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Studies on the anti-vasoconstrictor activity of BRL 34915 in spontaneously hypertensive rats; a comparison with nifedipine

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1 The blood pressure lowering and anti-vasoconstrictor effects of BRL 34915 and nifedipine were compared in female spontaneously hypertensive rats (SHR).

2 In conscious SHR, intravenous injection of BRL 34915 (0.1, 0.3 mg kg^{-1}) produced rapid, dose-related falls in mean arterial pressure of greater than 3 h duration. Nifedipine, at the same intravenous dose levels, also evoked rapid anti-hypertensive effects, though these responses were of lesser magnitude and duration than those observed for BRL 34915.

3 In anaesthetized, ganglion-blocked SHR, BRL 34915 (0.1, 0.3 mg kg^{-1} i.v.) dose-dependently antagonized the pressor responses to incremental intravenous infusions of noradrenaline (3.8–28.5 ng min^{-1}) or phenylephrine (120–907 ng min^{-1}) but did not inhibit pressor responses to incremental infusions of methoxamine (0.47–3.63 $\mu g\,min^{-1}$), angiotensin II (7.0–52.9 ng min^{-1}) or vasopressin (0.27–2.0 mu min^{-1}).

4 In anaesthetized, ganglion-blocked SHR, nifedipine (0.1, 0.3 mg kg^{-1} i.v.) antagonized the pressor responses to each of the infused vasoconstrictor agents, being most effective against responses to noradrenaline or angiotensin II.

5 In pithed SHR, both BRL 34915 and nifedipine (each at 0.3 mg kg^{-1} i.v.) reduced the basal blood pressure level and produced marked inhibition of frequency-dependent pressor responses evoked by electrical stimulation of the spinal cord sympathetic outflow (0.25–4.0 Hz). Restoration of the basal diastolic blood pressure to within the control range, using a continuous intravenous infusion of vasopressin (0.98 mu min^{-1}), prevented the inhibitory effect of BRL 34915. In the case of nifedipine, however, even raising the basal blood pressure to a level exceeding that recorded in control rats (with vasopressin, 2.0 mu min^{-1}), did not reverse the inhibitory effect of the drug on frequency-dependent pressor responses.

6 It is concluded that the anti-hypertensive properties of BRL 34915 in SHR are probably unrelated to an anti-vasoconstrictor action. In contrast, it is suggested that the broadly-based anti-vasoconstrictor properties of nifedipine may contribute substantially to the anti-hypertensive properties of this drug.

Introduction

Recent publications have indicated that BRL 34915, (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol, is a potent blood pressure lowering agent in laboratory animals (Buckingham *et al.*, 1986), and that the vascular relaxant properties of the drug are most probably related to an ability to hyperpolarize the smooth muscle cell membrane (Hamilton *et al.*, 1986; Weir & Weston, 1986). Furthermore, tissue bath experiments have shown that BRL 34915 can inhibit contractile responses to noradrenaline in rat aorta (Weir & Weston, 1986), rat portal vein (Hamilton *et al.*, 1986; Weir & Weston, 1986) and in rabbit mesenteric artery (Clapham & Wilson, 1986). These results and the findings of an earlier study that BRL 34915 antagonized contractile responses to 5-hydroxytryptamine in rabbit isolated mesenteric artery (Buckingham *et al.*, 1984), have raised the possibility that such anti-vasoconstrictor activity, if expressed *in vivo*, could contribute to the blood pressure lowering properties of the drug.

In the present paper this possibility has been

explored by use of doses of BRL 34915 which, when given intravenously to conscious, female spontaneously hypertensive rats (SHR), lowered mean arterial blood pressure for several hours. The effects of these doses of BRL 34915 on pressor responses to infusions of various vasoconstrictor agents were then determined in anaesthetized, ganglion-blocked SHR. Effects of BRL 34915 on responses to electrical stimulation of the spinal cord total sympathetic outflow were established in pithed female SHR. For comparison, nifedipine was also studied to illustrate the different properties of a typical dihydropyridine calcium slow channel blocking drug.

Methods

Experiments were conducted in female SHR, aged at least 18 wk, and reared within the Beecham Medicinal Research Centre at Harlow.

Conscious rat studies

Rats were anaesthetized with methohexitone sodium (Brietal), 45 mg kg^{-1} i.p., and implanted with polyethylene tube catheters in the abdominal aorta and vena cava by a method described by Buckingham (1976). Following recovery from the anaesthetic, animals were housed singly in a quiet room and used for experiment on the second post-operative day. Mean arterial blood pressure (1 mmHg $\approx$ 133 Pa) was recorded continuously in unrestrained rats with Bell and Howell pressure transducers connected to Ormed multichannel chart recorders. Instantaneous ratemeters triggered by the arterial pulse wave provided a continuous record of heart rate. Measurements of blood pressure and heart rate for each animal were obtained from the chart records before dosing commenced (time 0) and at fixed intervals after the completion of an intravenous injection of BRL 34915 (or nifedipine in a separate study), 0.1 or 0.3 mg kg^{-1}, or drug vehicle, 1 ml kg^{-1}.

Anaesthetized and pithed rat experiments

For experiments conducted wholly under anaesthesia, pentobarbitone sodium (Sagatal), 60 mg kg^{-1} i.p. was administered as the anaesthetic agent. Polyethylene catheters were inserted into a carotid artery, to allow continuous blood pressure monitoring, and into a jugular vein to facilitate intravenous drug injections and subsequent infusion of a vasoconstrictor agent. All rats in these experiments were treated with mecamylamine hydrochloride, 2 mg kg^{-1} i.v., to produce ganglion blockade. In the case of animals to be subsequently infused with noradrenaline or phenylephrine, vascular β_2-adrenoceptors were blocked with ICI 118,551 hydrochloride, 2 mg kg^{-1} i.v. For the noradrenaline study, neuronal uptake was also inhibited with desmethylimipramine hydrochloride, 2 mg kg^{-1} i.v. No such pretreatments were given in the methoxamine, angiotensin II or vasopressin infusion studies.

Following the administration of mecamylamine with or without these further pretreatments, blood pressure was allowed to stabilize and each animal was assigned to either a control or drug-treatment group on the basis of a matching basal diastolic pressure. BRL 34915 (or nifedipine in a separate experiment), at doses of 0.1 or 0.3 mg kg^{-1} i.v., or drug vehicle, 1 ml kg^{-1} i.v., were then injected and blood pressure was allowed to stabilize for a further 15 min before intravenous infusion of the vasoconstrictor agent was started (by use of a Harvard 975 Compact Infusion Pump). The rate of infusion was increased approximately two fold at 20 min intervals until four infusion periods had been completed.

