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α_2-Adrenoceptor agonists enhance responses to certain other vasoconstrictor agonists in the rat tail artery

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1 The effects of the α_2-adrenoceptor agonists clonidine, rilmenidine, TL99 and UK14304 on the vasoconstrictor response to sympathetic nerve stimulation and on the concentration-response curves to noradrenaline and phenylephrine were compared in two isolated, perfused vascular tissues: the rat tail artery (which has both postjunctional α_1- and α_2-adrenoceptors), and the rabbit ear artery (in which only α_1-adrenoceptors are present postjunctionally).

2 In the rabbit ear artery, the first observable effect of α_2-adrenoceptor agonists was inhibition of vasoconstrictor responses to sympathetic nerve stimulation. This occurred with concentrations of the α_2-adrenoceptor agonists which were far below those producing vasoconstriction. Responses to noradrenaline were not affected.

3 In contrast, in the rat isolated perfused tail artery, α_2-adrenoceptor agonists, in concentrations that produced no other observable effects, enhanced the vasoconstrictor responses to sympathetic nerve stimulation and to noradrenaline. Much higher concentrations of α_2-adrenoceptor agonists produced vasoconstriction in most preparations and only then reduced the response to sympathetic nerve stimulation. The enhancing effect of α_2-adrenoceptor agonists was blocked by idazoxan, but not by prazosin.

4 Vasoconstrictor responses in the rat tail artery to the relatively selective α_1-adrenoceptor agonist phenylephrine were enhanced by α_2-adrenoceptor agonists. The enhancement of the response to phenylephrine was greater than that to the mixed α_1- and α_2-adrenoceptor agonist noradrenaline.

5 Vasoconstrictor responses in the rat tail artery to vasopressin, ATP and KCl, like those to α_1-adrenoceptor agonists, were enhanced by α_2-adrenoceptor agonists. However, vasoconstrictor responses to 5-hydroxytryptamine and angiotensin II were not enhanced.

Introduction

α_2-Adrenoceptors were first identified on noradrenergic nerve terminals where their activation leads to inhibition of transmitter release (Langer, 1974; Starke & Langer 1979). They were subsequently demonstrated in vascular smooth muscle in which they subserve vasoconstriction, resulting in a pressor response in intact animals (Docherty *et al.*, 1979; Drew & Whiting, 1979; Timmermans *et al.*, 1979; Timmermans & Van Zwieten, 1980), and contraction in isolated preparations of certain blood vessels (Medgett *et al.*, 1981; Holtz *et al.*, 1982; Hicks *et al.*, 1983). Recently a number of authors have shown that postjunctional α_2-adrenoceptors are involved in several physiological functions in various tissues (Berridge *et al.*, 1983; Reid, 1985; Strandhoy, 1985; Skoglund *et al.*, 1987).

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Shepperson (1984) demonstrated that the contraction of the nictitating membrane of the cat evoked by α_1-adrenoceptor agonists was enhanced by predosing with α_2-adrenoceptor agonists. He suggested that, in some tissues, the action of α_2-adrenoceptor agonists may be modulation of the response to α_1-adrenoceptor activation rather than to evoke a discrete response. This was extended by our previous study on the rat tail artery (Xiao *et al.*, 1987b; Xiao & Rand, 1987), which gave rise to the hypothesis that α_2-adrenoceptor agonists do not directly produce vasoconstriction but provide an ancillary drive to the vasoconstrictor stimulus produced by α_1-adrenoceptor agonists.

We have now compared the effects of α_2-adrenoceptor agonists on responses to sympathetic nerve stimulation and α_1-adrenoceptor agonists in two different vascular tissues *in vitro*: the

rat tail artery, which has postjunctional α_2-adrenoceptors (Weiss *et al.*, 1983; Medgett & Langer, 1984), and the rabbit ear artery, which lacks postjunctional α_2-adrenoceptors (Hieble & Woodward, 1984). We have also studied the effects of α_2-adrenoceptor agonists on responses to various other agents producing vasoconstriction in the rat tail artery.

Methods

The animals used were male Sprague-Dawley rats from our own colony, weighing 250–350 g and New Zealand white rabbits (Tillotside Rabbit Stud, South Australia) of either sex weighing 2–3 kg. The rats were anaesthetized with pentobarbitone (30 mg kg^{-1}, i.p.), and the rabbits were killed by a blow to the neck and exsanguination. Segments of approximately 1 cm long of the rat tail artery and the rabbit central ear artery were dissected out as described by Medgett & Langer (1984, 1986) and de la Lande & Rand (1965), respectively.

