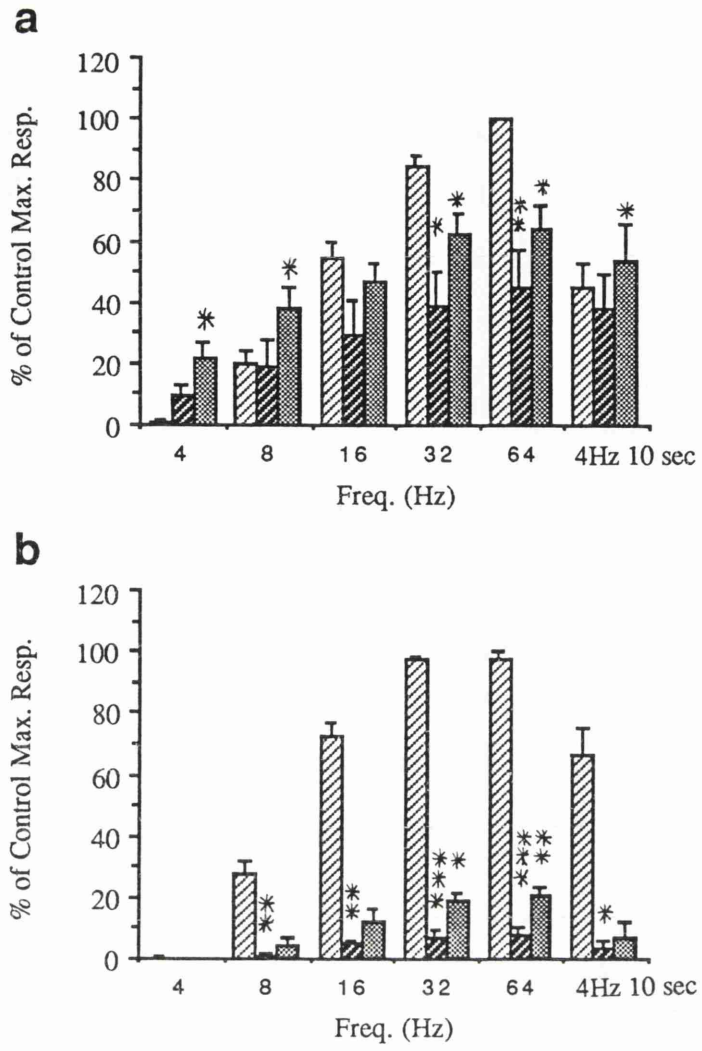

Figure 34. The effect of angiotensin II (0.03μM) on responses to EFS in the isolated plantaris vein, after α_1- & α_2-blockade, in either the absence (a) or presence (b) of cocaine (10μM). Light hatched bars represent the responses to the first frequency response curve (FRC 1). Dark hatched bars represent the responses to FRC 2, in the presence of prazosin (0.1μM) & rauwolscine (1μM). Dark stippled bars represent the responses to FRC 3 in the presence of AII and both antagonists. Values are given as mean ± s.e.m. (n=5, a; n=6 b). Statistical significance was tested using a paired t-test; column 2 vs column 1; column 3 vs column 2; $p<0.05$ (*); $p<0.01$(**); $p<0.001$(***).

The results with the plantaris vein strengthen the observation from the saphenous vein that AII-induced facilitation of nerve-mediated responses is selective for α_2-adrenoceptors. A possible prejunctional action of AII however cannot be ruled out altogether.

Effect of the endothelium on responses to agonist- and nerve-mediated responses in the isolated saphenous vein in the presence of cocaine and propranolol.

After removal of the endothelium, by gently rubbing the intimal surface with fine forceps, acetylcholine-mediated (1μM) relaxation of NA-induced (3μM) tone was abolished; relaxations mediated by sodium nitroprusside were unaffected. In endothelium-denuded segments of saphenous vein, the sensitivity to the non-selective α-adrenoceptor agonist NA was unchanged (Figure 35a). The sensitivities to amidephrine (selective α_1-adrenoceptor agonist) and xylazine (selective α_2-adrenoceptor agonist) were similarly unaffected by endothelial rubbing (Figure 35b&c). Removal of the endothlium does not therefore alter the sensitivity of the saphenous vein to α-adrenoceptor agonists.

When the responses were expressed in terms of absolute size of contraction, it was found that the absence of endothelium caused no increase in the maximum response to xylazine (Figure 36a). In contrast, with amidephrine and NA there was an increase in the maximum response in the absence of the endothelium (Figure 36b&c).

This suggests that the endothelium is capable of releasing an inhibitory factor (possibly nitric oxide, NO) which inhibits the function of postjunctional α_1-adrenoceptors.

Recently, the development of nitric oxide synthetase inhibitors such as L-NMMA and L-NAME have made it possible to inhibit the release of this particular EDRF without having to disrupt the endothelium and underlying smooth muscle.

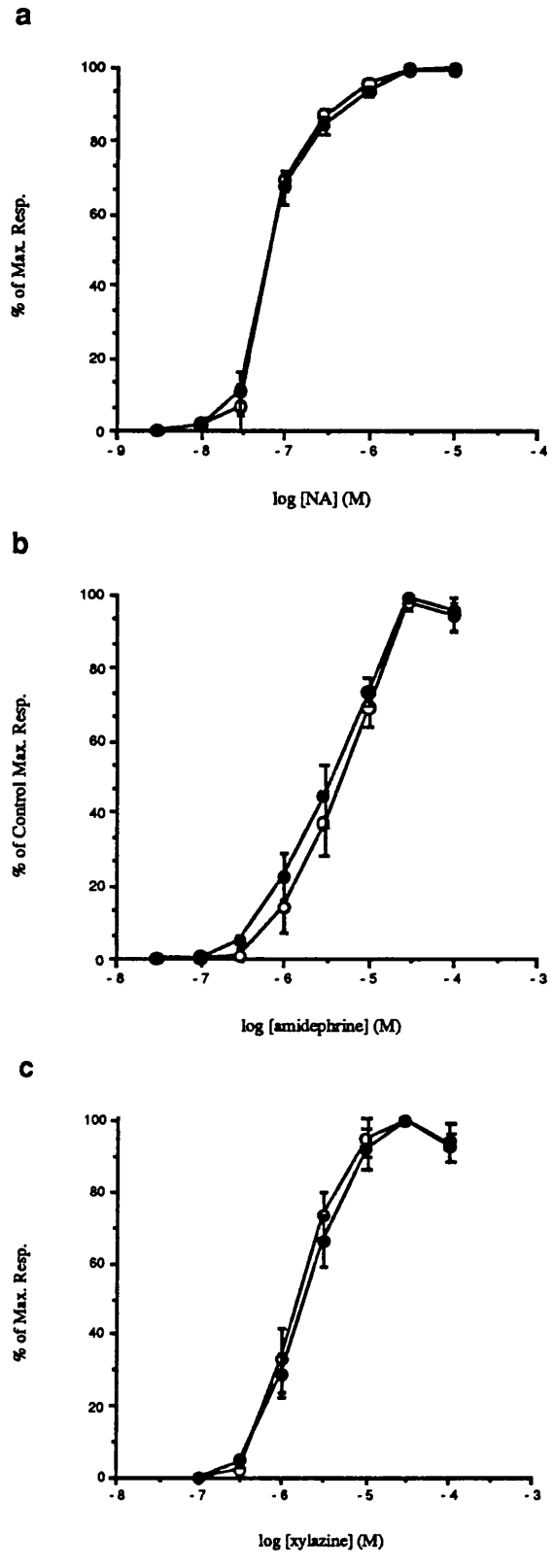

Figure 35. The effect of endothelium removal on the response to various α-adrenoceptor agonists in the isolated saphenous vein. Responses are expressed as a percentage of the maximum response to the agonist. Unfilled symbols represent control preparations, filled symbols represent endothelium denuded preparations. a) noradrenaline (non-selective), b) amidephrine (α_1-selective) and c) xylazine (α_2-selective). Vertical lines represent s.e.m. n=4.

