An examination of the sources of calcium for contractions mediated by postjunctional α_1 - and α_2 -adrenoceptors in several blood vessels isolated from the rabbit

C.J. Daly, W.R. Dunn, J.C. McGrath, D.J. Miller & V.G. Wilson

Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ, Scotland

1 The roles of intracellular and extracellular-derived Ca^{2+} in α -adrenoceptor-mediated contractions to noradrenaline (NA) have been investigated in several isolated blood vessels from the rabbit by examining responses in the presence of a modified Krebs-Henseleit saline with 2.5 mM Ca^{2+} and a Ca^{2+} -buffered saline with 0.1 μM free Ca^{2+} .

2 NA was tested in preparations of the abdominal aorta, distal saphenous artery, renal vein, lateral saphenous vein, plantaris vein and ear vein exposed to a Ca^{2+} -buffered saline with 0.1 μM [Ca^{2+}]. A concentration of NA which was maximally effective in modified Krebs-Henseleit saline, produced an initial transient contraction (ITC) followed by a relaxation towards baseline. This is evidence that α -adrenoceptor-mediated responses in all these blood vessels depend upon calcium from both sources.

3 The ITC was particularly pronounced in the arteries and was associated more closely with the α_1 -receptor subtype. In the abdominal aorta, distal saphenous artery and renal vein the ITC can almost exclusively be attributed to an α_1 -adrenoceptor (prazosin-sensitive, rauwolscline-resistant). In the ear vein, and to a lesser extent the plantaris vein, the ITC was mediated in part by an α_2 -adrenoceptor (prazosin-resistant, rauwolscline-sensitive).

4 α_2 -Adrenoceptors in the lateral saphenous vein largely account for the response to NA in modified Krebs-Henseleit saline, but α_1 -adrenoceptors mediate the ITC in Ca^{2+} -buffered saline. After selective inactivation of α_1 -adrenoceptors with a combination of phenoxybenzamine and rauwolscline, responses to NA in modified Krebs-Henseleit saline are slow in onset and there is no ITC in Ca^{2+} -buffered saline.

5 The possible significance of the coupling of postjunctional α_2 -adrenoceptors to dual sources of Ca^{2+} is discussed in relation to the interaction between α -adrenoceptor subtypes and the ease of demonstrating functional α_2 -adrenoceptors in isolated blood vessels.

Introduction

Postjunctional α_1 - and α_2 -adrenoceptors both mediate contraction of vascular smooth muscle (McGrath, 1982; Starke, 1987). Pressor responses mediated by each subtype exhibit differential sensitivity to calcium channel blockers. It has been suggested that α_1 -adrenoceptors are coupled to both the release of cellular-bound calcium and the opening of calcium channels, while α_2 -adrenoceptors are linked only to the latter (van Zwieten & Timmermans, 1987). There are, however, several reports not in accord with this view. For example, calcium entry blockers failed to exhibit any major differential sensitivity when examined against responses arising from the infusion of various selective agonists in the spinal dog (Morita *et al.*, 1985), pithed rat (O'Brien & McGrath, 1987) and conscious unrestrained rat (Lefevre-Borg *et al.*, 1988). In view of these conflicting observations and the difficulty in relating pressor responses to changes in peripheral resistance (Hiley & Thomas, 1987), we have now sought clarification by examining isolated blood vessels.

In contrast to the necessary reliance on drugs in *in vivo* experiments, the relative importance of extracellular Ca^{2+} for α_1 -adrenoceptor-mediated responses has been assessed by the omission of Ca^{2+} and the addition of calcium-chelating agents. In general this has provided support for the view that contractions elicited via this subtype are linked to two sources of Ca^{2+} (van Breemen *et al.*, 1986; McGrath & Wilson, 1988). Thus, in low Ca^{2+} salines containing EGTA, noradrenaline (NA) produced transient contractile responses via α_1 -adrenoceptors in the rabbit aorta, rat aorta and rat aorticocoegeus (Deth & van Breemen, 1974; McGrath *et al.*, 1984; McGrath 1985; Wilson *et al.*, 1987). In contrast, responses in the rat isolated portal vein were completely abolished by a similar procedure (Sigurdsson *et al.*, 1975). However, the difficulty associated with demonstrating functional

α_2 -adrenoceptors in isolated vascular smooth muscle (McGrath, 1982; Hieble *et al.*, 1986) has prevented a rigorous study of the Ca^{2+} -dependence of contractile responses mediated by this subtype. In two tissues examined to date, canine isolated saphenous vein (Jim & Matthews, 1985) and rat isolated femoral vein (Stubbs *et al.*, 1988), interventions known to prevent Ca^{2+} entry were found to abolish α_2 -adrenoceptor-mediated contractile responses.

