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Interactions between noradrenaline and α_2-adrenoceptor agonists in the superior mesenteric arterial bed of the rat

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1 Interactions between α_2-agonists and noradrenaline vasoconstrictor responses were studied in the superior mesenteric arterial bed of the rat by use of perfusion both *in situ* with blood and *in vitro* with Krebs-Henseleit solution.

2 Xylazine (1.9×10^{-6} mol) administered into the perfusion circuit reduced the maxium response to noradrenaline in the *in situ* preparation by 35% and decreased the pD_{50} for noradrenaline from 8.5 ± 0.01 to 7.9 ± 0.13 ($n=7$). Yohimbine (1 mg kg^{-1}, i.v.) gave a small parallel shift in the noradrenaline log dose-response curve and prevented the reduction in the maximum response by subsequent administration of xylazine.

3 *In vitro*, xylazine (1.9×10^{-6} mol) also gave a long-lasting reduction of 37% in the maximum response but did not affect the mid-point sensitivity to noradrenaline. Yohimbine (10^{-6}M) did not change either of these effects.

4 Clonidine (1.9×10^{-6} mol) did not affect the maximum response to noradrenaline *in vitro* but did reduce the pD_{50} from 7.72 ± 0.17 to 6.9 ± 0.17 ($n=6$). Yohimbine did not change these effects.

5 Guanfacine (1.8×10^{-6} mol) had no effect on the sensitivity of the *in vitro* preparation to noradrenaline but did reduce the maximum response by 20%. Yohimbine (10^{-6}M) prevented the depression of the maximum response.

6 It is concluded xylazine and clonidine interfere with noradrenaline induced vasoconstriction only to a limited extent through their interaction with α_2-adrenoceptors and that some other, as yet uncharacterised mechanism which may be activated by their aryl amidine structure, is responsible for their *in vitro* effects.

Introduction

Postjunctional vascular α-adrenoceptors are known to be of the α_1 and α_2-subtypes (McGrath, 1982) but possible interactions mediated by these two receptor subtypes have received little attention. Clonidine has been shown to antagonize the vasoconstrictor responses to noradrenaline in a variety of saline-perfused organs (Hepburn & Bentley, 1982), an effect ascribed to α_1-adrenoceptor antagonism by clonidine. However, a possible alternative explanation is that postjunctional α_2-adrenoceptor activation by clonidine, which alone does not produce vasoconstriction, produces a functional antagonism of the responses to noradrenaline. This view is supported by the observations of Fiotakis & Pipili (1983) who found that the α_2-adrenoceptor agonist UK-14,304 markedly attenuated the pressor responses to noradrenaline in the *in vitro* isolated perfused mesentery of the rat and that the responses to noradrenaline were enhanced by α_2-adrenoceptor blockade with rauwolscine. Arising from the renewed interest in the role of the endothelium in the maintenance of vascular tone, it has been shown that the pressor actions of a wide range of α-adrenoceptor agonists are potentiated by the removal of the endothelial cell layer (Miller *et al.*, 1984; Lues & Schumann, 1984; Eglème *et al.*, 1984). However from the extensive study undertaken by Lues & Schumann (1984) there seems to be some uncertainty as to the precise nature of the subtype of α-adrenoceptor involved.

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We have previously demonstrated that the *in situ* autoperfused superior mesenteric arterial bed of the rat appears to possess postjunctional vasoconstrictor α-adrenoceptors exclusively of the α_1-subtype (Nichols & Hiley, 1985). In addition we have observed that high doses of xylazine and clonidine, which although they produce little or no pressor responses in themselves, markedly attenuate the pressor response to noradrenaline (Hiley & Nichols, 1983). In this paper we set out to investigate the interactions between α_2-adrenoceptor agonists and the α_1-adrenoceptor-mediated pressor responses to noradrenaline in both the *in situ* autoperfused and Krebs-perfused *in vitro* preparations of the superior mesenteric arterial bed of the rat.