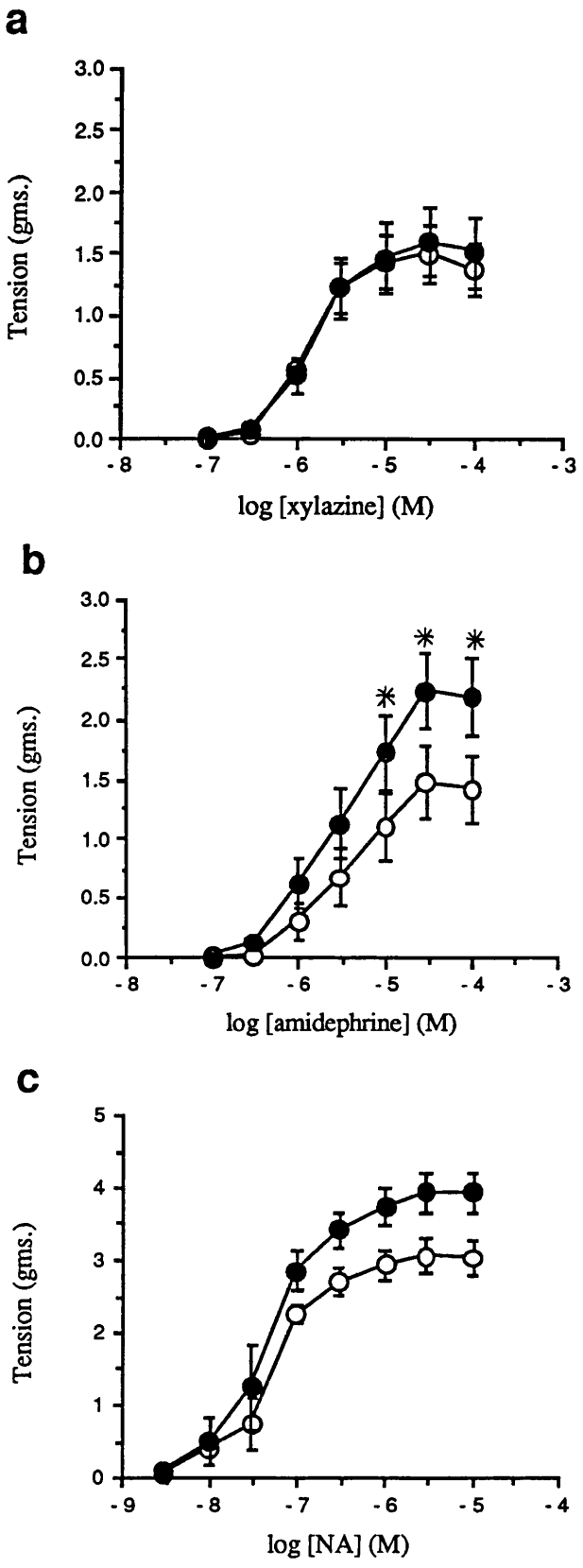

Figure 36. The effect of endothelium removal on the response to various α-adrenoceptor agonists in the isolated saphenous vein. Responses are expressed in terms of force generation in gms. Unfilled symbols represent control preparations, filled symbols represent endothelium denuded preparations. a) noradrenaline (non-selective), b) amidephrine (α_1-selective) and c) xylazine (α_2-selective). Vertical lines represent s.e.m. n=4.

Effect of L-NAME on Responses to E.F.S. in the Isolated Saphenous Vein.

Experiments were performed to establish whether inhibition of NO production would selectively enhance the α_1-adreneceptor mediated (first phase) component of the nerve mediated response in the saphenous vein.

L-NAME (0.1mM) alone caused no alteration in basal tone.

L-NAME (0.1mM) caused a marked potentiation of both phases of the response to 32Hz stimulation (Figure 37, upper and lower panels). The first phase of the responses to 16, 32 and 64Hz was potentiated by around 300% while the second phase was potentiated by up to 600% (Figure 38a).

In the presence of L-NAME, rauwolscine caused a slight contraction. Earlier experiments (in the absence of L-NAME) found that rauwolscine caused contraction only in 30% of the preparations. Rauwolscine alone caused a potentiation of the first phase and an inhibition of the second phase. L-NAME in the presence of rauwolscine caused a potentiation of both phases (Figure 38b).

Prazosin alone inhibited both phases of the response to E.F.S. L-NAME in the presence of prazosin potentiated only the second phase of the responses (Figure 38c).

These results suggest that L-NAME does not selectively potentiate a particular sub-type of α-adrenoceptor.

The maximum response to exogenously applied NA was potentiated by 40% in the presence of L-NAME (Figure 39).

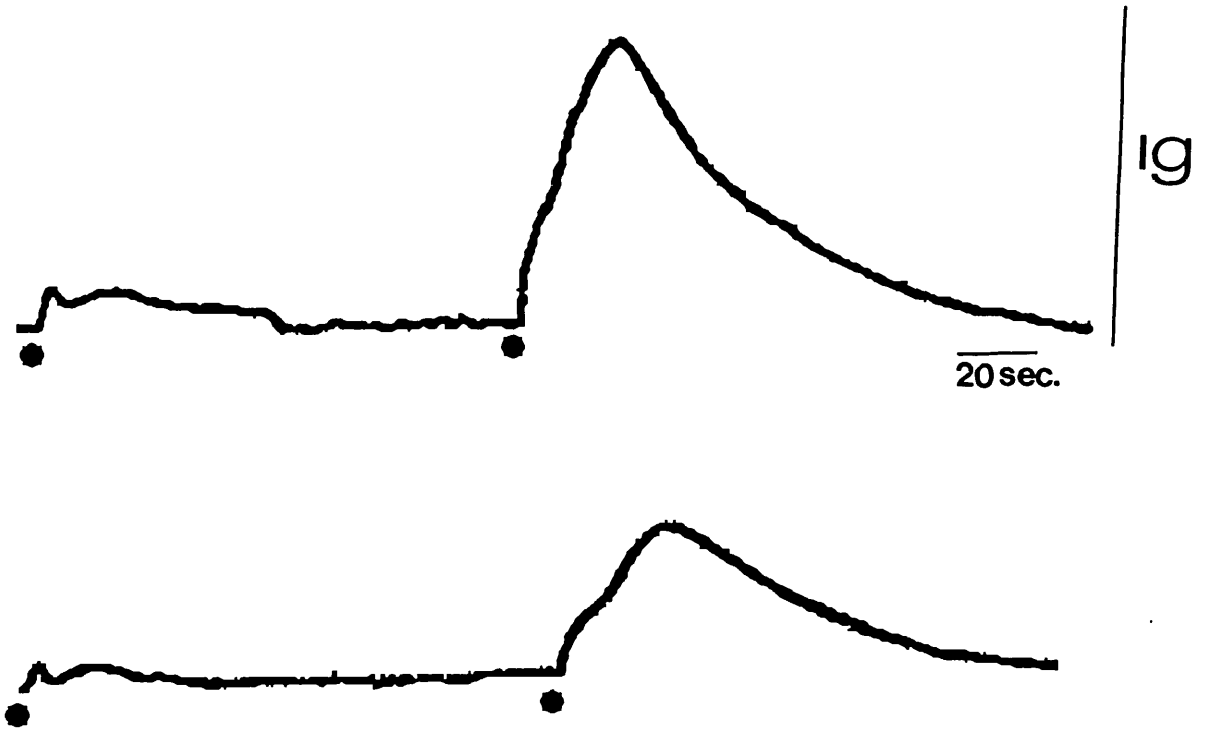

Figure 37. Representative tracings of the effect of L-NAME (0.1mM) on responses to 32 Hz stimulation in the rabbit isolated saphenous vein. Top and bottom panels show responses from different segments of the same vein. Control responses are shown on the left of both tracings, responses on the right are after addition of L-NAME.

