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α_2-Adrenoceptor agonists potentiate responses mediated by α_1-adrenoceptors in the cat nictitating membrane

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1 α_1 but not α_2-adrenoceptors mediate contractions of the cat nictitating membrane.

2 The contractions of this tissue evoked by α_1-adrenoceptor agonists, but not those evoked by angiotensin II, are potentiated by pre-dosing with α_2-adrenoceptor agonists.

3 This potentiation is reversed by the α_2-adrenoceptor antagonist, WY 26392.

4 Pressor responses evoked by α_1-adrenoceptor agonists or angiotensin II were not affected by α_2-adrenoceptor agonists.

5 Contractions of the nictitating membrane evoked by noradrenaline were reduced by pretreatment with WY 26392.

6 These results suggest that in some tissues the role of α_2- adrenoceptors may be to modulate responses to α_1-adrenoceptors, rather than to evoke a discrete response themselves.

Introduction

α-Adrenoceptors have been subdivided pharmacologically into two groups known as α_1- and α_2-adrenoceptors (Langer, 1980; Starke, 1981.). Both these receptor subgroups have been reported to exist both pre- and post junctionally in sympathetic neuroeffector junctions. (Timmermans & Van Zwieten, 1980; Docherty, 1983.) The majority of the published data concerning postjunctional α_2-adrenoceptors concentrates on those receptors found in vascular smooth muscle, where they mediate a contractile response (Timmermans & Van Zwieten, 1981.) The only non-vascular tissue in which postjunctional α_2-adrenoceptors have been reported to mediate a contractile response is the rat annococcygeus muscle. (Docherty & MaGrath, 1980).

The reason for, or the advantage of, having two subtypes of the same receptor on an effector is not apparent from the available experimental data. In vascular smooth muscle it has been suggested that these receptors may have different anatomical distributions (Langer *et al.*, 1980). A consequence of this is the suggestion that α_2-adrenoceptors may mediate responses to sympathetic nerve stimulation, whereas α_2-adrenoceptors may mediate responses to circulating catecholamines (Langer & Shepperson, 1982). The possibility that α_2-adrenoceptors might mediate smooth muscle responses other than, or in addition to, a contraction, has not been investigated, although there is at least one report that α_2-adrenoceptors inhibit the responses evoked by α_1-adrenoceptor agonists in the rat mesentery (Fiotakis & Pipili, 1983). The work described in this paper originated from a series of experiments in which the effects of α_2-adrenoceptor agonists were studied in a variety of smooth muscle preparations in order to look for responses other than muscular contraction. The cat nictitating membrane was chosen for these studies because it has previously been suggested that this tissue contains only α_1-adrenoceptors (Langer *et al.*, 1981).

A preliminary account of this work has been presented to the British Pharmacological Society (Shepperson, 1984).

Methods

Cats (2–2.5 kg, female) were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} i.t. and 6 mg $^{-1}h^{-1}$ i.v.). Following tracheotomy, the preparation was bivagotomised and a femoral artery and both femoral veins cannulated. The left nictitating membrane was attached to a Grass FT03 force displacement transducer by a cotton thread. A rest-

