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α_2-Adrenoceptor agonists enhance vasoconstrictor responses to α_1-adrenoceptor agonists in the rat tail artery by increasing the influx of Ca^{2+}

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1 The α_2-adrenoceptor agonists TL99 (2-(*NN*-dimethyl)amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) and UK14304 (5-bromo-6-[2-imidazoline-2-yl-aminol]-quinoxaline), in concentrations that are less than 1% of those producing vasoconstriction, enhance vasoconstrictor responses to noradrenaline and phenylephrine in isolated perfused preparations of the rat tail artery.

2 The enhancing effect was abolished when Ca^{2+} was absent and by the calcium channel blocking drug diltiazem.

3 α_2-Adrenoceptor agonists had no effect on the component of the responses to noradrenaline and phenylephrine that is attributable to mobilization of intracellular Ca^{2+}, but enhanced the component attributable to influx of extracellular Ca^{2+}.

4 These results suggest that the enhancing effect of α_2-adrenoceptor agonists on responses of the rat tail artery to α_1-adrenoceptor agonists involves an increase in Ca^{2+}-influx into smooth muscle cells through Ca^{2+} channels that are opened when α_2-adrenoceptors are activated.

Introduction

There is clear evidence for the presence of postjunctional α_2-adrenoceptors as well as α_1-adrenoceptors on the smooth muscle of many vascular preparations (McGrath, 1982). The presence of postjunctional α_2-adrenoceptors on the smooth muscle of the rat tail artery has been demonstrated in binding studies with selective radioligands (Weiss *et al.*, 1983; Dashwood & Jacobs, 1985; Nasseri *et al.*, 1985; Abel & Minneman, 1986), and activation of these α_2-adrenoceptors produces contractile responses (Medgett & Langer, 1984; Rajanayagam & Medgett, 1987; Abel & Minneman, 1986; Abe *et al.*, 1987).

Pressor responses to α_2-adrenoceptor agonists *in vivo* can be blocked by calcium channel blocking drugs such as verapamil, nifedipine and diltiazem (Van Meel *et al.*, 1981a,b; Cavero *et al.*, 1983; Timmermans *et al.*, 1984). Furthermore, contractions of isolated vascular smooth muscle elicited by α_2-adrenoceptor activation are reduced by lowering the Ca^{2+} concentration and by calcium channel blocking drugs in the rat tail artery (Medgett & Rajanayagam, 1984; Hicks *et al.*, 1985; Su *et al.*, 1986) and the saphenous vein of the rabbit (Schümann & Lues, 1983) and dog (Jim & Matthews, 1985). Hence, influx of Ca^{2+} appears to be involved in the vasoconstrictor action of α_2-adrenoceptor agonists.

In concentrations well below those producing vasoconstriction, α_2-adrenoceptor agonists enhance responses of the isolated perfused rat tail artery to sympathetic nerve stimulation and to a number of vasoconstrictor agents including α_1-adrenoceptor agonists, adenosine 5′-triphosphate (ATP), vasopressin and high K^+ solution (Xiao *et al.*, 1987a; Xiao & Rand, 1989a). The aim of the present study was to examine the possible involvement of Ca^{2+} entry in the enhancing effect of the α_2-adrenoceptor agonists TL99 (Hicks & Cannon, 1980) and UK14304 (Grant & Scrutton, 1980; Cambridge, 1981) on vasoconstrictor responses to noradrenaline and phenylephrine in the rat isolated perfused tail artery. A preliminary account of part of this work was presented at the 6th Symposium on Neurovascular Mechanisms (Xiao *et al.*, 1987b).

Methods

Male Sprague-Dawley rats weighing 250 to 350 g were anaesthetized with pentobarbitone (30 mg kg^{-1},

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i.p.) and approximately 1 cm segments of the proximal portion of the tail artery were dissected out and set up as isolated preparations perfused at 4 ml min^{-1} as described by Xiao *et al.* (1987a) and Xiao & Rand (1989a). The perfusion pressure was measured with a Statham P23Db pressure transducer and recorded on a Rikadenki chart recorder. Vasoconstriction was measured as an increase in perfusion pressure in mmHg. A 30 min stabilization period was allowed before any experimental procedures were carried out.