For pithed rat studies, methohexitone sodium (Brietal), 45 mg kg^{-1} i.p., was administered as the anaesthetic agent and intra-arterial and intravenous catheters were inserted as above. In these animals, however, the trachea was also cannulated. Rats were then pithed with a stainless steel rod through the orbit of the right eye and the pithing rod advanced to the caudal end of the spinal canal. The tracheal cannula was rapidly connected to a small animal respiratory pump (BioScience U.K.) set to deliver 80 cycles of oxygen-enriched (60 ml min^{-1}) room air each minute. The pre-set volume for each cycle of the pump was approximately 15 ml kg^{-1} bodyweight. A short length of steel rod was inserted under the ventral skin of the left hindlimb to serve as an indifferent electrode. Atropine methyl nitrate (2 mg kg^{-1} i.v.) and (+)-tubocurarine hydrochloride (2 mg kg^{-1} i.v.) were administered to inhibit peripheral muscarinic receptors and neuromuscular transmission, respectively. ICI 118,551 hydrochloride (2 mg kg^{-1} i.v.) was given to block vascular β_2-adrenoceptors. After blood pressure had stabilized, the spinal cord total peripheral sympathetic outflow of each animal was electrically stimulated via the pithing rod at a frequency of 2.0 Hz (20 s), with monophasic rectangular pulses of 0.5 ms duration at supramaximal voltage. The pressor response observed was used as a basis for assigning individual rats to matching control and drug-treatment groups. Once blood pressure had returned to baseline, BRL 34915 (or nifedipine in a separate experiment), 0.3 mg kg^{-1} i.v., or drug vehicle, 1 ml kg^{-1} i.v., were administered and 15 min later a frequency-response relationship was constructed over the range 0.25–4.0 Hz. Each stimulation frequency was applied for 20 s and blood pressure was allowed to return to baseline between periods of stimulation.

In a second series of experiments, the intravenous injection of BRL 34915 or nifedipine was made at the time of peak effect of a prolonged infusion of vasopressin (0.98 mu min^{-1} in the BRL 34915 study; 2.0 mu min^{-1} in the nifedipine experiment). Control rats in each study were infused continuously with a matching volume of 5% w/v dextrose solution. Pressor responses to electrical stimulation were started 15 min after administration of BRL 34915, nifedipine or control vehicle.

Drugs

The following drugs were used: BRL 34915 (Beecham Research Laboratories), nifedipine (Bayer), atropine methyl nitrate, noradrenaline bitartrate, phenylephrine hydrochloride, angiotensin II, vasopressin (all Sigma), methoxamine hydrochloride, (+)-tubocurarine hydrochloride (Tubarine) (both Burroughs Wellcome), desmethylimipramine hydrochloride (Ciba-Geigy), mecamylamine hydrochloride (Merck, Sharpe and Dohme), ICI 118,551 hydrochloride (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; ICI), pentobarbitone sodium (Sagatal; May and Baker), methohexitone sodium (Brietal; Lilly).

BRL 34915 was dissolved in sterile water and nifedipine in a solution of 50% v/v polyethylene glycol in sterile water. Each of the vasoconstrictor agents used for infusion was dissolved in a 5% w/v dextrose solution, and ascorbic acid (1 mg ml^{-1}) was included in the noradrenaline, phenylephrine and methoxamine infusates. Other drugs for intravenous injection were dissolved in sterile water or isotonic saline. Sagatal and Brietal were drawn from multi-dose vials and (+)-tubocurarine hydrochloride was diluted in sterile water from the concentrated solution (15 mg 1.5 ml^{-1}) contained in a glass vial.

Infusion concentrations of noradrenaline, phenylephrine and methoxamine have been expressed as free base, and other drug concentrations in the form stated in the text.

Statistical analysis

All experimental data were analysed with Student's *t* test for unpaired data. Probability values (*P*) of less than 0.05 were considered statistically significant.

Results

Comparison between BRL 34915 and nifedipine in conscious SHR

In the BRL 34915 study, pretreatment levels of mean arterial pressure (± s.e. mean) were as follows: for 0.1 mg kg^{-1}, 143 ± 4 mmHg; for 0.3 mg kg^{-1}, 147 ± 5 mmHg; for control, 147 ± 6 mmHg. As demonstrated in Figure 1a, BRL 34915, 0.1 or 0.3 mg kg^{-1}, lowered blood pressure promptly after intravenous injection in conscious, female SHR. The peak anti-hypertensive effect of both doses was observed within the first 5 min, and after the higher dose, mean arterial pressure fell to a hypotensive level (<80 mmHg). During the remainder of the 3 h time course, blood pressure in drug-treated rats gradually increased towards pretreatment levels. Compared with the mean arterial pressure observed in control rats, however, marked and statistically significant anti-hypertensive effects of both doses of BRL 34915 were still evident at the end of the experiment.

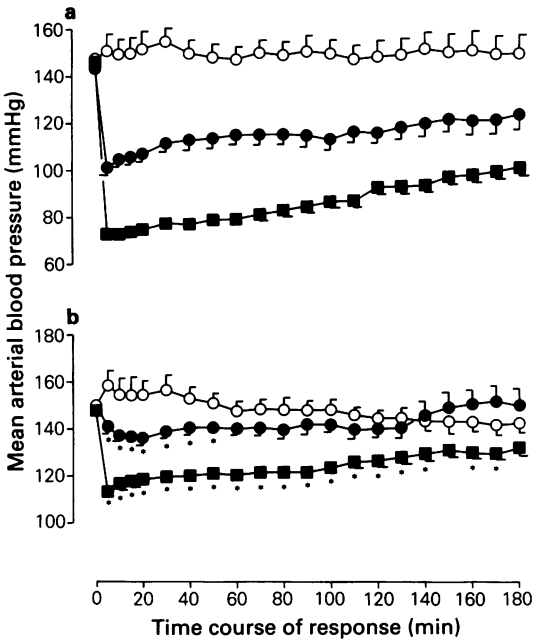

Figure 1 Effect of intravenously administered (a) BRL 34915 or (b) nifedipine on mean arterial blood pressure in conscious, female spontaneously hypertensive rats (SHR). The following symbols, which depict group mean arterial blood pressure values, are common to (a) and (b): control vehicle, 1 ml kg^{-1} (○); 0.1 mg kg^{-1} (●); 0.3 mg kg^{-1} (■). Group sizes were 11 or 13 animals in the BRL 34915 study and 8 animals in the nifedipine experiment. Vertical lines extending from the symbols depict standard error of the mean (s.e. mean). In (a) $P < 0.05$ for all points. In (b) and subsequent figures, statistically significant differences ($P < 0.05$) between the control group and drug-treated groups of rats are highlighted by an asterisk.

In a subsequent study of intravenous nifedipine,

pretreatment levels of mean blood pressure (± s.e. mean) were as follows: for 0.1 mg kg^{-1}, 150 ± 3 mmHg; for 0.3 mg kg^{-1}, 148 ± 4 mmHg; for control, 150 ± 4 mmHg. On a dose-for-dose comparison, nifedipine was a less potent antihypertensive agent than BRL 34915 (Figure 1b). Blood pressure was lowered only slightly by 0.1 mg kg^{-1} of nifedipine, and then only for 50 min. Following the higher dose of nifedipine (0.3 mg kg^{-1}), blood pressure fell to a nadir within 5 min and remained significantly lower than in control rats until 140 min. The overall effectiveness of this higher dose of nifedipine, however, appeared to be somewhat less than that of the lower dose of BRL 34915.