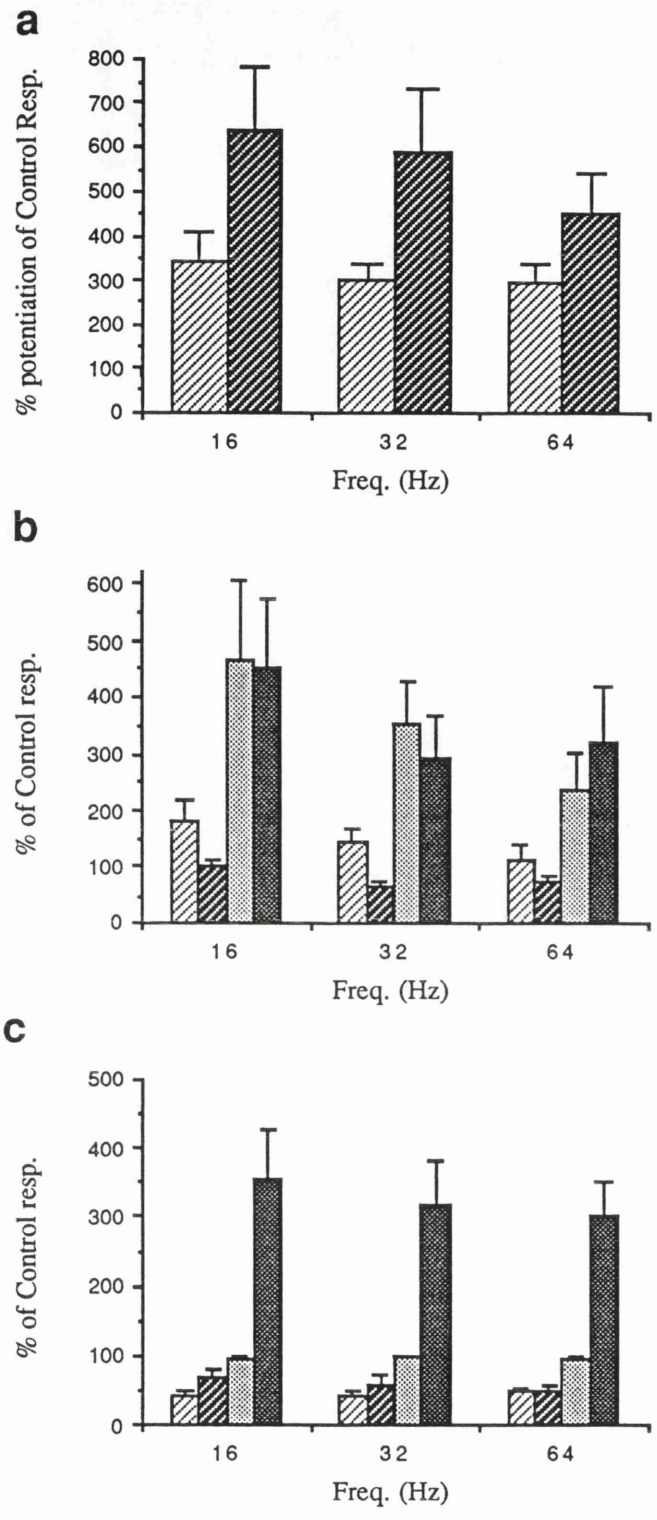

Figure 38. The effect of L-NAME (0.1mM) alone (a); after rauwolscine 1μM (b); and after prazosin 0.1μM (c), on responses to EFS in the isolated saphenous vein. Light hatched bars show first phase (*I*) and dark hatched bars the second phase (*II*) of response to EFS after L-NAME (a) or α-adrenoceptor antagonists rauwolscine (b) & prazosin (c). Light stippled bars show (*I*), dark stippled bars show (*II*) in the presence of L-NAME and adrenoceptor antagonists. Vertical lines show s.e.m. (a, n=6; b&c, n=4).

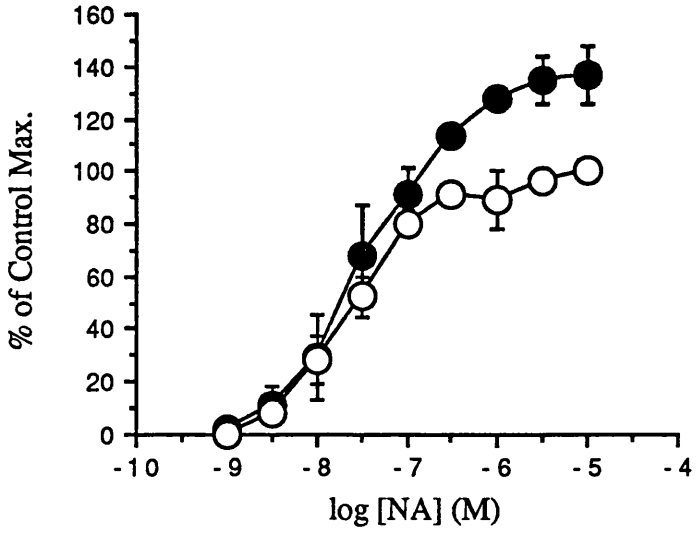

Figure 39. The effect of L-NAME on the concentration response curve (CRC) to noradrenaline (NA) in the rabbit isolated saphenous vein. Responses are expressed as a percentage of the maximum response to NA in the control CRC (○). Filled symbols (●) indicate responses to NA in the presence oh L-NAME (0.1mM). vertical lines indicate s.e.m. (n=6).

Discussion

The aim of this project was to establish the conditions required for the expression of post-junctional α_2-adrenoceptor mediated responses to electrical field stimulation in the rabbit isolated saphenous and plantaris veins. It is necessary therefore to first establish the presence of α_2-adrenoceptors on both of these vessels before attempting to study their participation in sympathetic neuroeffector transmission. This was achieved by determining the potency of selective and non-selective α-adrenoceptor agonists and antagonists.

UK-14304 is a selective α_2-adrenoceptor agonist (Cambridge, 1981), phenylephrine is a selective α_1-adrenoceptor agonist (McGrath, 1982) and noradrenaline (NA) is a non-selective α-adrenoceptor agonist. The relative potency of these three agonists, on any given tissue, provides an indication of the dominant α-adrenoceptor population. It is not sufficient to rely on agonist potency alone and therefore the potency of selective antagonists must also be considered. In this study the antagonists chosen for this purpose were: corynanthine (selective for α_1-adrenoceptors); prazosin (selective for α_1-adrenoceptors), and; rauwolscine (selective for α_2-adrenoceptors). Particular attention has been paid to the relative potencies of the di-stereoisomers, corynanthine and rauwolscine. The antagonist potencies were judged against contractions to the endogenous ligand NA.

α-adrenoceptor profile of the rabbit isolated saphenous vein

Published reports have provided conflicting evidence for the existence of postjunctional α_2-adrenoceptors on this vessel (Purdy *et. al.*, 1980; Schüman & Lues, 1983; Alabaster *et.al.*, 1985; Daly *et.al.*, 1988a,b). The conflict has arisen mainly through the use of prazosin (and its observed potency) in combination with synthetic 'selective' α-adrenoceptor agonists i.e. (BHT-920 & UK 14304). To try to resolve the differences in observed prazosin sensitivity, experiments were carried out with the

endogenous (naturally ocurring) agonist noradrenaline as well as other selective agonists and antagonists.

Based on the estimated pD_2 values, the order of agonist potency indicated the presence of a population of post-junctional α_2-adrenoceptors: UK 14304 > NA >> phenylephrine (Figure 1). The results obtained with the antagonists, however, were not as clear cut: the order of potency (based on pA_2 values) being rauwolscine = prazosin >> corynanthine. Although the potency of rauwolscine compared with corynanthine confirms the presence of α_2-adrenoceptors, the potency of prazosin (pA_2 8.44) indicates the presence of postjunctional α_1-adrenoceptors since its pA_2 value against NA, in the rabbit saphenous vein, lies within the lower limit of the expected range for prazosin at α_1-adrenoceptors (McGrath *et.al.*, 1991). Alternatively, the rabbit saphenous vein may contain a population of α_2-adrenoceptors which are similar to those found on the rat kidney which bind ^{3}[H]-rauwolscine with high affinity but are also sensitive to prazosin (Neylon & Summers, 1985).