The segment of artery was cannulated at the proximal end under a dissecting microscope. The distal end was tied and an opening was made in the wall just below the tie. The segment was mounted vertically, distal end uppermost, under a tension of 0.5 g and perfused, using an LKB peristaltic pump, with a modified Krebs solution at a rate of 4 ml min^{-1}, which has been shown previously to be satisfactory for preparations of both the rabbit ear artery (de la Lande & Rand, 1965) and the rat tail artery (Medgett & Rajanayagam, 1984). The perfusate, after passing through the lumen, was allowed to superfuse the adventitial surface of the vessel. The perfusion pressure was measured with a Statham P23Db pressure transducer and recorded on a Rikadenki chart recorder. Increases in perfusion pressure in mmHg were used to provide a measure of vasoconstrictor responses. Two platinum ring electrodes, 4 mm apart, were located around the proximal end of the artery segments for electrical stimulation of the periarterial sympathetic nerves. The preparations were surrounded by a water jacket maintained at 37°C. A 30 min period of perfusion-superfusion was allowed to elapse before any experimental procedures were carried out.

Stimulation of periarterial nerves was with monophasic square wave pulses of 1 ms duration and supramaximal voltage at a frequency of 2 Hz (delivered from a Scientific & Research Instruments Ltd stimulator). Each period of stimulation was continued until the rise in perfusion pressure had reached the peak level (usually 5–10 s). Vasoconstrictor responses to drugs were obtained by replacing the normal perfusing solution with one containing the required concentration of the agonist, which was subsequently perfused until the response reached a peak value (usually 1–2 min). Depolarization-evoked contractions were obtained by replacing the normal solution with one in which the equivalent amounts of NaCl were replaced by 24–64 mM KCl. Care was taken to limit rises in perfusion pressure to less than 300 mmHg to avoid damage and desensitization of the preparation (Medgett & Rajanayagam, 1984).

Rat tail artery segments were considered to be satisfactory if rises in perfusion pressure of 40 mmHg or more were elicited with stimulation at 2 Hz for 10 s or less. For the rabbit ear artery, the vasoconstrictor effect of noradrenaline was observed to establish that the responsiveness was satisfactory.

The effects of most vasoconstrictor agents were studied by constructing two non-cumulative concentration-response curves; the second was begun 30 min after completing the first. In experiments in which the effects of α_2-adrenoceptor agonists on vasoconstrictor agents were to be assessed, they were added to the perfusing solution after the control responses were established and remained present for the rest of the experiment.

Perfusion solution

The modified Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1.03, D-glucose 11.1, disodium edetate 0.067. The solution in the supply reservoir was continuously gassed with a mixture of 5% CO_2 in O_2. It passed through a heat-exchanger maintained at 37°C immediately before it entered the perfusion cannula.

Drugs

The following drugs were used: angiotensin II (AII; hypertensin, Ciba-Geigy); adenosine 5'-triphosphate (ATP), sodium salt (Sigma); clonidine hydrochloride (Boehringer Ingelheim); 5-hydroxytryptamine creatinine sulphate complex (5-HT, Sigma); idazoxan hydrochloride (Reckitt & Colman); (−)-noradrenaline hydrochloride (Sigma); rilmenidine (formerly oxaminozoline) phosphate (Servier); phenylephrine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); TL99 (2-(*NN*-dimethyl)amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, synthesized by Synthelabo, L.E.R.S.); UK14304 (5-bromo-6-[2-imidazoline-2-Yl-aminol]-quinoxaline, Pfizer); vasopressin (Parke-Davis). Stock solutions of noradrenaline, TL99 and 5-hydroxytryptamine were made in distilled water which contained ascorbic acid (0.05 mg ml^{-1}). Other drugs were dissolved in distilled water.

Statistical analysis

Values are given as mean ± s.e.mean and comparisons were by Student's paired or unpaired t test, as appropriate. The statistical significance of shifts of concentration-response curves was determined from comparison of the analyses of regression of response on log concentration for the linear portions of the curves; where slopes did not differ, horizontal displacements and their significances were calculated. Probability levels of less than 0.05 were taken as indicating significant differences.