Both BRL 34915 and nifedipine caused marked tachycardia in conscious rats, presumably as a reflex response to the falls in mean arterial pressure (data not shown).

Effects of BRL 34915 and nifedipine on the pressor responses to various vasoconstrictor agents infused into anaesthetized, ganglion-blocked SHR

Intravenous administration of BRL 34915 or nifedipine (0.1, 0.3 mg kg^{-1}) in anaesthetized, ganglion-blocked SHR caused dose-related falls in diastolic blood pressure, whereas injections of corresponding drug vehicle had little effect. For each of the experiments in this section the pre- and post-treatment levels of blood pressure are given in Tables 1 (for BRL 34915) and 2 (for nifedipine).

Noradrenaline infusion Pretreatment of rats with BRL 34915 resulted in a dose-related inhibition of the increments in diastolic blood pressure associated with intravenous infusion of noradrenaline at rates of 3.8, 7.4, 13.9 and 28.5 ng min^{-1} (Figure 2a).

Nifedipine was an even more potent inhibitor of

Table 1 Values of diastolic blood pressure (mmHg ± s.e. mean) before (pre) and after (post) treatment with drug vehicle or BRL 34915 in groups of anaesthetized, ganglion-blocked SHR

	Vehicle		*BRL 34915*			
			0.1 mg kg^{-1}		0.3 mg kg^{-1}	
Pressor agent to follow	Pre	Post	Pre	Post	Pre	Post
Noradrenaline	57 ± 3 (n = 10)	58 ± 3	58 ± 3 (10)	35 ± 2	59 ± 3 (10)	29 ± 2
Phenylephrine	70 ± 6 (n = 10)	70 ± 6	62 ± 3 (10)	38 ± 2	67 ± 5 (10)	30 ± 3
Methoxamine	65 ± 3 (n = 9)	68 ± 4	63 ± 5 (9)	39 ± 2	63 ± 4 (9)	27 ± 2
Angiotensin II	65 ± 5 (n = 12)	62 ± 6	61 ± 5 (13)	39 ± 3	66 ± 6 (12)	32 ± 4
Vasopressin	74 ± 5 (n = 9)	73 ± 4	71 ± 4 (10)	42 ± 2	74 ± 6 (9)	32 ± 3

n = number of rats in group

Table 2 Values of diastolic blood pressure (mmHg ± s.e. mean) before (pre) and after (post) treatment with drug vehicle or nifedipine in groups of anaesthetized, ganglion-blocked SHR

	Vehicle		*Nifedipine*			
			0.1 mg kg^{-1}		0.3 mg kg^{-1}	
Pressor agent to follow	Pre	Post	Pre	Post	Pre	Post
Noradrenaline	60 ± 5 (n = 8)	61 ± 4	57 ± 3 (8)	36 ± 2	60 ± 5 (8)	33 ± 2
Phenylephrine	66 ± 5 (n = 8)	66 ± 5	66 ± 3 (8)	52 ± 3	65 ± 4 (8)	38 ± 2
Methoxamine	61 ± 3 (n = 9)	64 ± 3	61 ± 3 (10)	38 ± 2	62 ± 2 (10)	35 ± 1
Angiotensin II	65 ± 5 (n = 8)	65 ± 5	70 ± 6 (8)	41 ± 3	66 ± 6 (8)	33 ± 1
Vasopressin	75 ± 6 (n = 8)	75 ± 6	70 ± 6 (9)	46 ± 3	73 ± 5 (9)	37 ± 1

n = number of rats in group

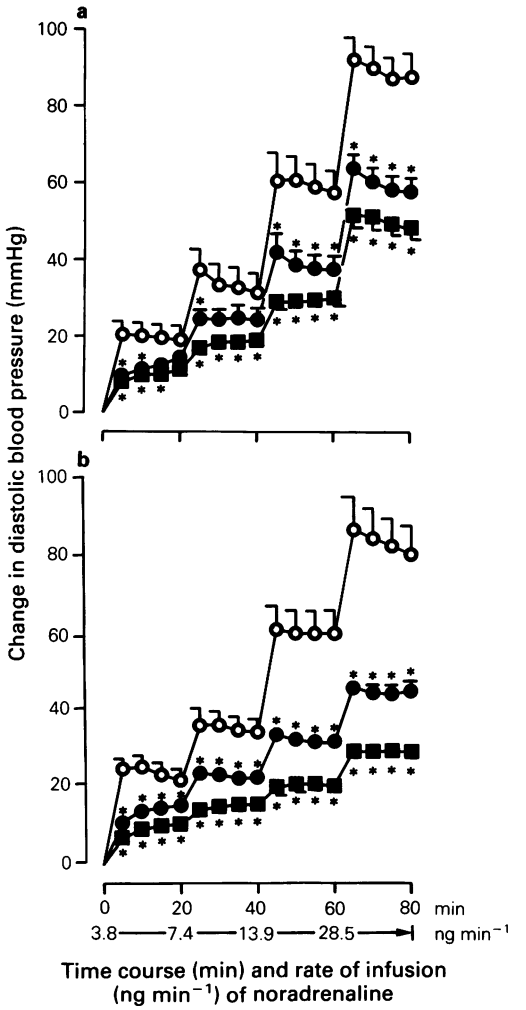

Figure 2 Effect of (a) BRL 34915 or (b) nifedipine on the pressor response to incremental intravenous infusion of noradrenaline in anaesthetized, ganglion-blocked, female spontaneously hypertensive rats. The following symbols, which depict group mean changes in diastolic blood pressure (± s.e. mean), are common to (a) and (b): control vehicle, 1 ml kg^{-1} (○); 0.1 mg kg^{-1} (●); 0.3 mg kg^{-1} (■). Each group contained 10 animals in the BRL 34915 experiment, and 8 animals in the nifedipine study.

noradrenaline-evoked pressor responses than BRL 34915 (Figure 2b). Thus the higher dose of BRL 34915 (0.3 mg kg^{-1}) and the lower dose of nifedipine (0.1 mg kg^{-1}) possessed approximately equivalent inhibitory activity against the pressor response to noradrenaline infused at 28.5 ng min^{-1}.