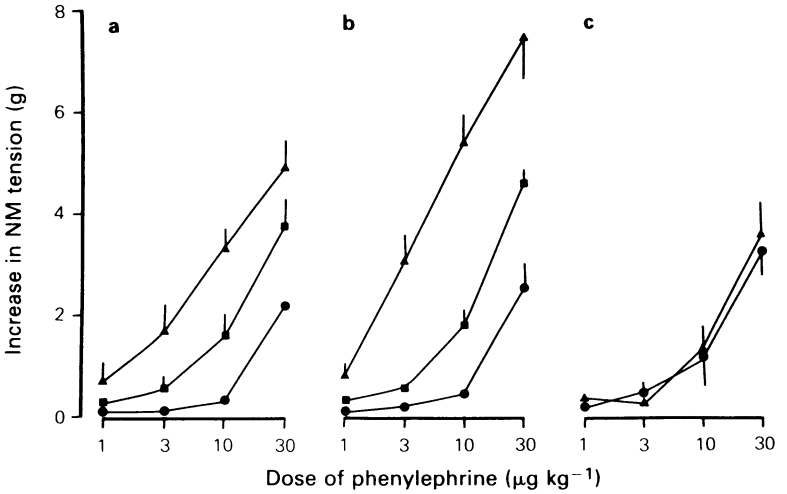

Figure 1 The contraction of the cat nictitating membrane (NM) evoked by intravenous doses of phenylephrine following, (a) saline 0.2 ml i.v. (●); clonidine, 3 $\mu g\,kg^{-1}$ i.v. (■) and clonidine 10 $\mu g\,kg^{-1}$ i.v. (▲); (b) saline 0.2 ml i.v. (●); UK 14304, 0.3 $\mu g\,kg^{-1}$ i.v., (■) and UK 14304, 3 $\mu g\,kg^{-1}$ i.v., (▲); (c) saline 0.2 ml i.v. (●); and following two further doses of saline 0.2 ml i.v., (▲). Each point represents the mean of four results with s.e. mean indicated by vertical lines. Both doses of clonidine and UK 14304 3 $\mu g\,kg^{-1}$ significantly enhanced the responses to phenylephrine (nested analysis of variance).

ing tension of 10–12 g was applied to the nictitating membrane and maintained as a basal tension throughout the experiment. Blood pressure was measured via the femoral artery by a Statham P23 1d pressure transducer. Nicitating membrane tension and blood pressure were recorded on a Grass model 7 polygraph. The preparations were treated with propranolol (1 kg^{-1} i.v.) to block β-adrenoceptors and chlorisondamine (1 mg kg^{-1} i.v.) to block ganglia.

All drugs were administered via a femoral vein whilst anaesthetic was infused via the second vein.

The preparation was allowed to stabilise for approximately 30 min. Following this period dose-response curves were constructed to either phenylephrine (1–30 $\mu g\,kg^{-1}$) or angiotensin II (0.03–0.3 $\mu g\,kg^{-1}$), and repeated until reproducible responses were obtained. An α_2-adrenoceptor agonist (clonidine, 3 or 10 $\mu g\,kg^{-1}$; or UK 14304, 0.3 or 3 $\mu g\,kg^{-1}$) or saline (0.2 ml), was then administered and 15 min later the dose-response curve to phenylephrine or angiotensin II was repeated.

In some experiments only one dose of UK 14304

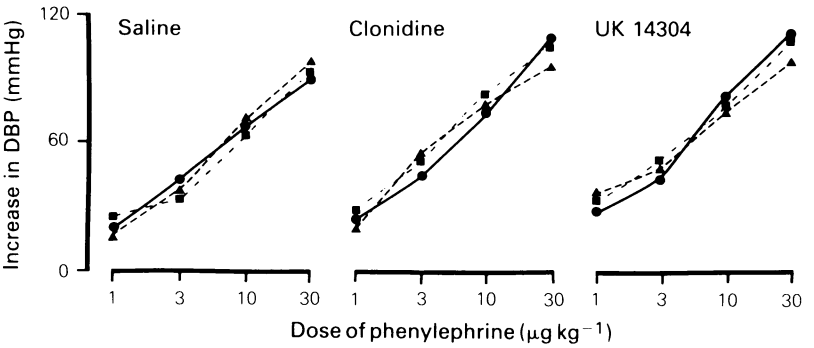

Figure 2 The diastolic pressor response (DBP) evoked by intravenous doses of phenylephrine following: Left hand panel; saline 0.2 ml i.v. (●); saline 0.2 ml i.v. (■); saline 0.2 ml i.v. (▲). Centre panel; saline 0.2 ml i.v. (●); clonidine, 3 $\mu g\,kg^{-1}$ i.v. (■); clonidine, 10 $\mu g\,kg^{-1}$ (▲). Right hand panel; saline 0.2 ml i.v. (●); UK 14304, 0.3 $\mu g\,kg^{-1}$ i.v. (■); UK 14304, 3 $\mu g\,kg^{-1}$ i.v. (▲). Each point represents the mean of four experiments with s.e.mean indicated by vertical lines. None of the treatments significantly altered the pressor responses from those obtained in the presence of the initial dose of saline.