Schild analysis showed that only corynanthine could be considered competitive, since the confidence limits for the slope of the line overlapped with unity. The selectivity of corynanthine (0.5μM-50μM) at α_1-adrenoceptors in this preparation may be questionable however since its pA_2 value (6.36) is intermediate between values expected at α_1-adrenoceptors (6.5 - 7.8) and α_2-adrenoceptors (4 -6.4) (McGrath *et.al.*, 1991). The more selective α_1-adrenoceptor antagonist prazosin, produced a line of slope 0.58. Rauwolscine produced a line closer to unity. However, it still could not be considered competitive. Comparisons of agonist and antagonist potencies in this tissue do not in themselves provide a clear picture of the adrenoceptor profile although they do indicate the presence of both α_1- and α_2-adrenoceptors.

The shape of the concentration response curve (CRC) for NA in the presence of rauwolscine provides a clue to the paradoxical nature of the drug potency orders. Low concentrations of NA were found to be resistant to rauwolscine (0.05 - 2.5μM, Figure

2c) and this caused the CRC to be biphasic. Similar results were obtained when the selective α_2-adrenoceptor antagonists CH-38083 and apo-yohimbine were tested against NA (Daly, *et.al.*, 1988b). Earlier studies found comparable results when phenylephrine was used as the agonist (Schüman & Lues, 1983; Alabaster *et. al.*, 1985). However the authors chose not to discuss the biphasic nature of the phenylephrine curve. In the present study, subsequent addition of prazosin (0.1μM) eliminated the resistant component and produced a parallel shift of the CRC (Figure 3). Using a combination of rauwolscine and phenoxybenzamine, a protocol was devised to irreversably block α_1-adrenoceptors. Under these conditions rauwolscine (0.05μM - 2.5μM) produced a parallel displacement of the CRC to NA, producing a Schild slope which was not significantly different from unity and a pA_2 (8.33) which falls within the expected range for an action at α_2-adrenoceptors (Daly *et.al.*, 1988c; McGrath *et.al.*, 1991). Taken together with the results of this study it is suggested that NA is activating both α_1- & α_2-adrenoceptors. The existence of a heterogeneous population of α-adrenoceptors, which displayss a functional interaction would explain the high potency of prazosin. A similar heterogeneous α-adrenoceptor system has been described in the dog saphenous vein (Hicks *et.al.*, 1991).

α-adrenoceptor profile of the rabbit isolated plantaris vein

Examination of the agonist pD_2 values alone indicates the presence of a population of post-junctional α_2-adrenoceptors: UK14304 > NA >>phenylephrine. Comparison of the antagonist pA_2 values against NA also confirmed the presence of post-junctional α_2-adrenoceptors: rauwolscine > corynanthine > prazosin. Careful analysis of the pA_2 values supports the presence of a population of α_2-adrenoceptors. Once again, like in the saphenous vein experiments, the selectivity of corynanthine (0.5μM - 50μM) is questionable since its pA_2 value (6.32) is on the border line for activity at α_1- and α_2-adrenoceptors (McGrath *et.al.*, 1991). Furthermore, corynanthine has a pA_2 of 6.22 against NA in the rabbit ear vein, a vessel known to

contain an almost homogeneous population of α_2-adrenoceptors (Daly *et.al.*, 1988a). This result coupled with the fact that prazosin was virtually ineffective (no pA_2 could be derived) in the plantaris vein points to the absence of functional α_1-adrenoceptors. The pA_2 for rauwolscine (7.56) lies at the high end of the expected range of activity at α_2-adrenoceptors. However, reported values for its activity at α_1-adrenoceptors in the rabbit ear artery (Hieble & Woodward, 1984), rabbit aorta and pulmonary artery (Wietzell *et.al.*, 1979) are in the 5-6 range.

It is interesting that, although UK 14304 was the most potent agonist, its intrinsic efficacy was less than that of either NA or phenylephrine which were both full agonists. It is possible that the lack of clear-cut evidence for α_1-adrenoceptors (a pA_2 value for prazosin could not be obtained) in some way corellates with the partial agonism observed with UK 14304; if α_2-adrenoceptors are indeed dependent on the functional α_1-adrenoceptors. In the saphenous vein UK 14304 had a higher intrinsic efficacy, relative to NA, than it did in the plantaris vein but in the saphenous vein prazosin was equipotent with rauwolscine against the NA CRC, i.e α_1-adrenoceptors are present and functional. While it may simply be that the receptor reserve for UK 14304 of the saphenous vein is greater, allowing more scope for partial agonist activity (Stephenson, 1956), it may also be that the lack of functional α_1-adrenoceptors in the plantaris vein diminishes the efficacy of the α_2-adrenoceptor agonist.

Overall, the data obtained for both veins using the various agonists and antagonists strongly suggests the presence of post-junctional α_2-adrenoceptors. On the saphenous vein the population is heterogeneous while, on the plantaris vein, the population is more homogeneous with respect to the α_2-subtype. It is conceivable therefore, that these α_2-adrenoceptors could contribute to neuroeffector transmission under certain circumstances.

α-adrenoceptor subtypes involved in the response to electrical field stimulation in the isolated saphenous vein

Electrical Field Stimulation (EFS) is routinely used to assess the effect and type of neurotransmitters liberated from sympathetic varicosities within the wall of blood vessels *in-vitro*. The saphenous vein of the rabbit (Harker & Vanhoutte, 1989), dog (Flavahan *et.al.*, 1987) and human (Docherty & Hyland, 1985) have all been used in this way to determine the adrenoceptor contribution to neuroeffector transmission. There appears though to be no general consensus on what is the optimal (i.e. most physiological) stimulus to deliver to *in-vitro* preparations of isolated blood vessels.

The activity of sympathetic nerves *in-vivo* is highly irregular in both humans and animals (Adrian *et.al.*, 1932; Delius *et. al.*, 1972). *In-vitro* experiments however are generally performed with very regular pulse patterns for reasons of simplicity and reproducibility.

Recordings of sympathetic nerve activity, which in some cases can be greater than 30 Hz (Burnstock & Costa, 1975), can be delivered to *in-vitro* preparations via a computer driven electrical stimulator. Using this technique to deliver 281 pulses at irregular intervals over a period of 154 seconds (85mA, 2 ms pulse width) Sjoblom-Widfeldt & Nilsson (1990) found the rat mesenteric artery to be 80% resistant to prazosin. These authors tested frequencies up to 32Hz and found that the greatest non-adrenergic responses were found at frequencies below 1.8Hz. In the rabbit ear artery (Kennedy *et.al.*, 1986) and illeocolic artery (Bulloch & Starke, 1990) co-transmision of ATP can also be demonstrated at low frequency stimulation.

It was assumed therefore that high frequency (>2Hz) stimuli would be more appropriate for for the study of α-adrenoceptor contribution to neuroeffector transmission since NA should be preferentially released at higher frequencies (Stjarne,

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1989; see introduction). It was decided that, for saphenous and plantaris vein, a stimulus protocol would be followed which was identical to that used in the rabbit saphenous artery by Burnstock & Warland (1987) to differentiate between adrenergic and non-adrenergic transmission.

Electrical field stimulation (EFS) of segments of rabbit isolated saphenous vein, for a one second duration, caused transient responses which were generally monophasic. The responses were frequency dependent and were maximal at between 32Hz and 64Hz (Figure 6). In some cases the 64Hz stimulus produced a response which was biphasic. However this was not a reliable feature. The response to 4Hz stimulation was very small and in some tissues was not measurable. A low frequency (4Hz), long duration (10 seconds) stimulus was tested on some preparations and was found to cause a slow response which was maintained only for the duration of the train of pulses.