Phenylephrine For the sake of clarity, the influence of only the higher dose of BRL 34915 on the pressor response to phenylephrine infusion is demonstrated in Figure 3a. At low infusion rates of phenylephrine (120 and 235 ng min^{-1}), BRL 34915 (0.3 mg kg^{-1}) exhibited scarcely any inhibitory effect on the progressive rise in diastolic blood pressure. However, the larger pressor effects elicited by 440 and

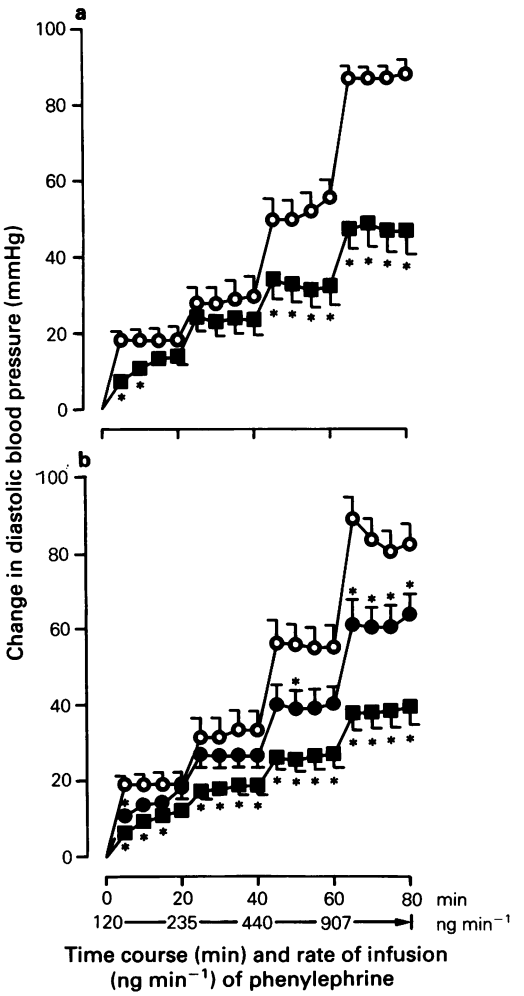

Figure 3 Effect of (a) BRL 34915 or (b) nifedipine on the pressor response to incremental intravenous infusion of phenylephrine in anaesthetized, ganglion-blocked, female spontaneously hypertensive rats. The following symbols, depicting group mean changes in diastolic blood pressure (± s.e. mean), are used in both (a) and (b): control vehicle, 1 ml kg^{-1} (○); 0.1 mg kg^{-1} (●); 0.3 mg kg^{-1} (■). Each group comprised 10 rats in (a) and 8 rats in (b).

907 ng min^{-1} of phenylephrine, were antagonized by BRL 34915. At each of the 5 min intervals during the highest infusion rate, the pressor response was significantly attenuated by BRL 34915, 0.1 mg kg^{-1}, though the pressor effects of lower infusion rates were scarcely influenced (data not shown).

The pressor responses to increasing infusion rates of phenylephrine were dose-dependently reduced by nifedipine (0.1, 0.3 mg kg^{-1}) (Figure 3b).

Methoxamine Increments in diastolic pressure produced by infusion of methoxamine at 0.47, 0.94, 1.76 and 3.63 μg min^{-1} were very similar in control rats and in those given either of the doses of BRL 34915. For the sake of clarity only the lack of effect of BRL 34915, 0.3 mg kg^{-1}, is depicted in Figure 4a.

In contrast to the lack of effect of BRL 34915 on pressor responses to methoxamine, nifedipine reduced the magnitude of the sequential increments. This inhibitory influence was most marked at the highest infusion rate of methoxamine (3.63 μg min^{-1}), though both doses of nifedipine were equally effective. For the sake of clarity only the effect of nifedipine, 0.3 mg kg^{-1}, is depicted in Figure 4b.

Angiotensin II Infusion of angiotensin II at rates of 7.0, 13.9, 26.0 and 52.9 ng min^{-1} caused stepwise increments in diastolic blood pressure in control rats and the magnitude of these increments was not significantly altered by pretreatment with BRL 34915. Only the lack of effect of BRL 34915, 0.3 mg kg^{-1}, is depicted in Figure 5a.

In contrast to the absence of effect of BRL 34915 on pressor responses to infused angiotensin II, nifedipine exhibited potent dose-related inhibitory effects against this pressor agent (Figure 5b). At each infusion rate of angiotensin II, nifedipine significantly reduced the magnitude of the pressor response and this effect was observed even at the lower dose (0.1 mg kg^{-1}) of the calcium antagonist.

Vasopressin Infusion of vasopressin at rates of 0.27, 0.52, 0.98 and 2.0 mu min^{-1} produced stepwise increments in diastolic blood pressure in both control and BRL 34915-treated groups. Figure 6a demonstrates the lack of an inhibitory effect of 0.3 mg kg^{-1} of BRL 34915 on these blood pressure increments. In fact, at a vasopressin infusion rate of 2.0 mu min^{-1}, the peak mean pressor response was significantly higher in BRL 34915-treated rats (83 mmHg) than in controls (65 mmHg). In the presence of BRL 34915, 0.1 mg kg^{-1}, the pressor responses to vasopressin infused at 0.27, 0.52 or 2.0 mu min^{-1} (but not 0.98 mu min^{-1}), were also significantly greater than those observed in the control group.

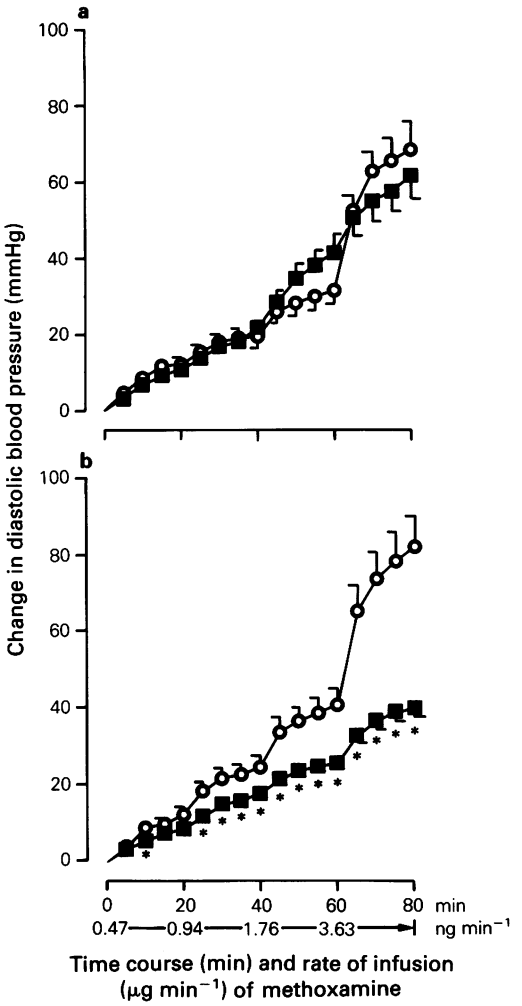

Figure 4 Effect of (a) BRL 34915 or (b) nifedipine on the pressor response to incremental intravenous infusion of methoxamine in anaesthetized, ganglion-blocked, female spontaneously hypertensive rats. The following symbols, depicting group mean changes in diastolic blood pressure (± s.e. mean), are used in both (a) and (b): control vehicle, 1 ml kg^{-1} (○); 0.3 mg kg^{-1} (■). Each group contained 9 rats in (a) and 9 or 10 in (b).