($3\,\mu g\,kg^{-1}$) was administered. After repeating the dose-response curve to phenylephrine in the presence of this dose of UK 14304, the α_2-adrenoceptor antagonist WY 26392 ($30\,\mu g\,kg^{-1}$, i.v.) was administered, and the response curve to phenylepherine repeated once more. In a second series of experiments the left common carotid artery was cannulated by puncturing the artery with a 26G needle with a cannula attached. Noradrenaline ($0.5-15\,\mu g\,kg^{-1}$) was administered by this route and dose-response curves were constructed in the absence of and 15 min after a dose of WY 26392 (30 and $100\,\mu g\,kg^{-1}$).

Drugs; the following drugs were used in this study: angiotensin II (Sigma), chlorisondamine chloride (Ciba-Giegy), clonidine HCl (Dept. of Chemistry, Wyeth Labs.), noradrenaline bitartrate (Sigma), phenylephrine HCl (Sigma), prazosin HCl (Pfizer U.K.), propranolol HCl (Sigma), UK 14304 (5-romo-6 - [2-imidazolin-2-ylamino] - quinoxaline Pfizer U.K.), WY 26392 (N-methyl-N-[1,3,4,6,7, 11bα-hexahydro-2H-benzo-[a]-quinolizin-2βyl]propan-1-sulphonamidehydrochloride Dept. of Chemistry, Wyeth Labs.).

Statistical methods: the results are expressed as the mean ± s.e.mean. The statistical significance of the data was evaluated by a paired t test for pairs of matched responses, or by nested analysis of variance for dose-response curves. $P<0.05$ was taken to be significant.

Results

Phenylephrine evoked a dose-related pressor response over the dose-range administered and a significant contraction of the nictitating membrane at doses of 10 and $30\,\mu g\,kg^{-1}$ (Figures 1 and 2). Repetition of the phenylephrine dose-response curve 15 min after clonidine (3 and $30\,\mu g\,kg^{-1}$) resulted in a significantly enhanced response of the nictitating membrane (Figure 1a). The pressor response to phenylephrine was not significantly affected by prior administration of clonidine (Figure 2).

Administration of UK 14304 (0.3 and $3\,\mu g\,kg^{-1}$) following the control dose-response curve to phenylephrine also resulted in an enhancement of the response of the nictitating membrane to subsequent doses of phenylephrine (Figure 1b). UK 14304 had no significant effect on the pressor responses to phenylephrine (Figure 2).

The administration of saline (0.2 ml) between phenylephrine dose-response curves in place of an α_2-adrenoceptor agonist had no significant effect on the response of the nictitating membrane (Figure 1c) or the pressor response evoked by subsequent doses of phenylephrine (Figure 2).

Angiotensin II evoked a dose-dependent pressor response and contraction of the nictitating membrane. Administration of UK 14304 ($3\,\mu g\,kg^{-1}$) had no significant effect on either response to angiotensin II (Figure 3).

In a separate series of experiments, UK 14304 ($3\,\mu g\,kg^{-1}$) was administered after a dose-response curve to phenylephrine and significantly potentiated the nictitating membrane response to a subsequent series of doses of phenylephrine. Administration of the α_2-adrenoceptor antagonist WY 26392 ($30\,\mu g\,kg^{-1}$) in the presence of UK 14304 significantly reduced the response of the nictitating membrane