Results

Effects of α_2-adrenoceptor agonists on responses to sympathetic nerve stimulation

In the rabbit ear artery, infusions of clonidine (10 to 300 nM) produced marked concentration-dependent reductions of vasoconstrictor responses to sympathetic nerve stimulation, as illustrated in Figure 1a. With concentrations of 10 and 30 nM, clonidine had no effect on the resting perfusion pressure, but the higher concentrations (100 and 300 nM) produced vasoconstriction; when this occurred, the inhibitory effect was taken from the raised level of perfusion pressure.

In the rat tail artery, infusions of rilmenidine in concentrations that had no direct vasoconstrictor action (0.01 to 3 μM) enhanced the vasoconstrictor responses to sympathetic nerve stimulation significantly (paired t test), as illustrated in Figure 1b. When the concentration of rilmenidine was raised to 10 μM, the mean vasoconstrictor response to sympathetic nerve stimulation did not differ significantly from the pre-rilmenidine level. In a few experiments, 10 μM rilmenidine increased the resting perfusion pressure. The highest concentration of rilmenidine used in this series of experiments (30 μM) usually produced vasoconstriction and significantly inhibited the response to sympathetic nerve stimulation. However, in other experiments it was noted that a vasoconstrictor effect only occurred when the concentration of rilmenidine was raised to 100 μM.

Similar patterns of effects to those described above for clonidine on the rabbit ear artery and rilmenidine on the rat tail artery were obtained with the other α_2-adrenoceptor agonists used in this study, but we have not as yet made systematic quantitative comparisons between the various agonists.

Effects of α-adrenoceptor antagonists in the rat tail artery

The relatively selective α_2-adrenoceptor antagonist idazoxan (300 nM) had no effect on the resting perfusion pressure. It reduced vasoconstrictor responses

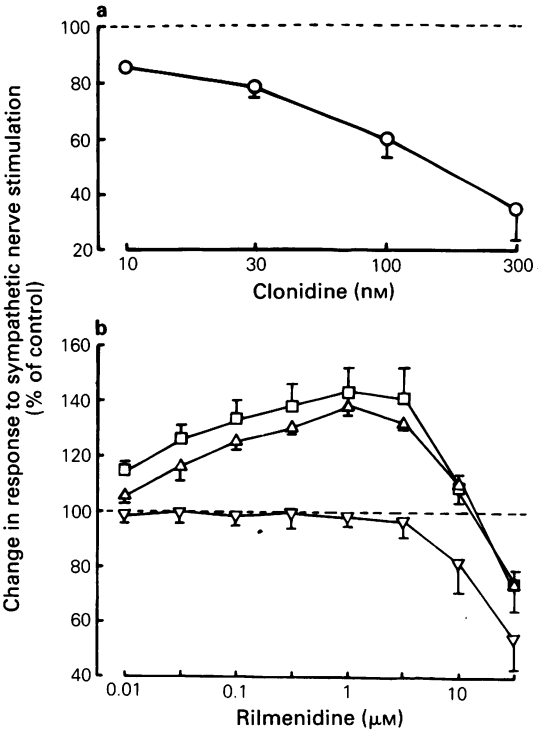

Figure 1 Effects of α_2-adrenoceptor agonists on vasoconstrictor responses to sympathetic nerve stimulation (1 ms pulses at 2 Hz for 10 s at 5 min intervals) in isolated perfused arteries. (a) Effects of clonidine in rabbit ear arteries ($n = 5$). (b) Effects of rilmenidine in rat tail arteries (□, $n = 8$), together with its effects in the presence of prazosin (0.3 nM, △, $n = 4$) or in the presence of idazoxan (300 nM, ▽, $n = 4$). Symbols indicate mean values and vertical lines show s.e. There were no significant differences between the means for rilmenidine alone and the corresponding means for rilmenidine in the presence of prazosin (t test).

to sympathetic nerve stimulation by 14 ± 8% ($n = 10$) of the control level, but this was not significant. Idazoxan (300 nM) abolished the enhancement by rilmenidine of the stimulation-induced vasoconstrictor responses, as illustrated in Figure 1b. The direct vasoconstrictor response to the higher concentrations of rilmenidine was not affected by idazoxan. The effect of the higher concentrations of rilmenidine in reducing responses to sympathetic nerve stimulation still occurred in the presence of 300 nM idazoxan (Figure 1b).