Although pressor responses to vasopressin infusion rates of 0.27 and 0.52 mu min^{-1} were not influenced by nifedipine, 0.3 mg kg^{-1}, responses to higher rates of infusion were significantly reduced (Figure 6b). Nifedipine, 0.1 mg kg^{-1}, did not influence the magnitude of the pressor response to vasopressin at any of the four infusion rates (data not shown).

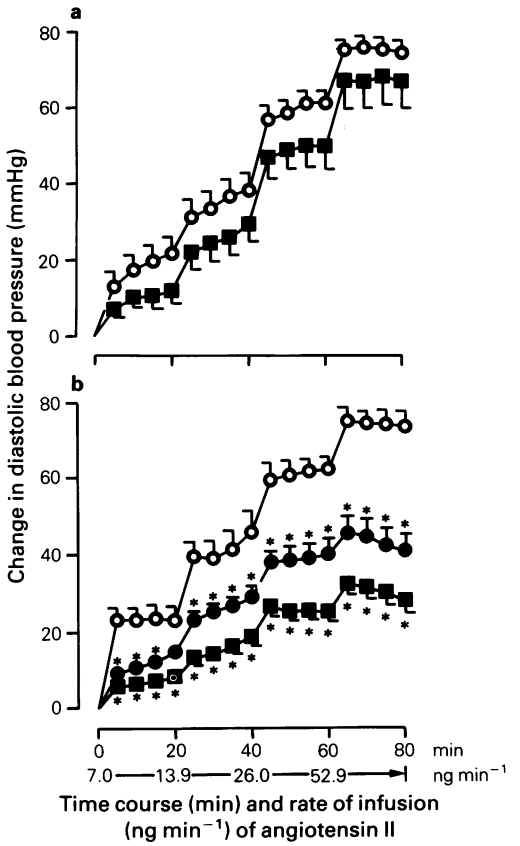

Figure 5 Effect of (a) BRL 34915 or (b) nifedipine on the pressor response to incremental intravenous infusion of angiotensin II in anaesthetized, ganglion-blocked, female spontaneously hypertensive rats. The following symbols, depicting group mean changes in diastolic blood pressure (± s.e. mean), are used in both (a) and (b): control vehicle, 1 ml kg^{-1} (○); 0.1 mg kg^{-1} (●); 0.3 mg kg^{-1} (■). Group sizes were 12 or 13 rats in (a) and 8 rats in (b).

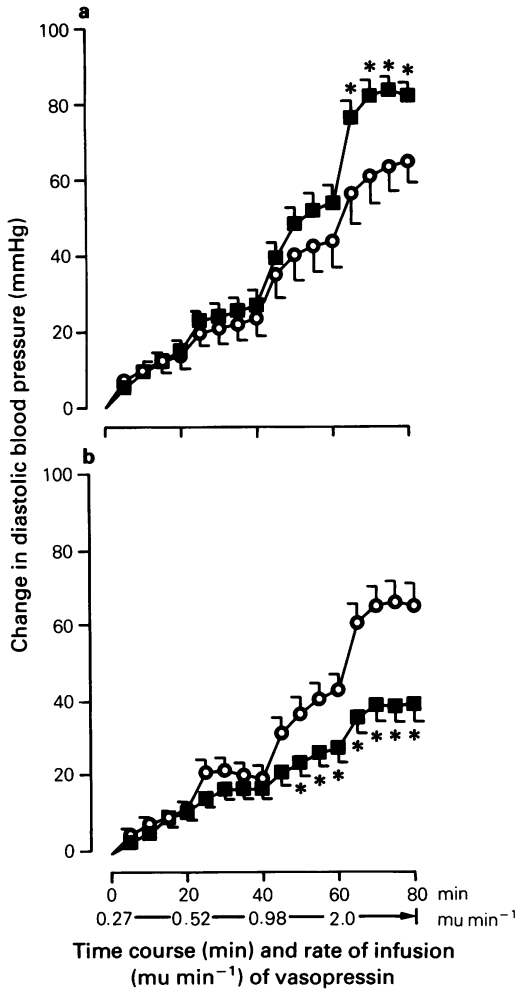

Figure 6 Effect of (a) BRL 34915 or (b) nifedipine on the pressor response to incremental intravenous infusion of vasopressin in anaesthetized, ganglion-blocked, female spontaneously hypertensive rats. The following symbols, common to both (a) and (b), depict group mean changes in diastolic blood pressure (± s.e. mean); control vehicle, 1 ml kg^{-1} (○); 0.3 mg kg^{-1} (■). Groups of 9 or 10 rats were used in (a) and 8 or 9 for (b).

Effects of intravenous BRL 34915 and nifedipine on the pressor responses to electrical stimulation of the total sympathetic outflow in pithed SHR

In all experiments in this section, rats were allotted to matching groups on the basis of their pretreatment pressor response to electrical stimulation at 2.0 Hz. In the first experiment, the group mean pressor responses to 2.0 Hz were 96 ± 5 mmHg in control rats and 100 ± 5 mmHg in rats to be treated with BRL 34915, 0.3 mg kg^{-1}. Fifteen minutes after treatment the mean basal diastolic blood pressure was 42 ± 4 mmHg in controls and 22 ± 1 mmHg in the BRL 34915 group ($P < 0.05$). Figure 7a demonstrates that the frequency-blood pressure response relationship as observed in control rats was displaced to the right in rats following BRL 34915 treatment. In a second experiment both the control group and the BRL 34915 (0.3 mg kg^{-1}) group had a mean pretreatment pressor response to electrical stimulation at 2.0 Hz of 94 ± 5 mmHg. In this study BRL 34915 injection was made at the time of peak

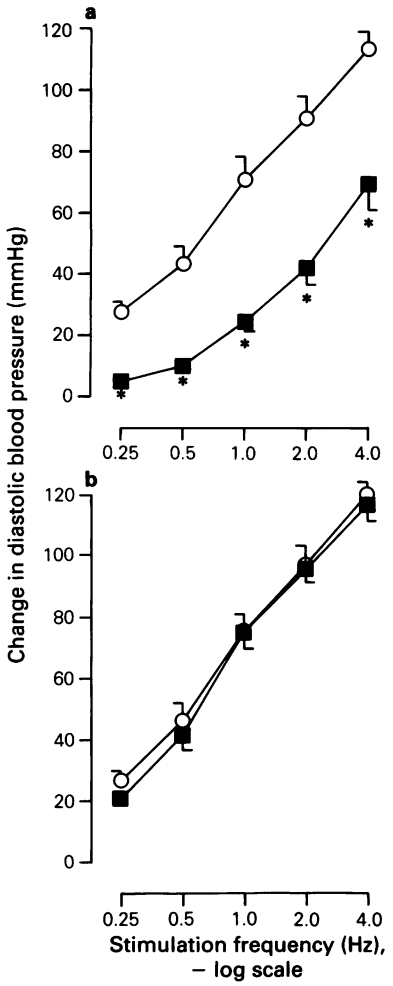

Figure 7 Effect of intravenously administered BRL 34915, either (a) alone or (b) in the presence of a continuous intravenous infusion of vasopressin (0.98 mu min^{-1}), on the pressor responses evoked by electrical stimulation of the spinal cord total sympathetic outflow in pithed, female spontaneously hypertensive rats. The following symbols, depicting group mean changes in diastolic blood pressure in response to each frequency (± s.e. mean), are used in both (a) and (b): control vehicle, 1 ml kg^{-1} (○); 0.3 mg kg^{-1} (■), In (a) the group size was 8 rats and in (b) 9 rats.

effect of a continuous infusion of vasopressin, 0.98 mu min^{-1}; control rats were infused with a matching volume of 5% w/v dextrose solution. Fifteen minutes after this injection, diastolic blood pressure was 43 ± 2 mmHg in controls and 42 ± 2 mmHg in rats given BRL 34915. In the presence of a continuous infusion of vasopressin, the inhibitory effect of BRL 34915 on the frequency-response relationship was abolished (Figure 7b).