In the present study we have examined the ability of NA to elicit contractions in the presence of a low concentration of extracellular Ca^{2+} in several isolated blood vessels taken from the rabbit. These include the saphenous and plantaris veins (where the response mediated by α_2 -adrenoceptors exceeds that produced by α_1 -adrenoceptors), ear vein ($\alpha_2 \gg \alpha_1$), left renal vein and abdominal aorta (α_1) (see: Daly *et al.*, 1988a,b) and saphenous artery ($\alpha_1 > \alpha_2$; Dunn *et al.*, 1989). Our findings provide further support for the view that α_1 -adrenoceptors are generally linked to two Ca^{2+} coupling mechanisms (release and entry), but we have identified two isolated veins in which postjunctional α_2 -adrenoceptors appear to be similarly coupled. The possible relationship between postjunctional α_2 -adrenoceptor-mediated release of cellular-bound Ca^{2+} and prazosin-sensitive, rauwolscline-sensitive contractile responses mediated by this receptor subtype in isolated blood vessels is also discussed.

Methods

White albino New Zealand rabbits of either sex weighing 2.3–3.0 kg were killed by stunning followed by exsanguination. Segments of the abdominal aorta, distal saphenous artery, the left renal vein, the ear vein, the lateral saphenous vein and the plantaris vein were cleaned of fat and connective tissue *in situ* and then placed in ice-cold modified Krebs-Henseleit physiological salt solution (Krebs-Henseleit saline, for composition

see below). The ear vein was defined as the 10 mm region either side of the first bifurcation of the vein running parallel to the ear artery. The plantaris vein was represented as the 15 mm distal segment of the continuation of the lateral saphenous vein measured from the ankle. The distal saphenous artery was taken as a 20 mm segment of the saphenous artery (medial side) between the knee and ankle. Segments 3 mm in length were taken from each vein (5 mm for the ear vein) or artery and suspended between two 0.2 mm thick stainless steel supports as previously described (Daly *et al.*, 1988a). The upper support was connected by surgical silk to a Grass FT03 isometric transducer while the lower support was connected to a rigid glass tissue holder. Preparations were then mounted in 30 ml isolated organ baths under an initial resting tension of 4 g wt. (aorta), 1.5 g wt. (distal saphenous artery), 0.5 g wt. (renal vein), 0.3 g wt. (ear vein), 1.5 g wt. (lateral plantaris vein), 2 g wt. (lateral saphenous vein) and allowed to relax. Each segment was bathed in modified Krebs-Henseleit saline maintained at 37°C and gassed with 5% CO₂ in O₂. No attempt was made to remove the endothelium in any preparation.

In one group of experiments postjunctional α_2 -adrenoceptors in the isolated lateral saphenous vein and the plantaris vein were isolated by the receptor protection procedure described previously (Daly *et al.*, 1988b). Briefly, preparations were exposed to 1 μ M rauwolscine 10 min before the addition of 0.3 μ M phenoxybenzamine. Both antagonists were applied to the tissue for 30 min and the irreversible antagonist was then washed out by replacing the bathing solution twice with modified Krebs-Henseleit saline containing 1 μ M rauwolscine only. Finally, 1 μ M rauwolscine was removed by replacing the bathing solution 5 times over 60 min.

After a 90 min equilibration period, during which a steady resting tension was achieved, each preparation was exposed to 3 μ M NA and allowed to contract for 10 min. After a complete washout, an additional one hour equilibration period was allowed before the experiment was commenced. This procedure was found to minimize changes in the sensitivity of the preparation to subsequent additions of the agonist. Basal tension after the sighting response remained stable for the rest of the experiment; 1.5–2.0 g wt. (aorta); 0.5–0.6 g wt. (distal saphenous artery); 0.15–0.2 g wt. (renal vein); 0.1–0.15 g wt. (ear vein); 0.2–0.3 g wt. (lateral plantaris vein, lateral saphenous vein). Isometric contractions were recorded by a Grass FT03 transducer connected to a Linseis 2065 pen recorder.