Methods

In vivo *preparations*

Male Wistar rats (230–250 g; Bantin & Kingman, Hull) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.; Sagatal, May & Baker). The animals were prepared for the *in situ* blood perfusion of the superior mesenteric arterial bed as described previously by Nichols & Hiley (1985). Briefly, the trachea was cannulated as were the jugular vein, for the administration of drugs and saline, and the right carotid artery, for measurement of systemic arterial blood pressure (Bell & Howell pressure transducer Type 4-422-0001) and heart rate (Grass 7P44 tachograph using the pressure trace as the trigger). The abdomen was opened, the abdominal aorta and the superior mesenteric artery dissected free and after a 20 min halt to allow haemostasis to occur, the animals were heparinised (1000 u kg^{-1}, i.v.). The abdominal aorta was cannulated with the inflow cannula of the perfusion circuit which consisted of a Harvard peristaltic perfusion pump (Type 2903), a trapped air system and a heat exchanger. After filling the extracorporeal circuit with blood the outflow cannula of the system was inserted into the superior mesenteric artery and perfusion was started at a rate of 2 ml min^{-1}.

Noradrenaline was given into the extracorporeal circuit in volumes of up to 50 μl every 2–5 min and the effects of xylazine were studied 10 min after its administration (0.5 mg in 50 μl) into the extracorporeal circuit. Yohimbine was administered intravenously 15 min before the construction of noradrenaline dose-response curves or 5 min before the administration of xylazine.

Rectal temperature was measured and maintained at 37 ± 1°C by means of a homeothermic blanket (Bioscience, Sheerness, Kent) and during perfusion the animals received an intravenous infusion of saline (0.9% NaCl w/v) at 0.1 ml min^{-1} to prevent volume depletion.

In vitro *preparations*

The Krebs-perfused, isolated mesenteric vascular bed of the rat was prepared essentially as described by McGregor (1965). Male Wistar rats (300–350 g; Bantin & Kingman, Hull) were anaesthetized as described above. Following heparinisation (1000 u kg^{-1} i.v.) the superior mesenteric artery was cannulated and the associated vascular arcade perfused at 2 ml min^{-1} with Krebs-Henseleit solution containing (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 5.5. The Krebs-Henseleit solution was gassed with 5% CO_2 in O_2 and maintained at 37 ± 1°C. The mesentery was then dissected clear of the abdomen and placed on a perspex water-heated block and maintained again at 37 ± 1°C. The tissue was overlayed with Nescofilm to minimize surface damage. Perfusions were carried out at constant flow (2 ml min^{-1}) by use of a Harvard peristaltic perfusion pump (Type 1203A). Prior to all experiments the preparation was allowed to stabilise for 45–60 min.

Noradrenaline was given into the perfusion system in volumes of either 30 or 100 μl. Yohimbine (1.0×10^{-6} M) was dissolved in the perfusion fluid reservoir and its effects studied after 20 min equilibration. α_2-Adrenoceptor agonists, xylazine, clonidine and guanfacine, were administered as bolus doses in 50 μl.

In both *in vitro* and *in vivo* experiments responses were recorded as changes in perfusion pressure measured with Bell and Howell pressure transducers (Type 4-422-0001) placed close to the end of the outflow cannulae and coupled to Grass model 79D polygraphs.

Drugs

Stock solutions (1 mg ml^{-1}) of noradrenaline bitartrate (Koch Light) were prepared in saline containing ascorbic acid (1 mg ml^{-1}). Stock solutions of yohimbine hydrochloride (1 mg ml^{-1} or 1 mM; Sigma), xylazine (1 mg ml^{-1}; the gift of Bayer UK) and clonidine hydrochloride (10 mg ml^{-1}; the gift of Boehringer Ingelheim) were prepared in saline. Guanfacine hydrochloride (10 mg ml^{-1}; the gift of Sandoz) was dissolved in ethanol. All drug solutions were prepared fresh daily. Drug dilutions were made in saline.

Data analysis

Each log dose-response curve was fitted to the logistic equation:

$$R = \frac{R_{max} \times (A)^n}{(ED_{50})^n + (A)^n}$$

in which R is the response and A is the concentration of agonist. The unknowns R_{max}, ED_{50} and n are respectively the maximum response, the dose producing half maximal response and the slope function. A modified Marquardt procedure was used as implemented in the Harwell routine VB01A on the Cambridge University IBM 3081 (Aceves *et al.*, 1985). For each preparation the midrange sensitivity was calculated as the negative log ED_{50} (pD_{50}). In the *in situ* experiments a true maximum was not obtained (see Figure 1) because, after such a response, the preparation rapidly deteriorated. Consequently, the highest response obtained within the range tested was taken as a maximum value. All the data are presented as the mean ± one standard error of the mean and the statistical significance of differences between means was determined by analysis of variance followed by the least significance difference procedure (Snedecor & Cochran, 1980).