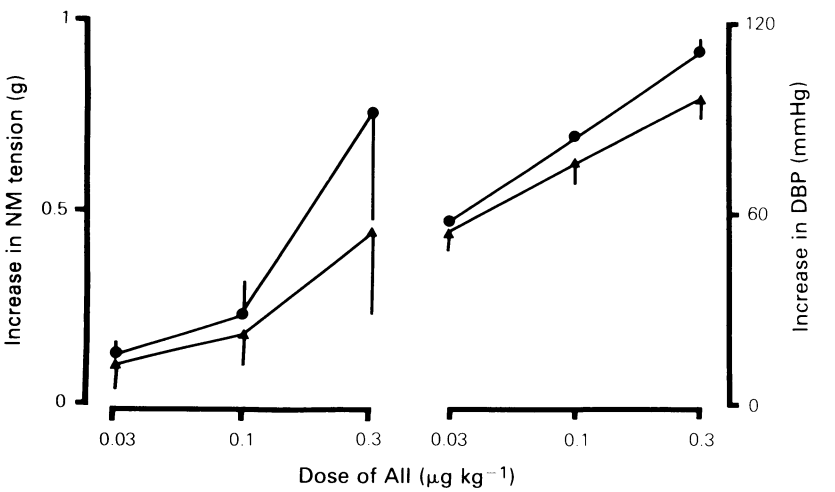

Figure 3 The contraction of the cat nictitating membrane (NM, left hand panel) and diastolic pressor response (DBP, right hand panel) evoked by intravenous doses of angiotensin II following saline 0.2 ml i.v., (●), and UK 14304, $3\,\mu g\,kg^{-1}$ i.v. (▲). Each point represents the mean of four experiments with s.e.mean indicated by vertical lines. UK 14304 had no significant effect on the responses to angiotensin II.

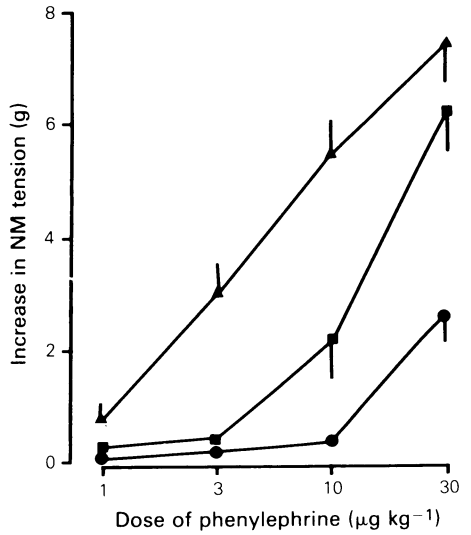

Figure 4 The contraction of the cat nictitating membrane (NM) evoked by intravenous doses of phenylephrine following saline 0.2 ml i.v., (●); UK 14304, 3 μg kg^{-1} i.v., (▲); and UK 14304, 3 μg kg^{-1} i.v. + WY 26392, 30 μg kg i.v., (■). Each point represents the mean of four experiments with s.e. mean indicated by vertical line. UK 14304 significantly enhanced the response to phenylephrine and WY 26392 significantly reduced this response (nested analysis of variance).

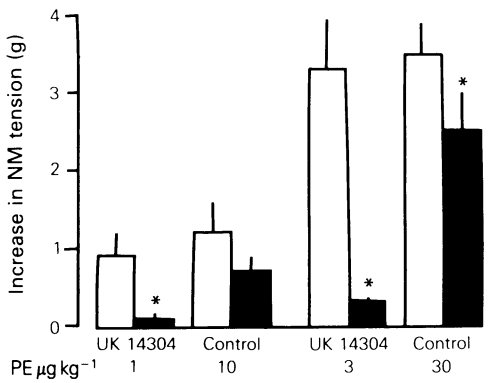

Figure 5 The effect of WY 26392 on two matched responses of the cat nictitating membrane to intravenous doses of phenylephrine in the absence and presence of UK 14304 (3 μg kg^{-1} i.v.). Preparations were pretreated with either saline (0.2 ml i.v., open columns) or WY 26392 (3 μg kg^{-1} i.v., filled columns) 15 min before injecting phenylephrine. Each bar represents the results from three experiments. There was no significant difference between the pairs of control responses. * Indicates a significant difference between the response in the presence of WY 26392 and the corresponding saline control (Paired t test, $P<0.05$).