The relatively selective α_1-adrenoceptor antagonist prazosin (0.3 nM) markedly reduced vasoconstrictor responses to sympathetic nerve stimulation by 32.8 ± 2.7 ($n = 13$) of the control level. The enhancement by rilmenidine of the responses to sympathetic nerve stimulation in the presence of prazosin did not

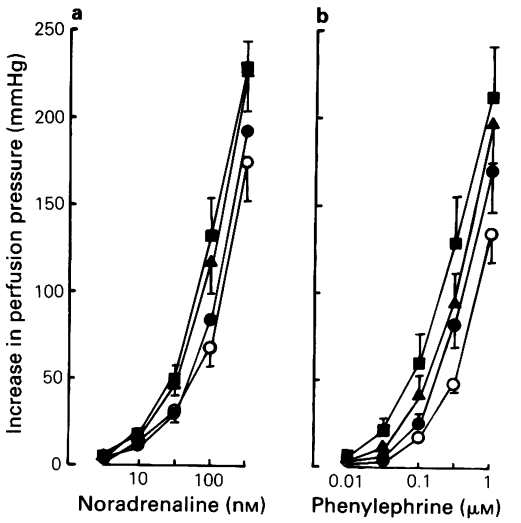

Figure 2 Concentration-response curves for (a) noradrenaline- and (b) phenylephrine-induced vasoconstriction in rat perfused tail arteries. (○) First curves for vasoconstrictor agonist alone (a: $n = 5$; b: $n = 6$). (●), (▲) and (■) second curves in the presence of 10, 100 or 1000 nM rilmenidine, respectively ($n = 6$ for each drug at each concentration). Symbols indicate mean values and vertical lines show s.e.

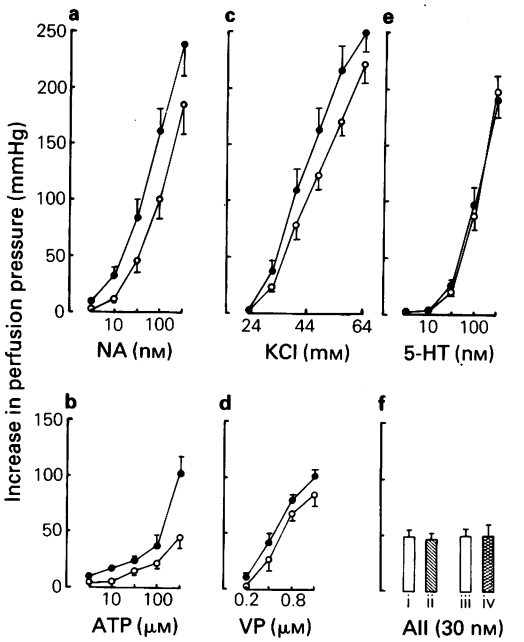

Figure 3 Effects of UK14304 on responses to vasoconstrictor agents in rat isolated perfused tail arteries. (a) Responses to noradrenaline (NA, $n = 6$) in the absence (○) and presence (●) of UK14304 (10 nM). (b–e) Responses to (b) ATP ($n = 4$), (c) KCl ($n = 4$), (d) vasopressin (VP, $n = 5$) and (e) 5-hydroxytryptamine (5-HT, $n = 4$) in absence (○) and presence (●) of UK14304 (30 nM). (f) Responses to 30 nM angiotensin II (AII, $n = 4$): (i) and (iii) are first responses; (ii) and (iv) are second responses in the absence and presence, respectively, of UK14304 (30 nM).

differ significantly from that in its absence (Figure 1b). The vasoconstrictor effect of the higher concentrations of rilmenidine was reduced by prazosin; with 30 μM rilmenidine, 0.3 nM prazosin reduced the vasoconstrictor response to 55 ± 2% ($n = 4$) of the control level. The inhibitory effect of the high concentration of rilmenidine on responses to sympathetic nerve stimulation was not affected by prazosin (Figure 1b).

Qualitatively similar effects of idazoxan and prazosin on the actions of the other α_2-adrenoceptor agonists used in this study have been observed, but no quantitative comparisons have been made.