Results

Noradrenaline produced dose-related increases in mesenteric arterial perfusion pressure in both *in vivo* and *in vitro* preparations. The responses were stable for up to 2 h *in vivo* and 5 h *in vitro* and have previously been shown to be mediated by α_1-adrenoceptors since they can be blocked with selective doses of prazosin (10 μg kg^{-1}) (Nichols & Hiley, 1985; Nichols, 1985).

In situ *blood perfusion of superior mesenteric arterial bed*

Xylazine (1.9×10^{-6} mol; 0.5 mg) administered into the superior mesenteric artery *in situ* produced a small transient increase in perfusion pressure of 6.0 ± 0.5 mmHg ($n = 7$). Figure 1a shows that this dose of xylazine produced a shift to the right of the noradrenaline log dose-response curve with reductions in the slope and the maximum response which persisted for the remaining course of the experiment (up to 1 h). When determined 10 min after the administration of xylazine, the mean systemic arterial pressure was reduced by 39 ± 6 mmHg (from 126 ± 4 mmHg) and heart rate was lowered by 81 ± 10 beats min^{-1} (from 456 ± 9 beats min^{-1}; $n = 7$). These effects persisted throughout the rest of the experiment.

The α_2-antagonist yohimbine, given at a dose of 1 mg kg^{-1}, produced a small parallel shift of 1.77 ± 0.04 ($n = 6$) in the noradrenaline log dose-response curve (Figure 1b). In another group of animals, administration of 1.9×10^{-6} mol xylazine 5 min after yohimbine increased perfusion pressure in the superior mesenteric arterial bed by 10 ± 2 mmHg ($n = 5$). Subsequent determination of the noradrenaline log dose-response curve gave a dose-ratio for the combined administration of yohimbine followed by xylazine of 2.92 ± 0.36 ($n = 5$; Figure 1c) relative to the curve obtained in untreated animals; this was

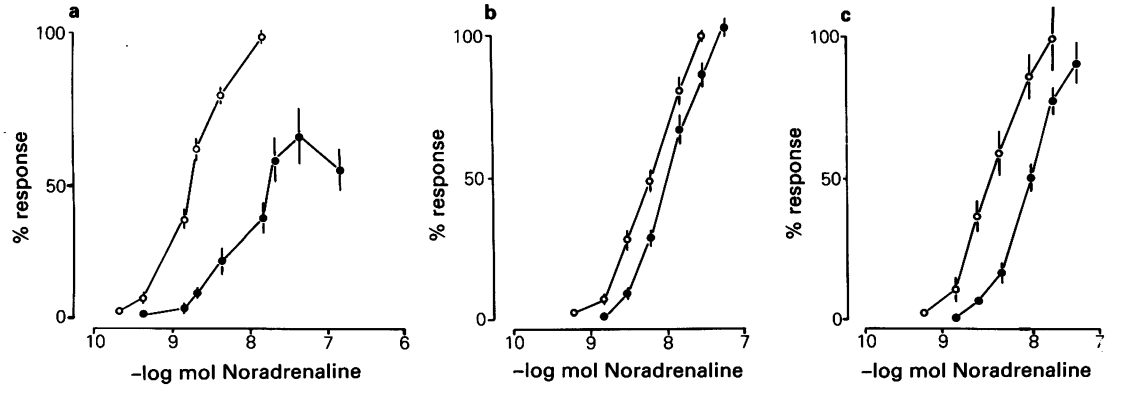

Figure 1 Percentage increases in perfusion pressure in response to noradrenaline administered into the blood-perfused superior mesenteric arterial bed of the rat *in situ*. (a) (○) Control; (●) responses after a bolus dose of xylazine (1.9×10^{-6} mol) administered into the perfusion circuit. (b) (○) Control; (●) responses after the i.v. injection of yohimbine (1 mg kg^{-1}). (c) (○) Control; (●) responses after the i.v. administration of 1 mg kg^{-1} yohimbine (i.v.) and 1.9×10^{-6} mol xylazine given into the perfusion circuit. Each point represents the mean of the results obtained in (a) 7, (b) 6 and (c) 5 experiments. Values are expressed as a percentage of the maximum response obtained in each preparation and the vertical bars indicate ± 1 s.e.mean.

significantly greater ($P < 0.01$) than that produced by yohimbine alone.