Perfusion solutions

The physiological salt solution (PSS) was of 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 and disodium edetate 0.067. For some experiments, the $CaCl_2$ concentration was reduced to 0.61 mM. For others, $CaCl_2$ was omitted and 2 mM EGTA (ethylene-*bis*-(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid) was added to the solution. The reservoirs of solutions were gassed with 5% CO_2 in O_2 and maintained at 37°C.

Vasoconstrictor responses

Responses to noradrenaline and phenylephrine were obtained by replacing the drug-free PSS with one containing the required concentration of an agonist, which was subsequently perfused until the response reached its peak value (usually less than 30 s) before returning to drug-free PSS. Two non-cumulative concentration-response curves were constructed; the second was begun 30 min after completing the first. When the effects of drugs or altered Ca^{2+} concentrations on concentration-response curves were to be determined, the PSS was modified after the first curve had been constructed and was used throughout the construction of the second curve. Only two curves were obtained from any one artery preparation.

Mobilization of intracellular Ca^{2+} and Ca^{2+} influx

The method of Manzini *et al.* (1982) was used to examine the effect of α_2-adrenoceptor agonists on the components of the responses to noradrenaline and phenylephrine attributable to mobilization of intracellular Ca^{2+} and to influx of extracellular Ca^{2+}. The procedure was to replace normal PSS with a Ca^{2+}-free solution containing EGTA 5 min before determining the response to noradrenaline or phenylephrine. When the response (due to the mobilization of intracellular Ca^{2+}) had been obtained, the solution was replaced with normal PSS and the secondary response (due to Ca^{2+} influx) then occurred. Perfusion with normal PSS was continued for a further 30 min before repeating the procedure since Casteels & Droogmans (1981) showed that at least 10 min period of perfusion with normal Ca^{2+}-containing solution was required for refilling the intracellular Ca^{2+} store in the rabbit ear artery.

Drugs

The following drugs were used: diltiazem hydrochloride (Synthelabo); idazoxan hydrochloride (Reckitt & Colman); (−)-noradrenaline hydrochloride (Sigma); phenylephrine hydrochloride (Sigma); 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). Stock solutions of noradrenaline and TL99 were dissolved in distilled water which contained ascorbic acid (0.05 mg ml^{-1}). Other drugs were dissolved in distilled water.

Statistical analysis

Differences between means were analysed by Student's paired or unpaired *t* test, as appropriate. Differences between lines (slopes or displacements) were analysed by comparing the parameters of regression equations. Probability levels of less than 0.05 were taken as indicating significant differences.

Results

Vasoconstrictor responses

Noradrenaline (3–300 nM) and phenylephrine (0.01–3 μM) produced concentration-dependent increases in perfusion pressure (Figure 1). The concentration-response curves were linear between perfusion pressures of 25 mmHg to about 200 mmHg. No attempt was made to obtain maximal responses because rises in perfusion pressure greater than about 250 mmHg resulted in loss of reproducibility of responses, presumably because of damage to the preparation. When log concentration-response relationships were analysed by determining the regression equations, only responses with a mean value of 25 mmHg or greater were used. In control experiments, there were no significant differences between two successive concentration-response curves to either noradrenaline or phenylephrine.

Vasoconstrictor responses to the α_2-adrenoceptor agonists were produced only when the concentrations reached 10 μM for UK14304 and 3 μM for TL99, as found previously by Medgett & Langer (1984).

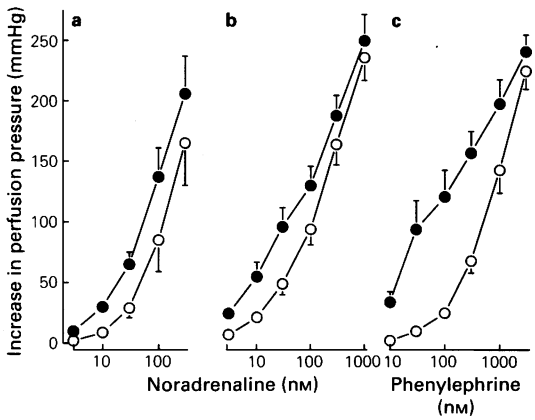

Figure 1 Concentration-response curves for noradrenaline (a and b) and phenylephrine (c): (○) first curves for the vasoconstrictor agonist alone; (●) second curves in the presence of 10 nM TL99 (a) or 30 nM UK14304 (b and c). Symbols indicate mean values and vertical bars show standard errors (a: $n = 7$; b, c: $n = 6$).