In the first parallel experiment with nifedipine instead of BRL 34915, the initial pressor response to electrical stimulation at 2.0 Hz was 105 ± 4 mmHg in control rats and 103 ± 3 mmHg in rats to be given nifedipine. Treatment with nifedipine, 0.3 mg kg^{-1}, resulted in a fall in basal diastolic blood pressure to 28 ± 2 mmHg, whereas the basal blood pressure in control rats was 49 ± 2 mmHg ($P < 0.05$). Compared with the control group, nifedipine caused a statistically significant reduction in the magnitude of frequency-dependent pressor responses resulting in a marked displacement of the frequency-response relationship to the right (Figure 8a). In a second experiment, initial electrical stimulation at 2.0 Hz produced pressor responses of 100 ± 6 mmHg in the control group and 98 ± 6 mm Hg in the drug-treatment group. Injection of nifedipine, 0.3 mg kg^{-1} i.v., at the time of peak pressor effect of a continuous intravenous infusion of vasopressin (2.0 mu min^{-1}) resulted in a fall in diastolic blood pressure to 60 ± 4 mmHg after 15 min. Infusion of control rats with 5% w/v dextrose solution resulted in a basal blood pressure level of 44 ± 3 mmHg ($P < 0.05$). Despite the fact that basal diastolic pressure was then significantly higher in nifedipine-treated rats than in the control group, pressor responses to a range of electrical frequencies were still markedly reduced in the nifedipine group (Figure 8b).

Discussion

The present studies illustrate important differences between the ability of blood pressure lowering doses of BRL 34915 and of nifedipine to influence responses to various vasoconstrictor agents when these were infused into anaesthetized, ganglion-blocked SHR. BRL 34915 proved to be highly selective in its anti-vasoconstrictor action, being effective as an inhibitor of the pressor responses to exogenous noradrenaline (Figure 2a) and phenylephrine (Figure 3a) but being ineffective in reducing responses to methoxamine (Figure 4a), angiotensin II (Figure 5a) or vasopressin (Figure 6a). The discrepancy between the influence of BRL 34915 on responses to phenylephrine and methoxamine, both relatively selective α_1-adrenoceptor agonists, is particularly intriguing. Although the reasons for this discrepancy are not clear, other authors have drawn attention to the possible existence of a subclassification of α_1-adrenoceptors in some vascular tissues (Holck *et al.*, 1983; Medgett & Langer, 1984; Toda *et al.*, 1984; Flavahan & Vanhoutte, 1986). Following experiments in rat isolated portal vein and aorta, Ham-

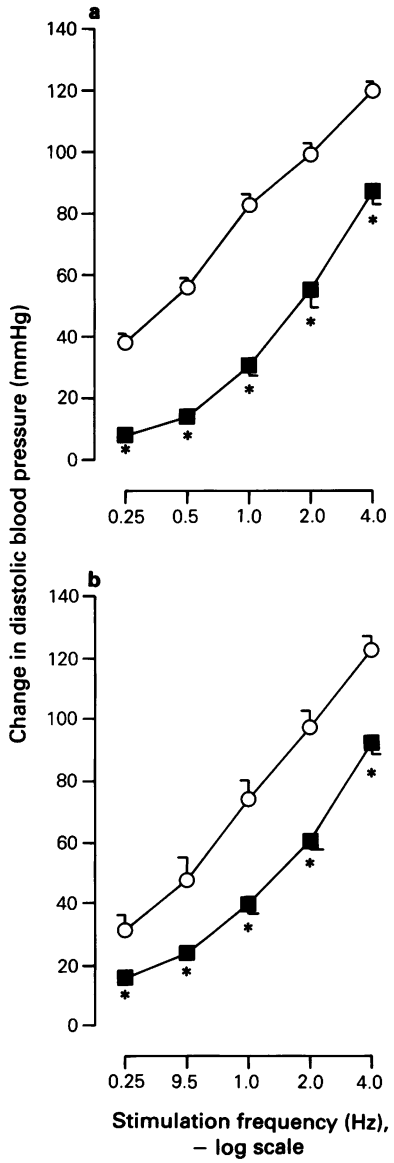

Figure 8 Effect of intravenously administered nifedipine, either (a) alone or (b) in the presence of a continuous intravenous infusion of vasopressin ($2.0\,mu\,min^{-1}$), on the pressor responses evoked by electrical stimulation of the spinal cord total sympathetic outflow in pithed, female spontaneously hypertensive rats. The following symbols, depicting group mean changes in diastolic blood pressure in response to each frequency (± s.e. mean), are common to (a) and (b): control vehicle, $1\,ml\,kg^{-1}$ (○); $0.3\,mg\,kg^{-1}$ (■). The group size in (a) was 10 rats, and 9 in (b).

ilton *et al.* (1986) and Weir & Weston (1986) concluded that inhibition by BRL 34915 of contractile responses to noradrenaline was related to an ability to open membrane potassium channels leading to either hyperpolarization of the cell or to the short-circuiting of a depolarizing stimulus. The same hypothesis could be advanced to explain the ability of the drug to reduce pressor responses to infused noradrenaline or phenylephrine in the present experiments *in vivo*. It would appear, however, that the coupling of methoxamine-sensitive α_1-adrenoceptors to vascular contraction in anaesthetized rats is not influenced by the activation of membrane potassium channels. Similarly, vascular smooth muscle contraction evoked by angiotensin II or vasopressin would also appear to be unaffected by the state of activation of BRL 34915-sensitive potassium channels. It should be added, however, that in recording pressor responses to vasoconstrictors *in vivo*, we are measuring the algebraic summation of contractile events in all vascular beds and it is conceivable that this overall response may not reflect the behaviour of each vascular bed.