It should be noted that after yohimbine, xylazine did not significantly lower mean systemic arterial pressure or produce a bradycardia; the mean changes were 4.0 ± 3.0 mmHg and 12 ± 4 beats min^{-1} ($n = 5$).

In vitro *perfusion of the isolated superior mesenteric arterial bed*

Administration of 1.9×10^{-6} mol xylazine into this preparation did not produce any increase in perfusion pressure. However, this single dose produced a long lasting (it was followed for up to 120 min) diminution of $37 \pm 7\%$ ($P < 0.01$) in the maximal response to noradrenaline but, in contrast to the *in vivo* preparation, there was no significant change in the mid-point sensitivity; the pD_{50} values were 7.98 ± 0.14 and 7.90 ± 0.08 respectively for the control and xylazine-treated log dose-response curves (Figure 2a).

Figure 2b shows that yohimbine, in the perfusing solution at a concentration of 1.0×10^{-6}M, caused a parallel shift in the noradrenaline log dose-response curve with a dose-ratio of 10.5 ± 4.3 ($P < 0.05$). When xylazine was administered after yohimbine there was still a reduction of $52 \pm 6\%$ ($P < 0.01$) in the maximal response to noradrenaline and there was no significant rightward displacement of the noradrenaline log dose-response curve ($pD_{50} = 6.96 \pm 0.32$) relative to that obtained in the presence of yohimbine alone ($pD_{50} = 7.02 \pm 0.13$).

The administration of clonidine (1.9×10^{-6} mol; 0.5 mg) as a bolus dose resulted in no reduction in the maximal response of the preparation to noradrenaline (Figure 3a). However, it was observed that clonidine caused a rightward displacement of the noradrenaline log dose-response curve; the pD_{50} in the absence and presence of clonidine were respectively 7.72 ± 0.17 and 6.91 ± 0.17 ($n = 6$; $P < 0.05$). Yohimbine (1.0×10^{-6}M) had little effect on the decreased sensitivity of the preparation to noradrenaline observed in the presence of clonidine alone (Figure 3b).

Guanfacine (1.8×10^{-6} mol; 0.5 mg) was without effect on the sensitivity of the tissue to noradrenaline. The pD_{50} values for the responses to noradrenaline after the ethanol vehicle and in the presence of guanfacine were 8.19 ± 0.15 and 8.01 ± 0.15 respectively (Figure 4a). Guanfacine did however cause a depression in the maximal response of the tissue to noradrenaline by $20 \pm 6\%$ ($P < 0.01$), but this did not occur in experiments with the prior addition of yohimbine (1.0×10^{-6}M) to the perfusion fluid. Yohimbine (1.0×10^{-6}M) caused a shift in the noradrenaline dose-response curve to the right, whilst the vehicle (ethanol) produced no further effect on the sensitivity of the tissue to noradrenaline (Figure 4b).

Discussion

This study clearly shows an interaction between a range of α_2-adrenoceptor agonists and the response to noradrenaline in the mesenteric vascular bed of the rat, which is a vasoconstriction previously shown probably to be mediated purely through α_1-adrenoceptors (Fiotakis & Pipili, 1983; Nichols & Hiley, 1985; Nichols, 1985).

When administered via the superior mesenteric artery to the intact animal, xylazine produced systemic haemodynamic effects which were maintained throughout the experiment. Since this indicates that