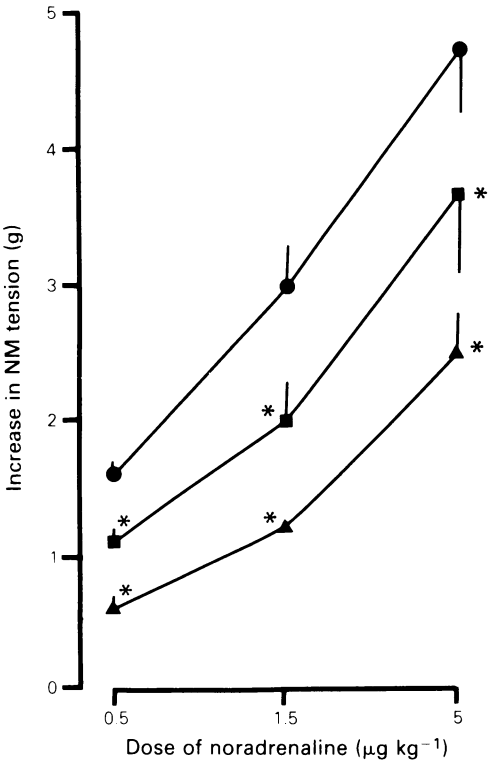

Figure 6 Contractions of the nictitating membrane (NM) evoked by injections of noradrenaline into the carotid artery of the anaesthetized cat. Noradrenaline was administered before (●) and after WY 26392, 30 μg kg^{-1} i.v., (■) and 100 μg kg^{-1} i.v., (▲). Each point represents the results from at least four experiments. Both doses of WY 26392 significantly inhibited the response to noradrenaline (nested analysis of variance).

to phenylephrine (Figure 4). This action of WY 26392 was compared with its effect upon the response of the nictitating membrane to phenylephrine in the absence of UK 14304. As can be seen from Figure 1, the response of the membrane to phenylephrine was enhanced by the α_2-adrenoceptor agonist. The effects of WY 26392 in the absence and presence of UK 14304 were therefore investigated using two matched responses rather than matched doses of phenylephrine. Under these experimental conditions, WY 26392 markedly inhibited the response to phenylephrine in the presence of UK 14304. In contrast, in the absence of UK 14304, WY 26392 produced only a small (though significant, $P<0.05$) inhibition of the response to the high dose of phenylephrine (30 μg kg), and had no significant effect on the response to the lower (10 μg kg^{-1}) dose (Figure 5).

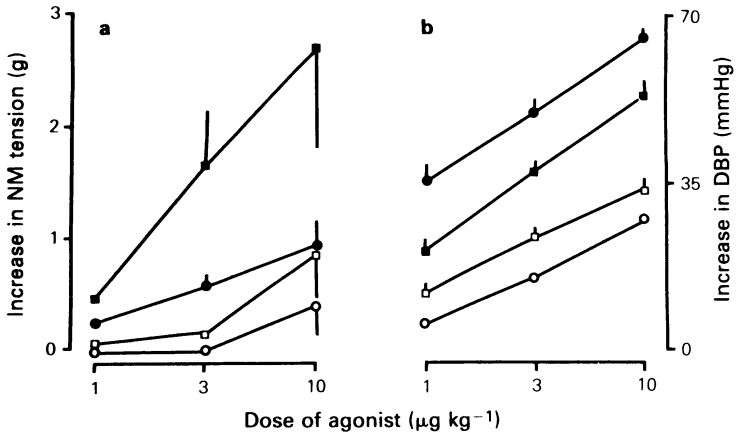

Figure 7 The contraction of the nictitating membrane (NM, a) and diastolic pressor response (DBP, b) evoked by intravenous doses of clonidine, (●), and UK 14304, (■); and of these agonists in the presence of prazosin, 30 μg kg^{-1} (open symbols). Each point represents the mean of four experiments. Prazosin significantly reduced both responses of the two agonists (nested analysis of variance).