Effects of α_2-adrenoceptor agonists on responses to noradrenaline and phenylephrine

In the rat tail artery, when two concentration-response curves for either noradrenaline (3–300 nM) or phenylephrine (0.01 to 1 μM) were obtained in succession, the second was virtually superimposable on the first. In the rabbit ear artery, the second concentration-response curve for noradrenaline (3–300 nM) exhibited a slight but not statistically significant time-dependent shift to the left.

When the second concentration-response curve for noradrenaline or phenylephrine in the rat tail artery was obtained in the presence of rilmenidine (0.01–1 μM), it was shifted to the left (Figure 2). The lowest concentration of rilmenidine used (0.01 μM) did not significantly shift the curve for noradrenaline but significantly shifted that for phenylephrine. The other α_2-adrenoceptor agonists used in this study had qualitatively the same effects as rilmenidine on concentration-response curves for noradrenaline and phenylephrine, but a systematic quantitative comparison has not yet been made. However, a comparison of the data in Figures 2a and 3a shows that 1000 nM rilmenidine and 10 nM UK14304 produced about the same shift to the left of the concentration-response curve for noradrenaline.

In the presence of α_2-adrenoceptor agonists at concentrations which significantly shifted the concentration-response curve for noradrenaline to the left in the rat tail artery, there was no significant effect on responses to noradrenaline in the rabbit ear artery.

Effect of α_2-adrenoceptor agonists on response to various types of vasoconstrictor agents in rat tail artery

The effects of α_2-adrenoceptor agonists, exemplified by UK14304, on vasoconstrictor responses to noradrenaline, KCl, 5-hydroxytryptamine, ATP, vasopressin, and angiotensin II are illustrated in Figure 3. UK14304 had no direct vasoconstrictor action at the concentrations used (10 and 30 nM).

The concentration-response curve for noradrenaline (3–300 nM) was significantly shifted to the left by 10 nM UK14304 (Figure 3a), and that for vasopressin (0.2–1.6 μM) was significantly shifted to the left by 30 nM UK14304 (Figure 3d).

Vasoconstrictor responses to ATP (10–300 μM) were significantly enhanced by 30 nM UK14304; the enhancement was greater with the highest concentration than with the lower concentrations of ATP (Figure 3b).

The concentration-response curve for KCl in the presence of 30 nM UK14304 departed significantly from parallelism with the control curve (Figure 3c), but vasoconstrictor responses to 40, 48 and 56 mM KCl were significantly increased (paired t test). The nature of the response to KCl was changed by UK14304: in its absence, there was a rapid initial vasoconstrictor effect followed by a lower secondary phase which slowly declined but, in the presence of UK14304, the initial peak was increased and the secondary phase declined more rapidly (Figure 4).

UK14304 had no effect on the concentration-response curve for 3–300 nM 5-hydroxytryptamine (Figure 3e) or on responses to single doses (30 nM) of angiotensin II (Figure 3f). Concentration-response curves for angiotensin II were not obtained because of the marked tachyphylaxis to its vasoconstrictor action.

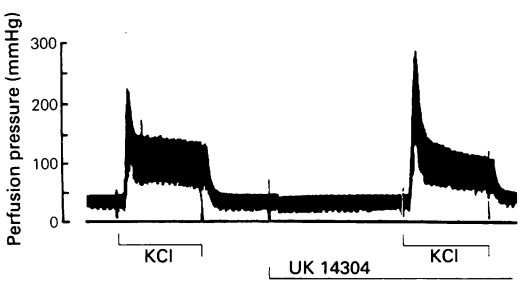

Figure 4 Effect of the addition of UK14304 (30 nmol^{-1}) on vasoconstrictor responses produced by exposure for 5 min to KCl (48 mmol l^{-1}) in a rat isolated perfused tail artery. Vertical axis: perfusion pressure in mmHg. Note that in this experiment the administration of KCl was for a longer period than described in Methods (1–2 min), in order to illustrate more clearly the change in the nature of the response.

Discussion

The aim of the present study was to confirm and extend our previous observations (Xiao & Rand, 1987; Xiao *et al.* 1987a,b), that clonidine and other α_1-adrenoceptor agonists enhanced vasoconstrictor responses elicited by activation of α_1-adrenoceptors in the rat tail artery preparation, and to compare them with those made in the rabbit ear artery preparation, since these two preparations have different populations of postjunctional α-adrenoceptor subtypes (see Introduction).