Effects of α_2-adrenoceptor agonists on vasoconstrictor responses

During infusions of concentrations of TL99 (10 nM) or UK14304 (30 nM) that were well below the thresholds for direct vasoconstrictor effects, the vasoconstrictor responses to noradrenaline and phenylephrine were enhanced. The log concentration-response line

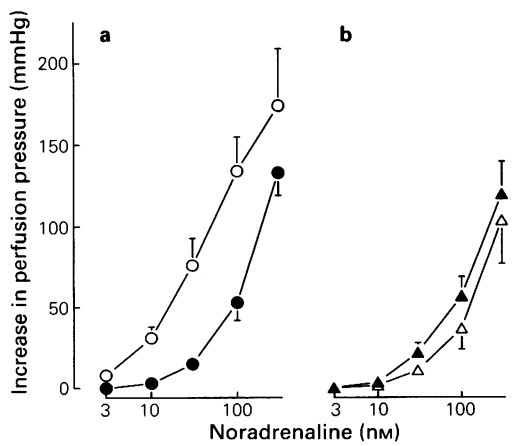

Figure 2 Concentration-response curves for noradrenaline: (a) (○) first curve for noradrenaline alone; (●) second curve in the presence of idazoxan (300 nM). (b) (△) First curve in the presence of idazoxan (300 nM); (▲) second curve in the presence of idazoxan (300 nM) plus TL99 (10 nM). Symbols are mean values and vertical bars show standard errors ($n = 6$).

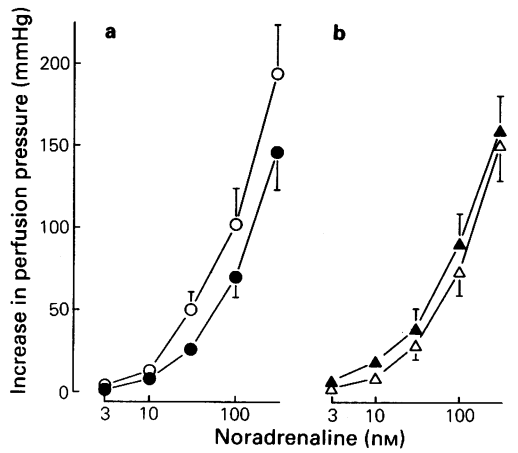

Figure 3 Concentration-response curves for noradrenaline: (a) (○) first curve for noradrenaline alone; (●) second curve in the presence of 1 μM diltiazem. (b) (△) First curve in the presence of 1 μM diltiazem; (▲) second curve in the presence of 1 μM diltiazem plus 10 nM TL99. Symbols are mean values and vertical bars show standard errors ($n = 6$).

for noradrenaline was significantly shifted to the left in the presence of TL99 (Figure 1a) and UK14304 (Figure 1b) by amounts corresponding to increases in potency of 2.4 fold and 2.1 fold, respectively. In the presence of UK14304, responses to the lower concentrations of phenylephrine were enhanced to a greater extent than were those to the higher concentrations, and the log concentration-response lines in the presence and absence of UK14304 departed significantly from parallelism (Figure 1c).

The α_2-adrenoceptor antagonist idazoxan (300 nM) had no effect on the resting perfusion pressure; however, it reduced vasoconstrictor responses to noradrenaline and significantly displaced the log concentration-response line to the right, to an extent amounting to a 5.1 fold decrease in potency (Figure 2a). When two successive curves for noradrenaline were obtained in the presence of idazoxan, they did not differ significantly. In the presence of idazoxan, TL99 had no significant effect on responses to noradrenaline and the log concentration-response lines did not depart significantly from coincidence (Figure 2b).