Administration of noradrenaline (0.5–5 μg kg) via the carotid artery evoked a dose-related contraction of the nictitating membrane. Pretreatment with WY 26392 (30 and 100 μg kg^{-1}) significantly inhibited the responses of the nictitating membrane to subsequent doses of noradrenaline (Figure 6).

Throughout these experiments it was noted that both UK 14304 and clonidine evoked small pressor responses and contractions of the nictitating membrane, the duration of these responses being 5–10 min. To investigate these effects further, dose-response curves were constructed for these agonists, (Figure 7). The response of the nictitating membrane to both of these agonists was markedly reduced or abolished by administration of prazosin (30 μg kg^{-1}) 15 min before constructing the agonist dose-response curves (Figure 7). In contrast, in the presence of prazosin these agonists evoked reduced but dose-related pressor responses (Figure 7).

Discussion

The vascular smooth muscle of several species contains both α_1- and α_2-adrenoceptors which mediate contractile responses (Timmermans & Van Zwieten, 1981). However the nature of the α-adrenoceptors in the vasculature of the cat has been the subject of some debate. In anaesthetized cats Drew & Whiting (1979) reported the presence of postjunctional α-adrenoceptors mediating pressor responses which were resistant to blockade by prazosin, but were unable to confirm that these receptors were α_2-adrenoceptors. More recent work *in vitro* employing selective agonists and antagonists has demonstrated that α_1- and α_2-adrenoceptors are located postjunctionally in the vasculature of the cat. The results presented in this paper support the findings of Langer & Shepperson (1982b). Thus, in the anaesthetized cat both the α_1-adrenoceptor agonist phenylephrine, and the α_2-adrenoceptor agonists clonidine and UK14304 evoked pressor responses and furthermore, the pressor responses to the α_2-agonists were resistant to blockade by prazosin.

In contrast to the pressor responses evoked by the α-adrenoceptor agonists, the contractions of the nictitating membrane evoked by either the preferential α_1- or α_2 agonists were abolished or markedly reduced by prazosin. This finding supports the hypothesis that these responses are mediated entirely by α_1-adrenoceptors (Langer *et al.*, 1981.). These results do not however exclude the possibility that α_2-adrenoceptors are also located on the smooth muscle of this preparation, merely that they do not mediate a contractile response. The fact that the reportedly highly selective α_2-adrenoceptor agonist UK14304 (Cambridge 1981) evoked a contraction of the nictitating membrane was unexpected and worth noting. This response, being readily blocked by prazosin, was clearly mediated via α_1-adrenoceptors. This result would suggest that UK 14304 is not a very selective α_2-adrenoceptor agonist under these experimental conditions. When using this agonist as an

experimental tool it is therefore important to verify its selectivity under any particular set of experimental conditions. It cannot be ruled out that this lack of selectivity is in fact due to the nature of the nictitating membrane itself. Several authors have reported that in both *in vivo* and *in vitro* preparations of the membrane, tolazoline and metanephrine are agonists evoking contractile responses (Arbilla & Langer, 1978). In most tissues tolazoline is a competitive α_2-adrenoceptor antagonist (Langer 1980), and metanephrine (a metabolite of noradrenaline) is an α-adrenoceptor agonist of low potency at postjunctional α-adrenoceptors (Arbilla & Langer, 1978).