Vasoconstrictor responses of rabbit ear artery elicited by sympathetic nerve stimulation were reduced by the α_2-adrenoceptor agonist clonidine in concentrations (10–30 nM) far below those producing vasoconstriction (100 and 300 nM). This result is consistent with the findings of Starke *et al.* (1975), using the rabbit pulmonary artery, and Steinsland & Nelson (1975), using the rabbit ear artery; they showed that clonidine inhibited responses to sympathetic nerve stimulation in much lower concentrations than those required to produce vasoconstriction. Both pulmonary and ear arteries from rabbit lack postjunctional α_2-adrenoceptors (Cambridge & Davey, 1980; Hieble & Woodward, 1984), so the only α_2-adrenoceptors on which clonidine can act are prejunctional, thus reducing the release of noradrenaline. The postjunctional α_1-adrenoceptors are only activated by high, non-selective concentrations of clonidine.

Vasoconstrictor responses of rat tail artery elicited by sympathetic nerve stimulation were enhanced by rilmenidine and other α_2-adrenoceptor agonists. Unlike the rabbit ear artery, the rat tail artery has both pre- and postjunctional α_2-adrenoceptors (Weiss *et al.*, 1983; Medgett & Langer, 1984), so it is possible that the enhancing effect of rilmenidine, and other α_2-adrenoceptors agonists, on responses of the rat tail artery to sympathetic nerve stimulation depends on the presence of postjunctional α_2-adrenoceptors. In accord with this, the enhancement of responses to sympathetic nerve stimulation by rilmenidine was abolished by the α_2-adrenoceptor antagonist idazoxan (300 nM) but not by the relatively selective α_1-adrenoceptor antagonist prazosin (0.3 nM).

Taking together the observations made in rabbit ear and rat tail artery preparations, it is possible that the enhancement of vasoconstrictor responses to sympathetic nerve stimulation produced in the latter preparation by α_2-adrenoceptor agonists contains a component attributable to a prejunctional inhibition of noradrenaline release, which partly offsets the

postjunctional augmentation of the contractile response of the vascular smooth muscle. However, we have not yet tested this possibility in experiments in which stimulation-induced release of noradrenaline was measured.

There is a clear dissociation between the direct vasoconstrictor effect of rilmenidine, which was markedly reduced by prazosin but not affected by idazoxan, and its enhancing effect on responses to sympathetic nerve stimulation, which was blocked by idazoxan and not significantly affected by prazosin. It appears, therefore, that its direct vasoconstrictor action is exerted on α_1-adrenoceptors, reflecting loss of selectivity in high concentrations.

The enhancement of responses to sympathetic nerve stimulation by rilmenidine was abolished by a concentration of idazoxan which did not abolish the inhibitory effect of rilmenidine on responses to sympathetic nerve stimulation. A similar finding came from a study in which TL99 was used (Xiao *et al.*, 1987b) and led us to suggest that it indicates a difference between pre- and postjunctional α_2- adrenoceptors. However, it may merely reflect an inadequate competitive blockade by idazoxan of the higher concentration of rilmenidine required for manifestation of the prejunctional inhibitory action.

In rat tail artery, vasoconstrictor responses elicited by noradrenaline and phenylephrine, like those to sympathetic nerve stimulation, were enhanced in the presence of α_2-adrenoceptor agonists. This is consistent with the finding of Shepperson (1984) with the cat nictitating membrane that the contractions evoked by α_1-adrenoceptor agonists were enhanced by pre-dosing with α_2-adrenoceptor agonists. We have demonstrated in this study and elsewhere (Xiao & Rand, 1987; Xiao *et al.*, 1987a,b) that a range of α_2-adrenoceptor agonists markedly enhance vasoconstrictor responses to α_1-adrenoceptor agonists in the rat tail artery in concentrations that are one-hundredth to one-thousandth of the concentrations directly producing vasoconstriction. These findings strongly suggest that the physiological role of postjunctional α_2-adrenoceptors is augmentation of the stimulus arising from activation of α_1-adrenoceptors, rather than direct production of a response.