Effect of diltiazem

The calcium channel blocking drug diltiazem (1 μM) had no effect on the resting perfusion pressure, but significantly reduced vasoconstrictor responses to noradrenaline and shifted the log concentration-response line to the right by an amount corresponding to a 2.1 fold decrease in potency (Figure 3a). When two successive curves for noradrenaline were

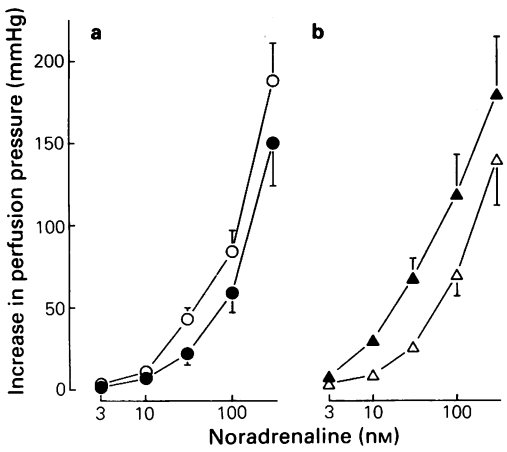

Figure 4 Concentration-response curves for noradrenaline: (a) (○) first curve for noradrenaline alone; (●) second curve after reducing the Ca^{2+} concentration from 2.5 to 0.61 mM. (b) (△) First curve in 0.61 mM Ca^{2+}; (▲) second curve in 0.61 mM Ca^{2+} with 10 nM TL99 present. Symbols are mean values and vertical bars show standard errors ($n = 6$).

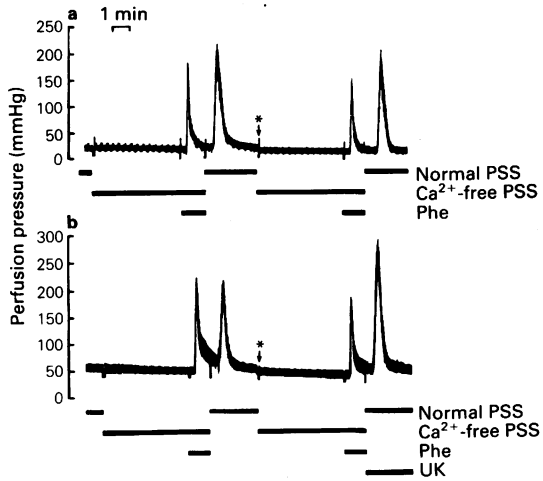

Figure 5 Records illustrating the transient vasoconstrictor responses to 10 μM phenylephrine (Phe) in Ca^{2+}-free PSS and after returning to normal PSS. The initial response in the Ca^{2+}-free PSS is due to mobilization of intracellular Ca^{2+}; the secondary response occurring when the Ca^{2+}-free solution was replaced by normal PSS is due to Ca^{2+} influx. (a): Two consecutive sets of responses (control); (b): when the second set of responses was elicited, 30 nM UK14304 (UK) was present in the normal PSS. There was a 30 min period of perfusion with normal PSS (indicated by *) between the first and second set of responses.

obtained in the presence of diltiazem, they did not differ significantly. In the presence of diltiazem, responses to noradrenaline were not significantly affected by TL99 (Figure 3b).

Effects of lowering the Ca^{2+} concentration

Reducing the concentration of Ca^{2+} in the PSS from 2.5 to 0.61 mM resulted in significant decreases in responses to noradrenaline, the log concentration-response line being shifted to the right by an amount corresponding to a 1.8 fold decrease in potency (Figure 4a). When first and second curves were obtained in a 0.61 mM Ca^{2+} solution, they did not differ significantly. When the second curve was obtained with TL99 (10 nM) present, responses to noradrenaline in a 0.61 mM Ca^{2+} solution were enhanced and the shift in the log concentration-response line corresponded to a 2.8 fold increase in potency (Figure 4b). The shifts caused by TL99 in the log concentration-response lines for noradrenaline to the left did not differ significantly between 0.61 and 2.5 mM Ca^{2+} solutions (*cf.* Figures 4b and 1a).