The inclusion of rauwolscine (1μM) had very little effect on responses to EFS. The response to 4Hz / 1 second was potentiated in individual experiments, but this was not statistically significant. The potentiation is probably due to the inhibition of prejunctional α_2-adrenoceptors, which inhibit transmitter release and have been shown to be present on this vessel (Levitt & Hieble, 1986). It is possible that the prejunctional action of rauwolscine is masking any postjunctional blockade and therefore analysis of the effect of rauwolscine based on overall size of the response may be complicated.

Prazosin (0.1μM) produced a significant inhibition of the responses at 16, 32 and 64Hz as well as of the 4Hz/10 seconds response. This indicates that α_1-adrenoceptors play a major role in the response to sympathetic neurotransmission. The further inclusion of rauwolscine in the presence of prazosin caused no further inhibition and actually reduced the statistical significance of the inhibition caused by prazosin

alone. This is probably due to the simultaneous pre- and postjunctional actions of rauwolscine, thus causing greater variability.

This set of experiments suggests that α_1-adrenoceptors play a major role in the neuroeffector response. However under these conditions the involvement of α_2-adrenoceptors cannot be assessed.

The effect of cocaine, propanolol and corticosterone on responses to EFS in the rabbit isolated saphenous vein.

The agonist studies of the first section employed cocaine and propranolol to inhibit uptake$_1$ and β-adrenoceptors respectively. Studies using normetanephrine suggested that extraneuronal uptake was not an important site for the removal of NA in saphenous and plantaris veins (Daly *et. al.*, 1988a,b). For a proper comparison therefore, the effect of adrenoceptor antagonists on responses to EFS should also be conducted in the presence of cocaine and propranolol. The effect of corticosterone was tested, in place of normetanephrine, to assess the importance of extraneuronal uptake since this compound is a more highly selective inhibitor of uptake$_2$ (Muscholl, 1961).

Propanolol (1μM) and corticosterone (30μM) which block β-adrenoceptors and extraneuronal uptake respectively had no effect , individually (Figure 9b,9c) or in combination (not shown), on the responses to EFS in the isolated saphenous vein. Cocaine (10μM) however, increased both the height and duration of the responses to EFS. This increase in duration was characterised by the emergence of a slow secondary phase of the response (Figure 10). In the presence of cocaine therefore, the time course of the responses to EFS were radically altered. The height of the second phase of the response varied and could exceed the size of the initial fast phase. The absolute size of the response to EFS was always enhanced in the presence of cocaine. This was

characterised by an increase in either the first or second or both phases of the response (Figure 11a). It does not follow though that responses in the presence of cocaine will always be larger than responses, of different segments, in its absence. A set of unpaired experiments showed that while cocaine potentiated each response judged by the height of one or more component or duration, these responses will not always be greater in size than those from tissues in the absence of cocaine (Figure 11b).

The biphasic nature of the response in the presence of cocaine clearly required detailed analysis of the adrenoceptor contribution (if any) to each phase. The resulting analysis is discussed in a later section.

While cocaine has a marked potentiating effect, inhibition of uptake$_1$ *per se* does not produce responses which are extraordinary. The next experiments were performed in the presence of cocaine. Propranolol was also included in the bathing medium to eliminate the participation of pre- or postjunctional β-adrenoceptors.

α-adrenoceptor contribution to EFS in the presence of cocaine and propoanolol in the rabbit isolated saphenous vein.

Rauwolscine had little effect in the absence or presence of cocaine and propranolol. Responses were slightly enhanced at all except the 64Hz response. The increase was statistically significant at the low frequency, long duration 4Hz / 10 seconds stimulus (Figure 13a). Interestingly, the response to 4Hz/10 seconds, in the presence of cocaine, was smaller relative to the maximum than the response in the absence of cocaine (Figure 8a). This difference was found to be statistically significant ($p = 0.013$, Student's unpaired t-test). During uptake$_1$ blockade, the increase in junctional NA concentration may result in increased autoinhibition (via pre-junctional α_2) and therefore a reduction in response. Cocaine has been shown to reduce purinergic responses in the rabbit illeocolic artery via an increase in autoinhibition

(MacDonald *et. al.*, 1992). Increased autoinhibition might also explain the greater effect of rauwolscine in the presence of cocaine.

Prazosin produced a powerful inhibition of responses at all frequencies and abolished the 4Hz / 1 second response. The combination of prazosin and rauwolscine caused a greater inhibition than prazosin alone. Responses to 8Hz and 16Hz were more susceptible to prazosin and rauwolscine in the presence of cocaine than in its absence (analysis of variance, $p<0.05$) The fact that α-blockade was more effective in the presence of cocaine suggests that the adrenergic component of the response is enhanced during uptake$_1$ blockade. This supports an earlier finding in the immature rat vas deferens, where cocaine was found to reveal an adrenergic component in an otherwise non-adrenergic response (MacDonald & McGrath, 1984). The finding in the present study that subsequent addition of rauwolscine, in the presence of prazosin, causes a reduction in responses, indicates a small post-junctional α_2- contribution in the presence of cocaine. In the rat tail artery blockade of uptake$_1$ enhances α_2-adrenoceptor nerve mediated vasoconstriction (Papanicolou & Medgett, 1986).

α-adrenoceptor subtypes involved in the response to electrical field stimulation in the isolated plantaris vein

Using exactly the same parameters of stimulation tested on the saphenous vein, EFS was found to produce frequency-dependent, transient, monophasic responses in the plantaris vein (Figure 14). Unlike the saphenous vein there was never any indication of a reliable or dominant secondary phase to the response.

Rauwolscine (1μM) had no significant effect versus any of the frequencies tested. Apart from the response to 4Hz/1 second, which was very slightly potentiated, all other responses were slightly inhibited. Once again prejunctional α_2- activation may be counter-balancing the effect of postjunctional α_2 blockade.

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Prazosin (0.1μM) caused a significant inhibition of the 16, 32 and 64 Hz responses. Subsequent addition of rauwolscine, however, failed to cause a further reduction suggesting that only α_1-adrenoceptors contribute to the response. It is possible that the α-blocker resistant (non-adrenergic) response may be mediated by a co-transmitter (possibly ATP) whose transmission has been enhanced by inhibiting auto-feedback with rauwolscine and whose contribution becomes more significant, by blocking the adrenergic response with prazosin. Further investigations of this are discussed later.

α-adrenoceptor contribution to EFS in the presence of cocaine and propranolol in the rabbit isolated plantaris vein.

Cocaine (10μM) itself caused the appearance of a secondary phase in the response to EFS, which tended to lengthen the duration of the response. This secondary phase, however, never dominated as was often the case in the saphenous vein.

Rauwolscine (1μM) caused a significant inhibition of the EFS induced response at all frequencies and abolished the 4Hz / 1 second response. A comparison (analysis of variance) of the effects of rauwolscine in both the absence and presence of cocaine gave significant differences ($p<0.05$) at each frequency (compare Figure 16a & 19a). This greater effect in the presence of cocaine strongly suggests that uptake$_1$-blockade can cause the recruitment of postjunctional α_2-adrenoceptors to the neuroeffector response. A similar conclusion was drawn by Papanicolaou & Medgett (1986) for the rat tail artery.

The effect of prazosin (0.1μM) in the presence and absence of cocaine was not markedly different although the data tended to be statistically more significant with

cocaine, perhaps due to the exaggerated adrenergic component which may result. This enhanced adrenergic component would account for the considerably greater action of prazosin and rauwolscine together, which occured in the presence of cocaine (compare Figure 16c & 19c).

It appears that, similar to the saphenous vein, cocaine augments the participation of α_2-adrenoceptors during EFS and increases the degree of adrenergic transmission.

Since there appeared to be a sizeable 'non-adrenergic' component in the absence of cocaine, experiments were performed to determine the nature of the α-blocker resistant component.