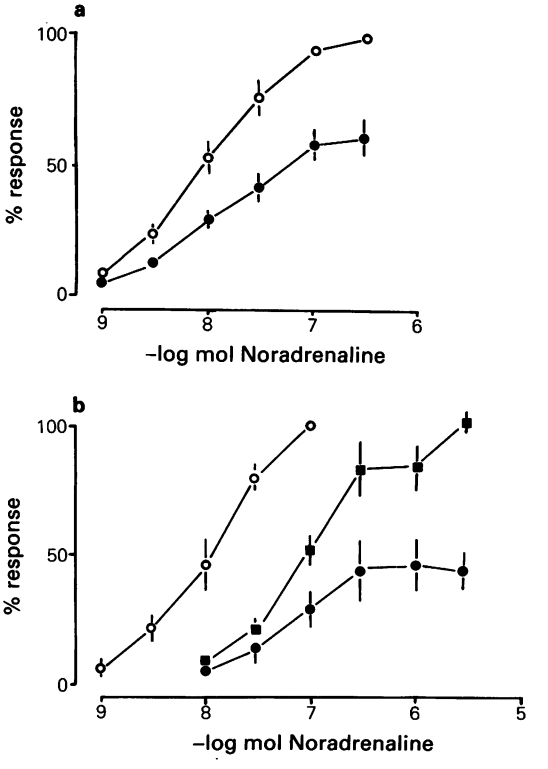

Figure 2 Percentage increases in perfusion pressure in response to noradrenaline in the superior mesenteric arterial bed of the rat perfused *in vitro* with Krebs-Henseleit solution. (a) (○) Control; (●) responses after a single dose of xylazine (1.9×10^{-6} mol). (b) (○) Control; (■) responses in the presence of 10^{-6}M yohimbine and (●) responses in the presence of 10^{-6}M yohimbine and after 1.9×10^{-6} mol xylazine given in the presence of the yohimbine. Each point is the mean of results obtained in 6 experiments and the vertical bars represent ± 1 s.e.mean. All values were calculated as a percentage of the maximum response to noradrenaline obtained in the control for each preparation.

xylazine was distributed throughout the vasculature in pharmacological levels it is impossible to state whether the actions of xylazine were due to an initial exposure to the drug or a consequence of its continued presence in the circulation. Parallel data obtained from *in vitro* Krebs-perfused mesenteric bed preparations suggest that part of the inhibitory effect of xylazine is due to an initial exposure to the drug, although only the maximum was altered *in vitro*. A similar observation was reported to occur in response to the α_2-adrenoceptor agonist UK-14,304 in the saline-perfused rat mesentery by Cambridge (1981) and by Fiotakis & Pipili (1983).

The actions of xylazine in mesenteric beds perfused *in vivo* and *in vitro* differed when yohimbine was used to discriminate the involvement of α_2-adrenoceptors. *In vivo*, pretreatment of the rats with $1\,mg\,kg^{-1}$ yohimbine, which produces dose-ratios of approx-

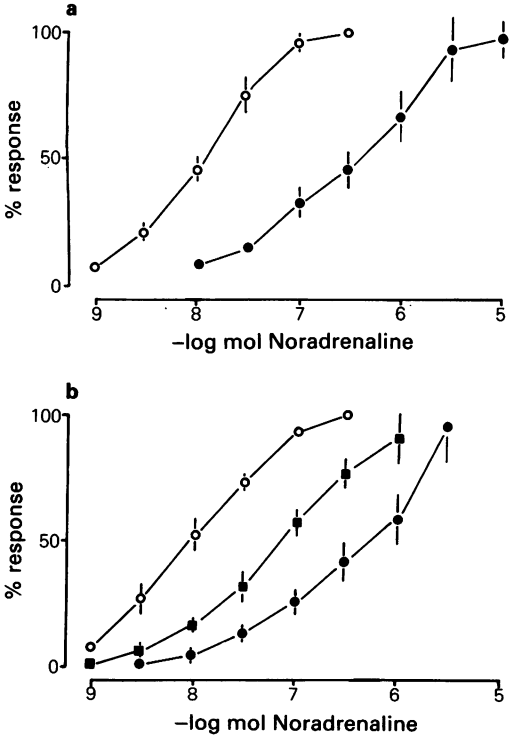

Figure 3 Percentage increases in perfusion pressure in response to noradrenaline in the superior mesenteric arterial bed of the rat perfused *in vitro* with Krebs-Henseleit solution. (a) (○) Control; (●) responses after a single dose of clonidine (1.9×10^{-6} mol). (b) (○) Control; (■) responses in the presence of 10^{-6} M yohimbine and (●) responses in the presence of 10^{-6} M yohimbine and after 1.9×10^{-6} mol clonidine given in the presence of the yohimbine. Each point is the mean of results obtained in 6 experiments and the vertical bars represent ± 1 s.e.mean. All values were calculated as a percentage of the maximum response to noradrenaline obtained in the control curve for each preparation.