The enhancement by rilmenidine of responses to noradrenaline was less than that of responses to phenylephrine. A possible explanation for this finding is that a larger proportion of the α_2-adrenoceptors were already activated by noradrenaline than by phenylephrine, since noradrenaline acts on α_1- as well as α_2-adrenoceptors, whereas phenylephrine is a relatively specific α_1-adrenoceptor agonist. Hence an additional α_2-adrenoceptor agonist could be expected to produce a greater effect in the presence of phenylephrine than in the presence of noradrenaline.

If the explanation for the greater enhancement of responses to noradrenaline than those to phenylephrine by rilmenidine is correct, it follows that an agonist acting on both α_1- and α_2-adrenoceptors would be producing a two fold effect; a primary stimulus for the response through the former, and a secondary enhancing effect through the latter. Such a concept has implications for studies on structure-action relationships and kinetic analysis of drug-receptor interactions for drugs acting on α-adrenoceptors. The rat isolated perfused tail artery is not a suitable preparation for exploration of a two fold effect of α-adrenoceptor agonists because large vasoconstrictor responses produce damage and erratic changes in responsiveness, precluding the obtaining of maximal responses and hence of ED_{50} values.

We have not set out systematically to compare α_2-adrenoceptor agonists in respect of their property of enhancing vasoconstrictor responses produced by activating α_1-adrenoceptors. However, the enhancement of responses to noradrenaline was greater with UK14304 than with rilmenidine. The difference may be due to the fact that UK14304 is a full agonist (Grant & Scrutton, 1980; Cambridge, 1981), whereas rilmenidine is a partial agonist (Li & Rand, 1988). Therefore we used UK14304 to determine whether or not α_2-adrenoceptor agonists enhanced vasoconstrictor responses of the rat tail artery produced by agents other than those activating α_1-adrenoceptors.

In rat tail artery, ATP elicited vasoconstrictor responses in concentrations of 3–300 μM. Responses of smooth muscle to ATP are usually due to activation of P_2-purinoceptors (Burnstock *et al.*, 1972; Reilly & Burnstock, 1987). The vasoconstrictor responses to ATP were enhanced by UK14304, especially with the highest concentration of ATP used (300 μM). Vasoconstrictor responses to vasopressin (0.2–1.6 μM) and KCl were also enhanced by UK14304. The increase in the vasoconstrictor responses to KCl produced by UK14304 indicates that α_2-adrenoceptor agonists can enhance not only responses to vasoconstrictor agonists acting on specific receptors, but also the response to a depolarizing agent. Therefore, some common mechanism which links the agents to the vasoconstrictor response may be involved.

A clue to the mechanism is provided by the findings that vasopressin-induced vasoconstriction depends on extracellular calcium and is inhibited by calcium antagonists (Fleckenstein, 1977; Altura & Altura, 1977; Hof, 1985). We have previously found that the enhancing effect of TL99 on vasoconstrictor responses to noradrenaline in the rat tail artery is reduced by diltiazem and abolished in a calcium-free solution (Xiao *et al.*, 1987a). It was suggested, therefore, that α_2-adrenoceptor agonists amplify the vaso-

constrictor responses to contractile agents by augmenting the influx of calcium.

The vasoconstrictor responses to 5-HT and AII were not enhanced by UK14304. The reasons for this are not clear. However, it may be relevant that AII and 5-HT, like α_2-adrenoceptor agonists, also enhance responses to α_1-adrenoceptor agonists (Nicholas, 1969; Medgett *et al.*, 1983; Meehan *et al.*, 1986) and other vasoconstrictor agonists (Van Nueten *et al.*, 1982; Xiao & Rand, 1988) and may have already augmented the influx of calcium to a maximal extent.

It is possible that an endothelium-derived factor contributes as a mediator in the interactions between α_2-adrenoceptor agonists and vasoconstrictor agents that we have observed. It has been established in our laboratory that both rabbit ear and rat tail artery preparations, set up as described in Methods, have a functional endothelium as evidenced by vasodilator responses to acetylcholine which are lost when the endothelium is removed. The enhancing effect of 5-hydroxytryptamine on vasoconstrictor responses to sympathetic nerve stimulation in the rabbit ear artery is not affected by removal of the endothelium, but the enhancement of responses to noradrenaline is attenuated (Meehan *et al.*, 1987). The effect of removal of endothelium on the enhancing effects of α_2-adrenoceptor agonists on vasoconstriction in the rat tail artery has not yet been investigated.

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