It is now widely accepted that many blood vessels exhibit responses to nerve stimulation which are the result of cotransmission of NA and ATP (see introduction). Proof of the existence of ATP as a cotransmitter is generally assessed through the actions of the P_{2x}-purinoceptor desensitising agent and stable analogue of ATP, α, β-methylene ATP. While the specificity of this compound may be questioned, it still remains a useful tool for the determination of purinergic transmission.

Effect of α, β-methylene ATP on the adrenergic and non-adrenergic responses to EFS in the isolated plantaris vein, in both the presence and absence of cocaine.

Application of 3μM α, β-methylene ATP caused a large transient contraction. Further addition of α, β-methylene ATP caused no response and therefore it was assumed that 3μM was sufficient to desensitise the P_{2x}-purinoceptors.

Responses to EFS were largely unaffected by 3μM α, β-methylene ATP. If anything, responses were slightly increased (Figure 20). α, β-methylene ATP has been shown to cause direct depolarisation of smooth muscle (Ishikawa *et. al.*, 1985) and this may account for the slight increase in responsiveness.

A similar α, β-methylene ATP induced potentiation of nerve induced responses has been reported in the rabbit isolated saphenous artery and guinea-pig vas deferens (Bulloch *et.al.*, 1990) as well as in the *in situ* rat vas deferens (Bulloch & McGrath, 1988). Evidence that the potentiation is caused by slight depolarisation was produced by Neild & Kotecha (1986). These authors observed that the potentiating effect of α, β-methylene ATP on nerve-induced contraction of the rat tail artery can be mimicked by causing a slight depolarisation with KCl.

Unpaired experiments were performed in the presence and absence of cocaine in an attempt to assess the transmitter responsible for mediating the non-adrenergic response in the absence of cocaine. Unfortunately, the striking difference observed in the earlier experiments (compare Figure 16 & 19) was not obtained. The degree of α-blockade was similar in the both the presence and absence of cocaine and both sets of tissues exhibited non-adrenergic responses. This resistant component was sensitive to α, β-methylene ATP. However, α, β-methylene ATP was able to abolish responses only in the absence of cocaine, again suggesting that adrenergic transmission is slightly enhanced in the presence of uptake$_1$ blockade.

The plantaris vein appears to receive a considerable degree of purinergic / adrenergic cotransmission. The superficial location of this vessel may indicate a role for cotransmission in thermoregulation. Recent experiments using the more superficial rabbit isolated dorsal cutaneous arteries have also uncovered responses to EFS which are resistant to prazosin (0.1μM) and sensitive to α, β-methylene ATP (Daly *et. al.*,

1992). It would be of interest to study the effect of cooling on cotransmission in these vessels.

Investigation of the biphasic responses obtained after uptake$_1$-blockade in the isolated saphenous vein.

After blockade of the uptake$_1$ mechanism, with cocaine (10μM), a secondary component developed in response to EFS of 16Hz and above in both the saphenous and plantaris veins. This secondary component was far more pronounced and reliably obtained in the saphenous vein. It was decided, therefore, to determine what receptor type was responsible for mediating this secondary 'slow' phase of contraction.

Since blockade of uptake$_1$ will increase the concentration of NA in and around the synaptic cleft, it is possible that this may cause stimulation of receptors which are located outside or on the periphery of the cleft. Alternatively, receptors which have a poor coupling mechanism and require persistent activation may be stimulated. It was of interest to determine whether the postjunctional α_2-adrenoceptors, known to be present from the effect of the agonists (Figure 1), were involved in this 'uncovered' response.

Addition of rauwolscine (1μM) caused a significant inhibition of the secondary component of the biphasic response, while potentiating the first phase. This can be explained by the simultaneous pre- and postjunctional inhibition of α_2-adrenoceptors.

CH-38083, which is a highly selective α_2-adrenoceptor antagonist (Vizi *et.al.*, 1986), also caused a marked reduction of the secondary component in the presence of cocaine. Interestingly, CH-38083 had very little apparent prejunctional activity and this may suggest a difference in the pre- and postjunctional α_2-adrenoceptors. A similar postjunctional selectivity has been recently described for SK&F 104856 *in-vivo*, which appears to have virtually no prejunctional α_2-activity (Hieble *et.al.*, 1992). The fore-

runner of this compound (SK&F 104078) was also reported to possess postjunctional α_2-selectivity (Ruffolo *et. al.*, 1987). In the rabbit saphenous vein SK&F 104078 caused inhibition of both phases (similar to corynanthine, Figure 24b) which may confirm its lack of postjunctional selectivity between α_1- and α_2- (Connaughton *et. al.*, 1988). Alternatively, it may suggest a small contribution from α_2-adrenoceptors to the first phase. However, the results with CH 38083 do not support this. Further development of postjunctional specific adrenoceptor antagonists will be crucial to the study of neuroeffector interactions where both pre- and postjunctional α_2-adrenoceptors are present.

Prazosin (0.1μM) inhibited both phases of the response. Corynanthine (1μM) caused a similar qualitative reduction in both phases of the response.

These results suggest that the secondary phase, which emerges in the presence of cocaine, is mediated via α_2-adrenoceptors which may require persistent stimulation or may be located extrajunctionally. The first phase of contraction is resistant to α_2-adrenoceptor antagonists but is sensitive to α_1-adrenoceptor antagonists and is therefore mediated by α_1-adrenoceptors. The fact that α_1-blockade can inhibit the α_2-meditated component suggests that the α_2-adrenoceptor is dependent on the function of the α_1-adrenoceptor. Such an interaction would also explain the ability of prazosin to inhibit responses to UK-14304 in this vessel. It has been proposed that the normally quiesent postjunctional α_2-adrenoceptors on the rabbit isolated saphenous artery are dependent (for their expression) on the function of another receptor, either α_1- or angiotensin II (Dunn *et. al.*, 1991).

Effects of O_2 and CO_2 on the biphasic response in saphenous vein.

In the pithed rat, the pressor response to α_1-adrenoceptor agonists is attenuated if the rats are ventilated on a low O_2 gas mixture and the response to α_2-adrenoceptor

agonists is increased if the CO_2 is raised (Grant *et. al.*, 1985). It would thus appear that the optimum conditions in which to study α_2-adrenoceptor function would be one of low O_2 and high CO_2. Since relative to arterial PO_2 and PCO_2, the normal environment of venous smooth muscle would favour α_2-adrenoceptors, it is necessary to establish the sensitivity to prazosin and rauwolscine under conditions which are more closely related to the physiological environment.

Firstly, the effect of lowering the O_2 from 95% to 16%, while maintaining the same rate of gassing, was assessed. Tissues were mounted in baths bubbled with 95% O_2 / 5% CO_2 and responses to EFS were obtained. Cocaine was omited from the Krebs solution and the responses were found to be monophasic. When the gassing was rapidly switched from 95% O_2 / 5%CO_2 to 16% O_2 / 5% CO_2 responses at 16Hz and above developed a secondary phase. This second phase emerged within the first 5 minutes of lowering the O_2. Switching back to the 95% O_2 mixture rendered responses monophasic, again within 5 minutes (Figure 26).

This suggests that the vasoconstriction pattern of the saphenous vein is altered in different O_2 levels and that the pattern can start to change within 5 minutes.

From the previous section it can be proposed that the secondary phase which emerges in 16% O_2 may be mediated by α_2-adrenoceptors. If the representative traces of figure 26 are examined closely, it can be seen that the secondary phase which appears is not at the expense of the first phase. It might have been expected, from the work of Grant *et. al.*, that the first phase would have been reduced since the α_1-adrenoceptors appear to be dependent on the level of O_2. It is possible that the O_2 concentration of 16% (still high by extracellular physiological standards) may not be low enough to inhibit the α_1-adrenoceptors of venous tissue, which will after all be acclimatised to low PO_2. Unfortunately the baths used in this study were over 1.5 inches wide in order to

accomodate the electrode assembly. For this reason it was very difficult to maintain a low (e.g. 4%) O_2 concentration.