Effects of α_2-adrenoceptor agonists on the components of responses to noradrenaline and phenylephrine dependent on mobilizing intracellular Ca^{2+} and on Ca^{2+}-influx

When a Ca^{2+}-free, EGTA-containing, modified PSS was perfused, vasoconstrictor responses to 1 μM noradrenaline or 10 μM phenylephrine rose sharply to peak values that were approximately equivalent in magnitude to those produced by one-tenth the concentrations in normal PSS. Then, in the continued presence of the agonist, the perfusion pressure declined towards the base-line value, as shown for phenylephrine in Figure 5. This initial transient vasoconstrictor response in Ca^{2+}-free medium was attributed by Manzini *et al.* (1982) to mobilization of intracellular Ca^{2+}. On changing to perfusion with drug-free PSS containing the normal (2.5 mM) concentration of Ca^{2+}, there was a second transient vasoconstrictor response: this secondary response was attributed by Manzini *et al.* (1982) to the influx of extracellular Ca^{2+} into the vascular smooth muscle cells.

Neither TL99 (10 nM) nor UK14304 (30 nM) had any significant effect on the initial responses to noradrenaline or phenylephrine in the Ca^{2+}-free modified PSS. However, the secondary transient vasoconstrictor responses that appeared on changing to normal PSS were increased by UK14304 (Figure 5) and TL99. The secondary response to phenylephrine decreased by 3.5 ± 5.6% ($n = 8$) from the first to

the second occasion in control experiments (as in Figure 5a), but increased by $23.7 \pm 5.6\%$ ($n = 6$) when UK14304 was added before the second occasion (as in Figure 5b). With noradrenaline there was a decrease by $12 \pm 4.4\%$ ($n = 4$) in control experiments, but an increase of $14.7 \pm 5.4\%$ ($n = 6$) when UK14304 was added before the second occasion.

Discussion

Vasoconstrictor responses elicited by noradrenaline or phenylephrine in the rat isolated perfused tail artery were enhanced by concentrations of the α_2-adrenoceptor agonists TL99 and UK14304 that were subthreshold for producing vasoconstriction. This is consistent with the findings of Xiao *et al.* (1987a) and Xiao & Rand (1989a), using the same preparation, that vasoconstrictor responses to α_1-adrenoceptor agonists are markedly enhanced by α_2-adrenoceptor agonists in concentrations that are one-hundredth to one-thousandth of the concentrations producing other prejunctional or postjunctional effects. Similar observations were made by Shepperson (1984), who found that UK14304 in doses producing only small contractions of the cat nictitating membrane markedly enhanced contractile responses to phenylephrine.

The enhancing effect of TL99 on vasoconstrictor responses to noradrenaline was abolished by the relatively selective α_2-adrenoceptor antagonist idazoxan (Doxey *et al.*, 1983), confirming our previous findings that idazoxan blocked the enhancement by α_2-adrenoceptor agonists of responses resulting from activation of α_1-adrenoceptors by sympathetic nerve stimulation (Xiao *et al.*, 1987a; Xiao & Rand, 1989a) and responses elicited by certain other vasoconstrictor agents (unpublished observations).

When the Ca^{2+} concentration was zero and the chelating agent EGTA was present, UK14304 had no effect on the responses to noradrenaline or phenylephrine. Furthermore, the enhancing effect of TL99 on vasoconstrictor responses to noradrenaline was abolished in the presence of the calcium channel blocking drug diltiazem. When the component of the responses to noradrenaline and phenylephrine attributable to the influx of Ca^{2+} was studied by the method of Manzini *et al.* (1982), this component was enhanced by UK14304, but the component attributable to mobilization of intracellular Ca^{2+} was not affected. From these results it would appear that an influx of extracellular Ca^{2+} is required for producing the enhancing effect of α_2-adrenoceptor agonists on responses to α_1-adrenoceptor agonists.