With the experimental protocol employed in this study, reproducible contractile responses of the nictitating membrane were obtained to intravenous doses of phenylephrine and angiotensin II. The responses to angiotensin II have been reported to be mediated via angiotensin receptors and to be independent of both cholinergic and adrenergic mechanisms (Block *et al.*, 1971). The dose-response curves to both agonists were limited by the magnitude of the pressor response which they evoked. Doses of the agonists were chosen therefore which allowed a comparison to be made between the effects of compounds on both pressor responses and responses of the nictitating membrane. Administration of an α_2-adrenoceptor agonist 15 min prior to constructing a dose-response curve, significantly potentiated the responses to phenylephrine but not those to angiotensin II. This potentiation of the response to phenylephrine was reversed by the selective α_2-adrenoceptor antagonist WY 26392. (Lattimer *et al.*, 1982). As a result of this reversal, WY 26392 markedly inhibited the response to phenylephrine in the presence of UK 14304. In contrast, in the absence of UK 14304, WY 26392 had no effect upon the response to a low dose of phenylephrine and produced only a small inhibition of the response to a higher dose. The small inhibition of the response to the higher dose of phenylephrine may be the result of this agonist acting upon α_2-adrenoceptors as it is not a totally selective α_1-adrenoceptor agonist. This result demonstrated that the inhibition of the response in the presence of UK 14304 was not due to α_1-adrenoceptor blockade *per se*. These findings indicate that postjunctional α_2-adrenoceptors are present in this tissue, and that their activation leads to a specific enhancement of the response to α_1-adrenoceptor stimulation.

The response of the nictitating membrane to noradrenaline was investigated by administering the agonist directly into the carotid artery. With this technique more responses were obtained from the nictitating membrane as lower doses of agonist could be employed, minimising the pressor response. Under these experimental conditions the response of the nictitating membrane to noradrenaline was reduced by doses of WY 26392 which had little effect on the responses to phenylephrine in the experiments described above. It would appear therefore that the response of the tissue to noradrenaline though mediated by α_1-adrenoceptors, is enhanced by the action of this relatively non-selective α-adrenoceptor agonist on α_2-adrenoceptors. This result raises the possibility that the enhancing effect of α_2-adrenoceptors may be of physiological significance since, noradrenaline (being an α_1- and α_2-adrenoceptor agonist) released from the sympathetic innervation of the tissue could act upon both post junctional α-adrenoceptor subtypes. The ability of neuronally released noradrenaline to act at both receptor subtypes will depend upon the distribution of the receptors at the neuroeffector junction. It has previously been demonstrated that in vascular smooth muscle only α_1-adrenoceptors are innervated (Langer *et al.*, 1980), the location of postjunctional α_2-adrenoceptors in other types of smooth muscle awaits investigation.

The pressor responses to phenylephrine and agiotensin II were not significantly affected by the α_2-agonists. Clearly therefore the link between the two subtypes of α-adrenoceptor found in the nictitating membrane is not found in all tissues. It is tempting to speculate that the enhancing effect of the α_2-agonists may only be manifest in situations where they themselves do not evoke a tissue response.

The mechanism of the potentiation has not been defined by the experiments reported here. It has been demonstrated in a number of preparations that α_2-adrenoceptors evoke contractions of vascular smooth muscle by promoting the influx of calcium across the cell membrane, whilst α_2-adrenoceptors are dependent upon intracellular calcium (Timmermans & Van Zwieten, 1981). It might be hypothesised therefore that the enhancing effects of α_2-adrenoceptor agonists could be the result of an increased availability of intracellular calcium. Were this the case however it might be expected that the responses to angiotensin II would also be enhanced and this was not the case. With regard to this criticism, it appears that there is some doubt as to the location of the calcium stores which are involved in the contraction of smooth muscle evoked by angiotensin II (Peach, 1981). A further criticism of the hypothesis is that preliminary experiments in this laboratory have shown that the calcium antagonist verapamil ($10\,\mu g\,kg^{-1}\,min^{-1}$) a dose sufficient to reduce pressor responses to α_2-agonists (Cavero *et al.*, 1983) had no significant effect on the enhancement by UK 14304 of responses to phenylephrine. It would seem unlikely therefore that an enhancement of calcium influx is the explanation for the effect of α_2-adrenoceptor agonists in this preparation.

In conclusion, the experiments described here demonstrate a positive interaction between α_1- and α_2-adrenoceptors in the cat nictitating membrane. This effect occurs despite the lack of any measurable response of the tissue to activation of α_2-adrenoceptors by selective agonists.

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