Although nifedipine was clearly less potent than BRL 34915 in lowering the mean arterial pressure of conscious SHR, the calcium antagonist was a more effective inhibitor of pressor responses to a range of vasoconstrictor agents. Responses to noradrenaline (Figure 2b) and angiotensin II (Figure 5b) were particularly sensitive in this respect. A voluminous literature has accumulated on the apparent selectivity of calcium antagonists for inhibiting pressor responses to bolus intravenous injections of α_2- as opposed to α_1-adrenoceptor agonists *in vivo* (Van Meel *et al.*, 1981a, b; 1982; Cavero *et al.*, 1983; Gerold & Hauesler, 1983; Timmermans *et al.*, 1983a). As a result of more recent studies, however, this distinction between α_2- and α_1-adrenoceptors based on calcium dependency has been shown to be inadequate and the reader is referred to publications by Timmermans *et al.* (1983b, c), Ruffolo *et al.* (1984), Pedrinelli & Tarazi (1985) and Timmermans *et al.* (1985) for further discussion on this topic.

Aside from this continuing controversy, the results of the present study are consistent with the notion that the sustained vascular contraction, produced by intravenous infusion of the α_1-adrenoceptor agonists phenylephrine or methoxamine in anaesthetized ganglion-blocked SHR, is dependent upon the influx of extracellular calcium through nifedipine-sensitive channels. Furthermore, we can assume that the maintenance of a vascular contractile response to infusion of angiotensin II or vasopressin is similarly dependent upon nifedipine-sensitive calcium influx. Goldberg & Schrier (1984) have reported previously on the ability of nifedipine to impair markedly the pressor effects of intravenously infused noradrena-

line, angiotensin II or vasopressin in conscious rats. Perhaps of greater significance, however, was the observation in the present study that nifedipine also reduced the magnitude of pressor responses to electrical stimulation of the spinal cord sympathetic outflow after pithing (Figure 8a). Moreover, this inhibitory effect of nifedipine persisted even when the basal blood pressure was raised above that in control animals by an infusion of vasopressin (Figure 8b). These results demonstrate that the anti-vasoconstrictor effect of nifedipine in this context is essentially independent of the prevailing level of basal blood pressure. It is not possible to conclude from the present results that inhibition by nifedipine of pressor responses to sympathetic nerve excitation was due exclusively to an action on the vascular smooth muscle membrane within the synapse. For example, no account has been taken of the possibility that nifedipine may have (i) suppressed increments in cardiac output or (ii) exerted an extra-synaptic effect to ameliorate the pressor effects of circulating catecholamines released from the adrenal medullae. Both of these possibilities do not seem very likely for the reasons given below. First, on the subject of cardiac output, Holck & Gerold (1985) demonstrated that nifedipine ($0.1\,mg\,kg^{-1}$ i.v.) did not diminish the increment in cardiac output evoked by electrical stimulation of the thoracic outflow (T_{7-9}) at 20 Hz. It should be said, however, that a higher dose of nifedipine ($0.3\,mg\,kg^{-1}$ i.v.) was used in the present study. Nonetheless, Buckingham & Mitchell (1981) demonstrated that the relatively selective β_1-adrenoceptor antagonist, atenolol, failed to influence the magnitude of frequency-dependent pressor responses in pithed, adrenal-demedullated SHR. This suggests that increased cardiac output contributes little to the blood pressure response during brief periods of electrical activation of the spinal sympathetic outflow, at least in the absence of the adrenal medullae. Secondly, on the question of a role for the adrenal medullae, although both Yamaguchi & Kopin (1979) and Borkowski & Quinn (1985) have shown that adrenal-demedullation of rats several weeks beforehand, produced a modest reduction in the size of frequency-dependent pressor responses after pithing, the latter authors reported that a major proportion of this inhibition was attributable to a loss of adrenaline-mediated facilitation of sympathetic neurotransmission via excitation of prejunctional β_2-adrenoceptors. The contribution of adrenaline to frequency-dependent pressor responses via this facilitatory mechanism may well have been blocked in the present experiments by our use of the β_2-adrenoceptor selective antagonist, ICI 118,551.

The possibility that nifedipine inhibited the electrically-evoked pressor responses by diminishing the neurotransmitter release is also unlikely since a considerable body of evidence suggests that electrically-induced noradrenaline release from sympathetic nerve terminals is resistant to inhibition by calcium entry blockers (see review by Eikenburg & Lokhandwala, 1986).

In agreement with the findings of the present study, Wilffert *et al.* (1984), Clapham (1984) and Holck & Gerold (1985) have all reported that nifedipine inhibited pressor responses to electrical stimulation of the sympathetic outflow in pithed rats. Overall, therefore, current evidence favours the view that nifedipine is able to uncouple intrasynaptic α_1-adrenoceptor-mediated contractile events in the rat vasculature.

By contrast, the ability of BRL 34915 to inhibit pressor responses to electrical stimulation of the spinal sympathetic outflow (Figure 7a) was highly dependent upon the prevailing level of blood pressure. In BRL 34915-treated rats, the restoration of basal diastolic pressure to a level similar to that recorded in control animals, with an infusion of vasopressin, abolished the inhibitory effect of the drug on electrically-evoked responses (Figure 7b).

The fact that intravenous doses of nifedipine which produced only modest anti-hypertensive effects in conscious SHR, were sufficient to produce quite marked inhibition of pressor responses to a variety of vasoconstrictor stimuli when rats were anaesthetized or pithed, suggests that the broadly-based anti-vasoconstrictor properties of nifedipine may contribute substantially to this drug's anti-hypertensive activity. In contrast, the same intravenous doses of BRL 34915 were more effective in reducing blood pressure in conscious rats, but generally less effective in inhibiting responses to vasopressor stimuli during ganglion-blockade under anaesthesia or after pithing. It would seem unlikely, therefore, that the anti-hypertensive properties of BRL 34915 are related to an ability to uncouple the effects of vasoconstrictor hormones. Instead, the key to the blood pressure lowering action of BRL 34915 may lie in its ability to decrease basal vascular tone. The further fall in blood pressure produced by BRL 34915 in anaesthetized, ganglion-blocked or pithed SHR demonstrates that the drug does not depend for its action on an intact autonomic nervous system. Moreover, the inability of this drug to affect pressor responses to incremental infusions of angiotensin II in anaesthetized, ganglion-blocked SHR suggests that the fall in blood pressure observed in these and in pithed rats is not due to an inhibition of the vasoconstriction produced by high circulating levels of endogenous angiotensin II, found under severe hypotensive conditions. An inhibitory effect of BRL 34915 on vascular tone has already been observed in the rat isolated portal vein by Hamilton

et al. (1986). These workers demonstrated that even at a concentration which did not influence the resting membrane potential of vascular smooth muscle cells, BRL 34915 caused inhibition of both spike potentials and the rhythmic contractile activity of this tissue. As yet it is unproven whether spontaneously contracting rat portal vein constitutes a suitable model for the arterial microvasculature.

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References

BORKOWSKI, K.R. & QUINN, P. (1985). Adrenaline and the development of spontaneous hypertension in rats. *J. Auton. Pharmacol.*, **5**, 89–100.

BUCKINGHAM, R.E. (1976). Indwelling catheters for direct recording of arterial blood pressure and intravenous injection of drugs in the conscious rat. *J. Pharm. Pharmacol.*, **28**, 459–461.