Abe *et al.* (1987) found that the relatively selective α_1-adrenoceptor agonists methoxamine and phenylephrine produced an influx of Ca^{2+} into smooth muscle cells of the rat tail artery. More recently, Abe *et al.* (1988) reported that activation of α_1-adrenoceptors results in mobilization of intracellular Ca^{2+} and influx of extracellular Ca^{2+}, whereas activation of α_2-adrenoceptors results only in the influx of extracellular Ca^{2+}. Since idazoxan in a concentration that selectively blocks α_2-adrenoceptors reduces vasoconstrictor responses to methoxamine and phenylephrine in the rat tail artery (unpublished observations), it is possible that the Ca^{2+} influx produced by them could have been due to the activation of α_2-adrenoceptors. If this were the case, then the primary effects of methoxamine and phenylephrine on α_1-adrenoceptors resulting in mobilization of intracellular Ca^{2+} would be augmented by their secondary effect on α_2-adrenoceptors resulting in influx of extracellular Ca^{2+}.

TL99 enhanced vasoconstrictor responses to the relatively selective α_1-adrenoceptor agonist phenylephrine to a greater extent than those to noradrenaline, which acts on α_2- as well as α_1-adrenoceptors. We have previously found that another α_2-adrenoceptor agonist, rilmenidine, also enhanced responses to phenylephrine to a greater extent than those to noradrenaline (Xiao & Rand, 1989a). Furthermore, UK14304 enhanced the component of the response attributable to Ca^{2+} influx evoked by phenylephrine to a greater extent than that evoked by noradrenaline. The greater enhancement of responses to phenylephrine than to noradrenaline by α_2-adrenoceptor agonists may be because the noradrenaline had already activated a greater proportion of the α_2-adrenoceptor population than had phenylephrine. Responses to low concentrations of both phenylephrine and noradrenaline were enhanced to a greater extent than were those to high concentrations (Figure 1b,c). The decreasing enhancement with increasing concentrations, which was particularly marked with phenylephrine, could be due to increasing α_2-adrenoceptor activation.

Vasoconstrictor responses of the rat tail artery were reduced by idazoxan (Figure 2), diltiazem (Figure 3) and lowering the Ca^{2+} concentration of the perfusing solution from 2.5 to 0.61 mM (Figure 4). It is possible that these reductions may be partly due to elimination of an enhancement resulting from activation of α_2-adrenoceptors of the primary vasoconstrictor response produced by activating α_1-adrenoceptors. The reductions of responses to noradrenaline produced by idazoxan and diltiazem were accompanied by loss of the enhancing effect of TL99, but the enhancing effect of TL99 was still present when the Ca^{2+} concentration was decreased to 0.61 mM, suggesting that the increased Ca^{2+} influx produced by TL99 more than compensated for the decrease in the extracellular concentration.

We have previously suggested that the function of postjunctional α_2-adrenoceptors may be to augment the stimulus produced by activation of α_1-adrenoceptors (Xiao & Rand, 1989a). A similar suggestion has been made by Shepperson (1984) on the basis of experiments with the cat nictitating membrane. Such an ancillary role for postjunctional α_2-adrenoceptors might account for the functional interaction between α-adrenoceptor subtypes observed by Daly *et al.* (1988) in isolated vascular preparations, and for the suggestion by Sulpizio & Hieble (1987) that a circulating factor was necessary for the expression of pressor responses to α_2-adrenoceptor agonists *in vivo*.

5-Hydroxytryptamine in concentrations below those causing vasoconstriction produces a similar enhancement of vasoconstrictor responses to that produced by α_2-adrenoceptor agonists, and this effect also appears to be due to an increased Ca^{2+} influx in the rat tail artery (Xiao & Rand, 1989b) and rabbit ear artery (Meehan *et al.*, 1988). These observations lead us to suggest that the vasodilator and antihypertensive actions of calcium channel blocking drugs could be largely or even entirely due to inhibition of Ca^{2+} influx through channels that are opened by endogenous agonists that enhance vasoconstriction, rather than to blockade of calcium channels that are opened by the primary vasoconstrictor stimulus.