Experiments were performed to assess the sensitivity of the saphenous vein to prazosin and rauwolscine in Krebs bubbled with 16% O_2 / 5% CO_2. After initial inhibition of responses in 95% O_2 / 5% CO_2 with prazosin (0.1μM), switching the gas to 16% O_2 / 5% CO_2 potentiated the responses, returning them almost to control values (Figure 27a). If indeed the function of α_2-adrenoceptors is greater in high concentrations of O_2, this would explain the greater resistance to prazosin of tissues receiving 16% O_2. In a different set of experiments the initial inhibition caused by prazosin in 16% O_2 was found to be less than that obtained in the first set of experiments where the initial inhibition was obtained in 95% O_2 (compare light columns of figure 27a & 27b). Switching to 95% O_2, in the presence of prazosin, did not however cause a further reduction.

The effect of rauwolscine was similar in both 95% and 16% O_2. This perhaps points to the non-dependency of the α_2-adrenoceptors on O_2 concentration since CO_2 was maintained at 5% throughout the experiments. It has been shown that in the pithed rat the pressor responses to α_2-adrenoceptor agonists are more dependant on the level of CO_2 (Grant *et. al.*, 1985).

Although the results obtained after altering PO_2 did not achieve statistical significance (perhaps due to low numbers), certain features of the data are worthy of at least a brief mention. The change in shape of the response and the resistance to prazosin observed when tissues are aeriated with 16% O_2 raises the possibility that the α_2-subtype may play a greater role in the saphenous vein *in situ*, where venous PO_2 and PCO_2 should favour α_2-adrenoceptor expression.

If PO_2 and PCO_2 are important factors in the functional expression of both α-adrenoceptor subtypes the routine use of 95% O_2 / 5% CO_2, to provide O_2 saturation, may tend to exaggerate the importance of the α_1-adrenoceptor. In particular, perhaps experiments on veins should be performed under venous conditions.

The effects of angiotensin II on responses to EFS in the isolated saphenous vein in both the presence and absence of cocaine.

PO_2 and PCO_2 are not the only constituents of blood which can influence α-adrenoceptor function. Angiotensin II (AII) is a blood borne hormone which has facilitatory effects on a variety of biological tissues. It is well established that there is a close interaction of the sympathetic nervous system with the renin-angiotensin system. Adrenoceptors mediate the release of renin from the renal juxtaglomerular cells (Miller & Vander, 1965). More recent work has focused on the facilitatory effects of AII on i) the release of NA from sympathetic nerve terminals (Maclean & Unger, 1986) and ii) the activity of postjunctional α-adrenoceptors (Schuman & Lues 1983; Dunn *et. al.*, 1989; 1991a,b).

The facilitatory effect of AII on postjunctional α-adrenoceptors has been studied on a variety of isolated blood vessels which possess different α-adrenoceptor profiles. Schuman & Lues (1983) have described a postjunctional AII receptor on the rabbit saphenous vein which can augment contractions mediated via the α_2-adrenoceptor agonist B-HT 920. The potency of prazosin in the rabbit saphenous vein is reduced in the presence of AII while the potency of rauwolscine is unaffected (Dunn *et. al.*, 1991a). AII facilitates responses to NA in the rabbit ear vein, which contains an almost homogeneous population of postjunctional α_2-adrenoceptors (Dunn *et. al.*, 1991a). Responses to NA in the left renal vein, which has mainly α_1-adrenoceptors, are unaffected by AII. The facilitatory effect on all the vessels described was blocked by the AII receptor antagonist saralasin (0.1μM). AII also uncovers responses to

UK14304 in the rabbit femoral artery. However, a recent report has suggested that NA can facilitate responses to AII in this vessel and that the facilitation is mediated via an α_1-adrenoceptor (Prins *et. al.*, 1992). Consequently, a tissue which contains α_1-adrenoceptors, α_2-adrenoceptors and AII receptors may rely on an extremely complex and intimate interaction of all three receptor types.

AII (0.03μM) caused a powerful, transient contraction in the saphenous vein, confirming the presence of postjunctional AII receptors.

The presence of AII in the bathing medium did not affect the control responses to EFS. Since responses were not enhanced stimulation of prejunctional AII receptors seems unlikely.

In the absence of cocaine and after selective blockade of α_1- and / or α_2-adrenoceptors, AII was unable to significantly enhance the responses. However, in the presence of cocaine and after α_1-blockade with prazosin, AII caused a significant potentiation of the responses to 16, 32 & 64 Hz . In the presence of cocaine and rauwolscine responses were unaffected by AII. In the presence of both prazosin and rauwolscine only the response to 16Hz was significantly enhanced (compare Figures 29 & 30).

Since AII had a significant effect only in the presence of cocaine and prazosin this supports the view that AII can potentiate postjunctional α_2-adrenoceptor function. Furthermore, AII can enhance the α_2-adrenoceptor contribution to neuroeffector transmission only under certain conditions (e.g. α_1- and uptake$_1$ blockade).

The effect of angiotensin II on responses to EFS in the isolated plantaris vein in both the presence and absence of cocaine.

The plantaris vein has a population of α_2-adrenoceptors, revealed by the action of agonists, which can participate in the response to EFS, particularly in the presence of cocaine (Figure 19a). It is possible then that AII may be able to influence the balance of α-adrenoceptor subtype contribution to the response to EFS in this tissue under certain conditions.

AII (0.03μM) caused a transient contraction and subsequently potentiated the response to EFS. The potentiation was significant at all except the 8Hz and 4Hz/10 second responses. In the presence of cocaine, AII itself caused a transient contraction but failed to potentiate responses to EFS. If AII was acting at a prejunctional site then it might be expected to potentiate responses regardless of the presence of cocaine. No functional evidence (by the use of antagonists) could be found for the existence of prejunctional α_2-adrenoceptors in this tissue and so increased NA concentration in the synaptic cleft cannot be assumed to be concurrently promoting autoinhibition.

If AII is acting postjunctionally to enhance the α_2-adrenoceptor contribution then the presence of cocaine (which itself enhances α_2-contribution) may override the action of AII. This would explain the apparent lack of effect in the presence of cocaine i.e there is a limit to the α_2-contribution which either AII or cocaine can allow.

After α_1-blockade with prazosin (0.1μM), AII caused a significant potentiation of all responses with the exception of the 4Hz / 1 second response. The potentiation was greater in the absence than in the presence of cocaine.

After α_2-blockade with rauwolscine (1μM) AII caused no potentiating effect in the presence or absence of cocaine.

These results parallel the finding in the saphenous vein that AII can enhance α_2-adrenoceptor mediated neuroeffector responses, probably via a postjunctional mechanism.

The influence of the endothelium on the α-adrenoceptor contribution to EFS and responses mediated via exogenously applied agonists.

The endothelium is known to liberate several factors which modulate the activity of vascular smooth muscle. Some of the endothelium derived factors are vasoconstrictor (e.g. endothelin). However many are vasodilators (e.g nitric oxide, endothelium derived relaxing factor (EDRF), endothelium derived hyperpolarising factor and prostacyclin). Most vascular scientists have concentrated on the actions of nitric oxide (NO) and EDRF. It is currently being forwarded by many workers that EDRF and NO are the same factor.

To determine the modulating role of the endothelium, tissues were gently rubbed on their intimal surface. This procedure was found to inhibit acetylcholine mediated relaxation but did not affect relaxations to sodium nitroprusside (data not shown). Although damage to the inner layers of smooth muscle is inevitable, the responses after rubbing are commonly larger than without rubbing so much viable tissue evidently remains.

The sensitivity to amidephrine (α_1-agonist), xylazine (α_2-agonist) and NA was unchanged in the absence of the endothelium. The maximum response to both amidephrine and NA but not xylazine was increased indicating a potentiation of responses mediated by α_1-adrenoceptors in the absence of the endothelium. If basal release of a relaxing factor from the endothelium inhibits α_1-adrenoceptors then it might

be expected that the first phase of contraction to EFS in the saphenous vein should be enhanced in the absence of the endogenous vasodilator.