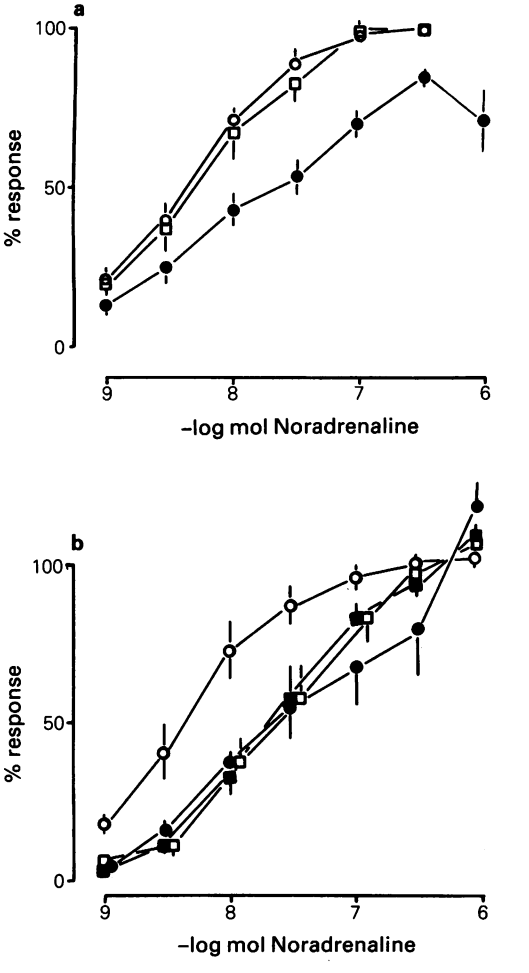

Figure 4 Percentage increases in perfusion pressure in response to noradrenaline in the superior mesenteric arterial bed of the rat perfused *in vitro* with Krebs-Henseleit solution. (a) (○) Control; (□) responses after a bolus injection of ethanol vehicle (50μl) and (●) responses after a single dose of guanfacine (1.8×10^{-6} mol). (b) (○) Control; (■) responses in the presence of 10^{-6} M yohimbine; (□) responses in the presence of yohimbine and after 50μl ethanol vehicle and (●) responses obtained in the presence of 10^{-6} M yohimbine obtained after administration of 1.8×10^{-6} mol guanfacine in the presence of the yohimbine. Each point is the mean of results obtained in 6 experiments and the vertical bars represent ± 1 s.e.mean. All values were calculated as a percentage of the maximum response obtained to noradrenaline in the control curve for each preparation.

imately 15 for α_2-adrenoceptor agonists and 2 for α_1-adrenoceptor agonists in pithed rats (van Meel *et al.*, 1981), resulted in a small (2 fold) parallel rightward shift of the noradrenaline dose-response curve. It was also observed that the very small vasoconstrictor response to xylazine was not reduced. This supports the earlier conclusion of Nichols & Hiley (1985) that, under these conditions, there do not appear to be any postjunctional α_2-adrenoceptors mediating vasoconstriction in this vascular bed. The small increase in perfusion pressure following xylazine administration may therefore be mediated by weak or partial agonist action on α_1-adrenoceptors (Kobinger & Pichler, 1981). Yohimbine appeared to prevent the depression of the noradrenaline maximum pressor response in the mesentery after combined administration of yohimbine and xylazine. Also the log dose-response curves we obtained were parallel, but it must be noted that we did not record true maxima in these experiments. However, yohimbine abolished the systemic hypotension and bradycardia observed following xylazine administration which would support the conclusion that these effects are all mediated by xylazine acting on α_2-receptors.

By contrast, yohimbine had quite different actions on the effects of xylazine *in vitro*. At the dose used, it was observed to produce a rightward shift in the noradrenaline dose/pressor-response curve, but its effect on the depressant action of xylazine on the noradrenaline response contrasted with the reversal observed *in vivo*. In the *in vitro* preparation, xylazine did not change the pD_{50} value for the noradrenaline response, but depressed the maximal response. In the presence of yohimbine there was again no change in the pD_{50} value of the noradrenaline dose-response curve following xylazine administration but the depression of the maximal response persisted. This failure of yohimbine to affect the actions of xylazine would suggest that the depression of the noradrenaline response by xylazine was not mediated by α_2-adrenoceptors.