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References

ABE, K., MATSUKI, N. & KASUYA, Y. (1987). Pharmacological and electrophysiological discrimination of contractile responses to selective α_1- and α_2-adrenoceptor agonists in rat tail artery. *Jpn. J. Pharmacol.*, **45**, 249–261.

ABE, K., MATSUKI, N. & SAITO, H. (1988). Mobilization of Ca^{2+} in postsynaptic α_1- and α_2-adrenoceptor-mediated vasoconstriction. *Proceedings of the 5th South East Asian/Western Pacific Regional Meeting of Pharmacologists*, Beijing. Abstract O 39.06.

ABEL, P.W. & MINNEMAN, K.P. (1986). Alpha-1 adrenergic receptor binding and contraction of rat caudal artery. *J. Pharmacol. Exp. Ther.*, **239**, 678–686.

CAMBRIDGE, D. (1981). UK14304, a potent and selective α_2-agonist for the characterisation of α-adrenoceptor subtypes. *Eur. J. Pharmacol.*, **72**, 413–415.

CASTEELS, R. & DROOGMANS, G. (1981). Exchange characteristics of the noradrenaline-sensitive calcium store in vascular smooth muscle of rabbit ear artery. *J. Physiol.*, **317**, 263–279.

CAVERO, I., SHEPPERSON, F., LÉFEVRE-BORG, F. & LANGER, S.Z. (1983). Differential inhibition of vascular smooth muscle responses to α_1- and α_2-adrenoceptor agonists by diltiazem and verapamil. *Circulation Res.*, **52** (suppl. 1), 69–76.

DALY, C.J., McGRATH, J.C. & WILSON, V.G. (1988). Pharmacological analysis of postjunctional α-adrenoceptors mediating contractions to (−)-noradrenaline in the rabbit isolated lateral saphenous vein can be explained by simultaneous activation of α_1- and α_2-adrenoceptors. *Br. J. Pharmacol.*, **95**, 485–500.

DASHWOOD, M. & JACOBS, M. (1985). Autoradiographic study of the alpha-adrenoceptors of rat aorta and tail artery. *Eur. J. Pharmacol.*, **115**, 129–130.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.G. (1983). Studies on RX 781094, a new selective potent specific antagonist of α_2-adrenoceptors. *Br. J. Pharmacol.*, **78**, 489–495.

GRANT, J.A. & SCRUTTON, M.C. (1980). Interaction of selective alpha-adrenoceptor agonists and antagonists with human and rabbit blood platelets. *Br. J. Pharmacol.*, **71**, 121–134.

HICKS, P.E. & CANNON, J.G. (1980). Cardiovascular effect of 2-(*NN*-dimethyl)amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene in pithed rat: differential antagonism by yohimbine and prazosin. *J. Pharm. Pharmacol.*, **32**, 786–788.

HICKS, P.E., TIERNEY, C. & LANGER, S.Z. (1985). Preferential antagonism by diltiazem of α_2-adrenoceptor mediated vasoconstrictor responses in perfused tail arteries of spontaneously hypertensive rats. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 388–395.

JIM, K.F. & MATTHEWS, W.D. (1985). Role of extracellular calcium in contractions produced by activation of postsynaptic alpha-2 adrenoceptors in the canine saphenous vein. *J. Pharmacol. Exp. Ther.*, **234**, 161–165.

MANZINI, S., MAGGI, C.A. & MELI, A. (1982). A simple procedure for assessing norepinephrine-induced cellular and extracellular Ca^{2+} mobilization in rabbit ear artery. *J. Pharmacol. Meth.*, **8**, 47–57.

McGRATH, J.C. (1982). Evidence for more than one type of postjunctional α-adrenoceptor. *Biochem. Pharmacol.*, **31**, 467–484.

MEDGETT, I.C. & LANGER, S.Z. (1984). Heterogeneity of smooth muscle alpha-adrenoceptors in rat tail artery *in vitro*. *J. Pharmacol. Exp. Ther.*, **229**, 823–830.

MEDGETT, I.C. & RAJANAYAGAM, M.A.S. (1984). Effects of reduced calcium ion concentration and of diltiazem on vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in rat isolated tail artery. *Br. J. Pharmacol.*, **83**, 889–898.