Recently, the development of NO synthase inhibitors (L-NAME & L-NMMA) has made it possible to eliminate the effect of EDRF / NO without removing the endothelium and thus damaging the inner layers of smooth muscle.

L-NAME has been shown to increase the tone and decrease the cross-sectional area of large veins *in-vivo* (Schwarzacher & Raberger, 1992). This work indicates that endogenous NO plays an important role in normal venous function.

In the isolated saphenous vein L-NAME (0.1mM) itself caused no alteration in baseline tension. Addition of rauwolscine, in the presence of L-NAME, caused a variable but reliable contraction. After pre-treatment with rauwolscine L-NAME also caused a variable but reliable contraction. Rauwolscine has been shown to be a partial agonist at α_1-adrenoceptors (Guan *et. al.*, 1991) and 5-HT_1-like receptors (Shimamoto *et. al.*, 1993) in the dog mesenteric vein. In the rabbit saphenous vein rauwolscine alone only caused contraction in 30% of tissues. The interplay between NO and α-adrenoceptors and / or 5-HT receptors requires further study.

The potentiation of nerve mediated responses caused by L-NAME can only be described as colossal. Both phases were potentiated, the first phase by around 300% and the second by about 500%. The degree of potentiation suggests that endogenous NO markedly suppresses the response to neuroeffector transmission.

In the presence of rauwolscine both phases of the response appeared to be potentiated. It should be noted though that the magnitude of the potentiation raised the possibility that the first phase may have been so augmented as to mask the secondary phase. In the presence of prazosin the secondary phase was potentiated to a far greater

degree than the first, showing a marked enhancement of the α_2-adrenoceptor mediated response. Since the maximum response to exogenous NA is only potentiated by around 40% it is possible that the endogenous NO is acting at a prejunctional site. Overflow studies will be required to confirm this. NO released from the endothelium will degrade quickly in the biophase and is unlikely to reach the sympathetic nerve varicosities. Perhaps the outer layers of smooth muscle can produce NO, the target for which would be a prejunctional site on the varicosity which inhibits further transmitter release. Another possibility is that macrophage cells which are present in the adventitia (and are known to produce NO) may be releasing NO around the synaptic cleft. This would explain why after endothelial removal, L-NAME is still capable of potentiating the response to EFS in the saphenous vein (Dr. J.F. Gordon, personal communication).

In summary, both the saphenous and plantaris veins of the rabbit contain functional populations of postjunctional α_2-adrenoceptors. These receptors make a significant contribution to neuroeffector transmission when neuronal uptake is blocked. This may indicate an extrajunctional location for these receptors or they may be intrajunctional but requiring persistent activation. Physiological factors such as O_2 / CO_2 concentration, and endothelium derived factors can alter α_2-adrenoceptor contribution to neuroeffector transmission in the saphenous vein. Angiotensin II can enhance α_2-adrenoceptor contribution to neuroeffector transmission in both the saphenous and plantaris veins.

The results of this study emphasise the importance of the physiological environment in the relative expression of α_2-adrenoceptors during sympathetic neuroeffector transmission in the rabbit isolated saphenous and plantaris vein.

Conclusion

The potency order of selective α-adrenoceptor agonists and antagonists can be used to assess the receptor profile in a particular tissue. This has confirmed the presence of a heterogeneous population of α_1- and α_2-adrenoceptors in the rabbit isolated saphenous vein. Low concentrations (10 - 300nM) of, the endogenous ligand, noradrenaline act via the α_1-subtype while higher concentrations (>300nM) can recruit the α_2-subtype. Further experiments are required to determine whether it is the location of the receptors within the vessel wall which varies or whether it is a function of the receptor itself.

The rabbit isolated plantaris vein has a more homogeneous population of α_2-adrenoceptors. The pA_2 value for corynanthine against noradrenaline and the lack of effect of prazosin suggests the absence of functional α_1-adrenoceptors.

Electron microscopy confirmed the presence of adrenergic nerves in both vessels. Electrical field stimulation was therefore used in both veins to determine whether the α_2-adrenoceptor subtype, known to be present, is involved in sympathetic neuroeffector transmission. When neuronal uptake is not inhibited, by inclusion of cocaine, the neuroeffector response is mediated via α_1-adrenoceptors in both veins. When cocaine is present α_2-adrenoceptors contribute to the neuroeffector response in both veins. Additional experiments are required to determine if the α_2-adrenoceptor requires persistant activation (by the increased junctional concentration of NA) or if the receptors are simply located outwith the neuroeffector junction.

In the saphenous vein the contribution of both adrenoceptor subtypes to neuroeffector transmission, in the presence of cocaine, can be clearly identified from the biphasic nature of the response to high frequency stimulation. The first phase of the response is sensitive to prazosin and is mediated via α_1-adrenoceptors. The second (more prolonged) phase is sensitive to rauwolscine or CH 38083 and is mediated via α_2-adrenoceptors. Inhibiting the first phase also has an inhibitory effect on the second phase which suggests that the α_2-adrenoceptor is in some way dependent on the α_1-adrenoceptor. Further work is required to determine if the interaction of these receptors is at the level of the second messenger or if it is within the membrane.

The saphenous vein exhibits a mainly adrenergic response to electrical field stimulation (i.e. α-blockers can almost abolish the response). The plantaris vein on the other hand has a significant non-adrenergic (α-blocker resistant) component of the response to electrical field stimulation. The non-adrenergic component is sensitive to inhibition by α, β-methylene ATP and is therefore mediated by P_{2x} receptors.

By lowering the Po_2 the shape of the response to electical field stimulation in the saphenous vein is altered. A secondary component appears which resembles the response in the presence of cocaine. It is not clear if this is the result of α_2-subtype recruitment since the effect of the antagonists were variable. There is evidence that the adrenoceptors are sensitive to changes in Po_2 and pH (see discussion). A further study is required to examine this during neuroeffector transmission. Such experiments would require a re-designed bath which would house the electrode assembly but would also allow accurate maintenance of gas tensions.

Angiotensin II significantly potentiates the response to electrical field stimulation in both veins when cocaine and prazosin are present. This potentiation is due to facilitation of the α_2-adrenoceptor mediated response. The absence of Angiotensin II, which is present in the blood, from *in-vitro* experiments may contribute to the difficulty in demonstration postjunctional α_2-adrenoceptors in isolated vessels. There are now many reports of interactions between AII and α-adrenoceptors (see discussion). Further experiment are required in both saphenous and plantaris vein to establish the mechanism of this interaction during neuroeffector transmission.

The biological importance of the vasodilator molecule nitric oxide (NO) has become very apparent in the past two years. The rabbit saphenous vein has a functional endothelium which can release NO and modulate the effect of exogenously applied α-agonists. Inhibition of NO synthetase with L-NAME causes a large increase in the response to electrical field stimulation in the rabbit saphenous vein. The augmentation of the response is not due to selective facilitation of either adrenoceptor subtype. It is not certain where the NO which influences the neuroeffector response is produced. Experiments are required to determine if it is the macrophage cells of the adventitia, the smooth muscle cells themselves or if it is endothelium derived NO.

In conclusion, the rabbit isolated saphenous and plantaris veins both contain populations of functional α_2-adrenoceptors which can contribute to neuroeffector transmission if neuronal uptake is blocked. The saphenous vein recieves mainly adrenergic transmission while the plantaris vein recieves adrenergic / purinergic co-transmission. Angiotensin II facilitates the α_2-adrenoceptor mediated component of the neuroeffector response in both veins. Changes in oxygen tension can alter the shape of the response in the saphenous vein as can inhibition of NO synthetase.

These results emphasise the importance of the physiological conditions under which *in-vitro* experimentation is performed. If the function of adrenoceptors are to be assesed on any vascular or non-vascular tissue it is crucial that the *in-vivo* condition is re-created as accurately as possible.

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