Analysis of the effects of yohimbine on the actions of clonidine shows that the combination of yohimbine and clonidine produces the same rightward shift of the noradrenaline dose-response curve as did yohimbine alone, suggesting that yohimbine has no effect on the actions of clonidine. It is therefore doubtful that the antagonistic action of clonidine on the pressor response to noradrenaline in this preparation is the result of activation of α_2-adrenoceptors. Conversely, the weak antagonistic effect of guanfacine on the maximum pressor response to noradrenaline was blocked by the introduction of yohimbine to the perfusate suggesting at least an α_2-adrenoceptor-mediated component to this effect of guanfacine.

There are some apparent disparities between the actions of xylazine observed *in vivo* and *in vitro* with the superior mesenteric arterial bed of the rat. These may be explained by the central actions of xylazine and yohimbine in the intact animal. Yohimbine antagonizes the central α_2-adrenoceptor-mediated depressor response to xylazine,as shown here by the lack of a hypotension of bradycardia upon xylazine administration when the animals were pretreated with yohimbine. Animals pretreated with yohimbine therefore showed little or no change in sympathetic outflow from the central nervous system when xylazine was administered. In addition yohimbine competitively antagonizes presynaptic α_2-adrenoceptors thus potentiating the actions of 'normal' sympathetic outflow wherever feedback is present. These positive actions, when summated with those of exogenously added noradrenaline, could well surmount the inhibitory actions of xylazine in the mesenteric vasculature which, as suggested by the results of *in vitro* experiments, are unlikely to be mediated via α_2-adrenoceptors. This could possibly explain why yohimbine was capable of reversing the depressant action of xylazine only in the *in vivo* preparations. However, it must be noted that in preliminary experiments, we found that sympathetic influence on perfusion pressure in the mesenteric arterial bed of the rat was slight, since pithing or deepening of anaesthesia caused only 5 mmHg fall in the resting perfusion pressure.

In the *in vitro* study, it is also noteworthy that the most potent antagonists of the pressor response to noradrenaline were the aryl amidine containing compounds, whereas guanfacine, having a different structure, was on a molar basis much less potent in its actions. It is therefore possible that the antagonistic actions of xylazine and clonidine on the noradrenaline pressor response are possibly a consequence of their azathiozolidine/imidazolidine structure, rather than their binding to α_2-adrenoceptors. On the other hand, there does appear to exist a functional postsynaptic α_2-adrenoceptor in the superior mesenteric arterial bed which is inhibitory; this was suggested by Fiotakis & Pipili (1983) who reported that rauwolscine potentiated the vasoconstrictor effects of noradrenaline and is supported by our observations with the non-imidazolidine guanfacine. It should be noted that Fiotakis & Pipili (1983) also conclude that prostaglandins play a significant role in the responses to noradrenaline in this preparation. It is therefore of interest to note that 1-*n*-butylimidazole is a potent inhibitor of thromboxane synthetase (Blackwell *et al.*, 1978) thus, as suggested by Blackwell *et al.* (1978), there may be diversion of the metabolism of arachidonic acid towards the production of prostacyclin (PGI_2), a vasodilator (Armstrong *et al.*, 1978).

Another possibility for this high potency of the aryl amidine containing compounds (which include the imidazolidine, clonidine, and the azathiozolidine, xylazine) in antagonizing the pressor responses to

noradrenaline could be the existence of a binding site, separate and discrete from the α_2-adrenoceptor binding site, as described for clonidine by Bond *et al.* (1985). As a consequence of either of these proposed mechanisms other related compounds should display similar properties to those described for clonidine and xylazine. It is worth noting here that Lues & Schumann (1984) concluded that the receptor on endothelial cells of rat aorta which modulated constrictor responses to noradrenaline was not an α_1-adrenoceptor nor was it clearly of the α_2-subtype.

In conclusion, it is clear that xylazine and clonidine have a complex depressant effect on vasoconstrictor responses to noradrenaline in the rat superior mesenteric arterial bed and that, *in vitro*, these effects do not appear to be mediated by α_2-adrenoceptors. The precise nature of these effects is difficult to determine, but may involve humoral factors of either eicosanoid origin or endothelial-derived factors of as yet an undetermined nature such as endothelium-derived relaxant factor (EDRF; Furchgott, 1983). Such mechanisms have been previously associated with the vascular actions of xylazine, clonidine and guanfacine (Lues & Schumann, 1984).

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