MEEHAN, A.G., MEDGETT, I.C. & STORY, D.F. (1988). Involvement of Ca^{2+} mobilization in the amplifying effect of serotonin on responses of rabbit isolated ear artery to exogenous noradrenaline. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **337**, 500–503.

NASSERI, A., BARAKEH, J.F., ABEL, P.W. & MINNEMAN, K.P. (1985). Reserpine-induced post-junctional supersensitivity in rat vas deferens and caudal artery without

changes in alpha-adrenergic receptors. *J. Pharmacol. Exp. Ther.*, **234**, 350–357.

RAJANAYAGAM, M.A.S. & MEDGETT, I.C. (1987). Greater activation of smooth muscle alpha$_2$-adrenoceptors by epinephrine in distal than in proximal segments of rat tail artery. *J. Pharmacol. Exp. Ther.*, **240**, 989–997.

SCHÜMANN, H.-J. & LUES, I. (1983). Postjunctional α-adrenoceptors in the isolated saphenous vein of the rabbit: characterization and influence of angiotensin. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **323**, 328–334.

SHEPPERSON, N.B. (1984). α_2-Adrenoceptor agonists potentiate responses mediated by α_1-adrenoceptors in the cat nictitating membrane. *Br. J. Pharmacol.*, **83**, 463–469.

SU, C.M., SWAMY, V.C. & TRIGGLE, D.J. (1986). Postsynaptic α-adrenoceptor characterization and Ca^{2+} channel antagonist and activator actions in rat rail arteries from normotensive and hypertensive animals. *Can. J. Physiol. Pharmacol.*, **64**, 909–921.

SULPIZIO, A. & HIEBLE, J.P. (1987). Demonstration of α_2-adrenoceptor-mediated contraction in the isolated canine saphenous artery treated with Bay K 8644. *Eur. J. Pharmacol.*, **135**, 107–110.

TIMMERMANS, P.B.M.W.M., MATHY, M.-J., THOOLEN, M.J.M.C., DE JONGE, A., WILFFERT, B. & VAN ZWIETEN, P.A. (1984). Invariable susceptibility to blockade by nifedipine of vasoconstriction to various α_2-adrenoceptor agonists in pithed rats. *J. Pharm. Pharmacol.*, **36**, 772–775.

VAN MEEL, J.C.A., DE JONGE, A., KALKMAN, H.O., WILFFERT, B., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981a). Organic and inorganic calcium antagonists reduce vasoconstriction *in vivo* mediated by postsynaptic α_2-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **316**, 288–293.

VAN MEEL, J.C.A., DE JONGE, A., KALKMAN, H.O., WILFFERT, B., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981b). Vascular smooth muscle contraction initiated by postsynaptic α_2-adrenoceptor activation is induced by an influx of extracellular calcium. *Eur. J. Pharmacol.*, **69**, 205–208.

WEISS, R.J., WEBB, R.C. & SMITH, C.B. (1983). Alpha$_2$-adrenoceptors on arterial smooth muscle: selective labelling by [^{3}H]clonidine. *J. Pharmacol. Exp. Ther.*, **225**, 599–605.

XIAO, X-H., MEDGETT, I.C. & RAND, M.J. (1987a). The α_2-adrenoceptor agonists clonidine TL99 and DPI enhance vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in the rat tail artery preparation. *Clin. Exp. Pharmacol. Physiol.*, **14**, 903–909.

XIAO, X-H., MEDGETT, I.C. & RAND, M.J. (1987b). α_2-Adrenoceptor agonists amplify vasoconstrictor responses to other agonists by a calcium-dependent mechanism. *Blood Vessels*, **24**, 228.

XIAO, X-H. & RAND, M.J. (1989a). α_2-Adrenoceptor agonists enhance response to certain other vasoconstrictor agonists in the rat tail artery. *Br. J. Pharmacol.*, **96**, 539–546.

XIAO, X-H. & RAND, M.J. (1989b). Amplification by serotonin of responses to other vasoconstrictor agents in the rat tail artery. *Clin. Exp. Pharmacol. Physiol.*, **16**, (in press).

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