BUCKINGHAM, R.E., CLAPHAM, J.C., COLDWELL, M.C., HAMILTON, T.C. & HOWLETT, D.R. (1984). Differences in vascular effects of BRL 34915 and nifedipine in vitro. *Abstr. 9th IUPHAR Congress*, London, 1720P. London: Macmillan.

BUCKINGHAM, R.E., CLAPHAM, J.C., HAMILTON, T.C., LONGMAN, S.D., NORTON, J. & POYSER, R.H. (1986). BRL 34915, a novel antihypertensive agent: comparison of effects on blood pressure and other haemodynamic parameters with those of nifedipine in animal models. *J. Cardiovasc. Pharmacol.*, **8**, 798–804.

BUCKINGHAM, R.E. & MITCHELL, P.J. (1981). Studies on the contribution of peripheral presynaptic β-adrenoceptor blockade to the anti-hypertensive effect of single doses of (±)-propranolol and atenolol in adrenal-demedullated spontaneously hypertensive rats. *Br. J. Pharmacol.*, **74**, 260–261P.

CAVERO, I., SHEPPERSON, N., LEFÈVRE-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. *Circ. Res.*, **52** (Suppl. 1), 69–76.

CLAPHAM, J.C. (1984). Interaction between nifedipine and postsynaptic α_1- and α_2-adrenoceptors following oral pretreatment of spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, **6**, 668–671.

CLAPHAM, J.C. & WILSON, C. (1986). Effects of the novel anti-hypertensive agent BRL 34915 in comparison with nifedipine on rabbit isolated mesenteric artery. *Br. J. Pharmacol.*, **87**, 77P.

EIKENBURG, D.C. & LOKHANDWALA, M.F. (1986). Calcium antagonists and sympathetic neuroeffector function. *J. Auton. Pharmacol.*, **6**, 237–255.

FLAVAHAN, N.A. & VANHOUTTE, P.M. (1986). α_1-Adrenoceptor subclassification in vascular smooth muscle. *Trends Pharmacol. Sci.*, **7**, 347–349.

GEROLD, M. & HAEUSLER, G. (1983). α-Adrenoceptors in rat resistance vessels. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **322**, 29–33.

GOLDBERG, J.P. & SCHRIER, R.W. (1984). Effect of calcium membrane blockers on in vivo vasoconstrictor properties of norepinephrine, angiotensin II and vasopressin. *Min. Electr. Metab.*, **10**, 178–183.

HAMILTON, T.C., WEIR, S.W. & WESTON, A.H. (1986). Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br. J. Pharmacol.*, **88**, 103–111.

HOLCK, M. & GEROLD, M. (1985). Calcium entry blockers inhibit vasoconstrictor responses to sympathetic nerve stimulation mediated by α_1-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **330**, 22–32.

HOLCK, M.I., JONES, C.H.M. & HAUESLER, G. (1983). Differential interactions of clonidine and methoxamine with the postsynaptic α-adrenoceptor of rabbit main pulmonary artery. *J. Cardiovasc. Pharmacol.*, **5**, 240–248.

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

PEDRINELLI, R. & TARAZI, R.C. (1985). Calcium entry blockade by nitrendipine and alpha adrenergic responsiveness in vivo: comparison with non calcium entry blocker vasodilators in absence and presence of phenoxybenzamine pretreatment. *J. Pharacol. Exp. Therap.*, **233**, 636–642.

RUFFOLO, R.R., MORGAN, E.L. & MESSICK, K. (1984). Possible relationship between receptor reserve and the differential antagonism of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses by calcium channel antagonists in the pithed rat. *J. Pharmacol. Exp. Therap.*, **230**, 587–594.

TIMMERMANS, P.B.M.W.M., DE JONGE, A., VAN MEEL, J.C.A., MATHY, M-J. & VAN ZWIETEN, P.A. (1983a). Influence of nifedipine on functional responses in vivo initiated at α_2-adrenoceptors. *J. Cardiovasc. Pharmacol.*, **5**, 1–11.

TIMMERMANS, P.B.M.W.M., MATHY, M-J., WILFFERT, B., KALKMAN, H.O., THOOLEN, M.J.M.C., DE JONGE, A., VAN MEEL, J.C.A. & VAN ZWIETEN, P.A. (1983c). Differential effect of calcium entry blockers on α_1-adrenoceptor-mediated vasoconstriction in vivo. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **324**, 239–245.

TIMMERMANS, P.B.M.W.M., THOOLEN, M.J.M.C., MATHY, M-J., WILFFERT, B., DE JONGE, A. & VAN ZWIETEN, P.A. (1983b). SGD 101/75 is distinguished from other selective α_1-adrenoceptor agonists by the inhibition of its pressor responses by calcium entry blockade and vasodilation in pithed rats and cats. *Eur. J. Pharmacol.*, **96**, 187–192.

TIMMERMANS, P.B.M.W.M., THOOLEN, M.J.M.C., MATHY, M-J., WILFFERT, B., DE JONGE, A. & VAN ZWIETEN, P.A. (1985). Effects of the irreversible α-adrenoceptor antagonists phenoxybenzamine and benextramine on the effectiveness of nifedipine in inhibiting α_1- and α_2-adrenoceptor mediated vasoconstriction in pithed rats. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **329**, 404–413.

TODA, N., OKAMURA, T. & NAKAJIMA, M. (1984). Modification by yohimbine and prazosin of the mechanical response of isolated dog mesenteric, renal and coronary arteries to transmural stimulation and norepinephrine. *Eur. J. Pharmacol.*, **98**, 69–78.

VAN MEEL, J.C.A., DE JONGE, A., KALKMAN, H.O., WILFFERT, B., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981a). 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.

VAN MEEL, J.C.A., DE JONGE, A., KALKMAN, H.O., WILFFERT, B., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981b). 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 ZOETEN, K., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1982). Impairment by nifedipine of vasopressor responses to stimulation of postsynaptic α_2-adrenoceptors in ganglion-blocked rabbits. Further evidence for the selective inhibition of postsynaptic α_2-adrenoreceptor-induced pressor responses by calcium antagonists. *J. Auton. Pharmacol.*, **2**, 13–20.

WEIR, S.W. & WESTON, A.H. (1986). The effects of BRL 34915 and nicorandil on electrical and mechanical activity and on ^{86}Rb efflux in rat blood vessels. *Br. J. Pharmacol.*, **88**, 121–128.

WILFFERT, B., DAVIDESCO, D., DE JONGE, A., THOOLEN, M.J.M.C., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1984). Interaction between the calcium entry blocker nifedipine with the α_1-adrenoceptor-mediated increase in diastolic pressure elicited by catecholamines. *J. Auton. Pharmacol.*, **4**, 87–92.

YAMAGUCHI, I. & KOPIN, I.J. (1979). Plasma catecholamine and blood pressure responses to sympathetic stimulation in pithed rats. *Am. J. Physiol.*, **237**, H305–H310.

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