Preparations were then exposed to either 55 mM KCl (without equimolar replacement of NaCl) or NA and the responses allowed to proceed to equilibrium. The concentration of NA employed was approximately 30–100 fold greater than the EC₅₀ determined from the equilibrium concentration-response curve (Daly *et al.*, 1988a,b); 3 μ M NA (aorta, distal saphenous artery, ear vein, saphenous vein and plantaris vein), 10 μ M NA (post-phenoxybenzamine/rauwolscine plantaris vein) and 30 μ M NA (renal vein and post-phenoxybenzamine/rauwolscine saphenous vein). In general high concentrations of NA are required for the release of cell-derived Ca²⁺ (see: McGrath *et al.*, 1984; McGrath 1985; Wilson *et al.*, 1987). The agonist was removed by repeated washing with modified Krebs-Henseleit saline. Preparations were then allowed to re-equilibrate for a further 60 min. The bathing solution was then replaced twice, separated by 1 min, with a modified Krebs-Henseleit saline incorporating 0.1 μ M free Ca²⁺, buffered by the combination of 0.5 mM Na₂EGTA and 0.25 mM CaCl₂ (0.1 μ M Ca²⁺ buffered saline), and 9 min later the response to NA or KCl tested.

Three different protocols were then performed. (i) *Abdominal aorta, distal saphenous artery, saphenous vein and plantaris vein*: preparations were repeatedly washed with 0.1 μ M Ca²⁺-buffered saline over 40 min to effect complete removal of NA, i.e. the preparations were not re-exposed to 2.5 mM Ca²⁺-modified Krebs-Henseleit saline. The effect of NA was then re-examined. (ii) *Abdominal aorta, distal saphenous artery, saphenous vein and plantaris vein*: preparations were then washed repeatedly with 2.5 mM Ca²⁺-modified Krebs-Henseleit saline

to remove NA and to allow 're-filling' of cellular Ca²⁺ stores. The effect of NA was re-examined 40 min later after a 10 min exposure to either 0.01 μ M Ca²⁺-buffered saline (0.025 mM CaCl₂ and 0.5 mM Na₂EGTA) or 1 μ M Ca²⁺-buffered saline (0.475 mM Ca²⁺ and 0.5 mM Na₂EGTA). (iii) *All preparations*: NA was again removed by repeated washing with modified Krebs-Henseleit saline and the preparations exposed to 0.1 μ M prazosin, 1 μ M rauwolscine or a combination of both antagonists for a minimum of 40 min. These concentrations of the antagonists have previously been shown to be selective for α_1 - (prazosin-sensitive) and α_2 -adrenoceptor (rauwolscine-sensitive) subtypes and are 30–100 fold greater than the antilog of the negative pA₂ value (Daly *et al.*, 1988a,b). At the end of this period, the response to NA was tested in the 0.1 μ M Ca²⁺-buffered saline incorporating the appropriate concentration of the antagonist(s) using the previous cycle.

In experiments with 55 mM KCl, responses in modified Krebs-Henseleit saline and then 0.1 μ M Ca²⁺-buffered saline were evoked after the initial reference response to 3 μ M NA in modified Krebs-Henseleit saline. Unless stated otherwise, the first response in 0.1 μ M Ca²⁺-buffered saline was expressed as a percentage of the maximum response to NA in modified Krebs-Henseleit saline (mean $\pm$ s.e.mean). Subsequent responses in 0.1 μ M Ca²⁺-buffered saline (with and without antagonists) were expressed as a percentage of the first response to the particular agonist in 0.1 μ M Ca²⁺-buffered saline. Differences between means were considered statistically different if $P < 0.05$ for either paired or unpaired observations (Student's *t* test). In all experiments one preparation of each blood vessel was not exposed to an antagonist and served as a time control.

The composition of the modified Krebs-Henseleit saline was (mM): NaCl 118.4, KCl 4.7, CaCl₂ 2.5, MgSO₄ · 7H₂O 1.2, NaHCO₃ 24.9, KH₂PO₄ 1.2 and glucose 11.1. Na₂EDTA 23 μ M was included in all experiments to prevent oxidative degradation of NA and 1 μ M propranolol and 10 μ M cocaine were also included to inhibit β -adrenoceptors and uptake₁, respectively. A 0.1 M stock solution of EGTA in 0.1 M NaOH, prepared fresh each week, was used to produce 0.5 mM Na₂EGTA in the Ca²⁺-buffered saline. The final concentration of 'free' Ca²⁺ in each of the solutions was calculated by the method outlined by Miller & Smith (1984).

The following drugs were used: (–)-noradrenaline bitartrate (Sigma), prazosin HCl (Pfizer), rauwolscine HCl (Roth), phenoxybenzamine HCl (SK&F) ($\pm$)-propranolol HCl (Sigma) and cocaine HCl (Macarthy's). With the exception of NA, which was dissolved in 23 μ M Na₂EDTA, all drugs were dissolved in distilled water and added to the organ baths in 0.1 ml volumes.

Results

The effect of Ca²⁺-buffered saline on responses to 55 mM KCl and NA

Contractile responses to the addition of 55 mM KCl to modified Krebs-Henseleit saline in three venous preparations were 85.5 $\pm$ 7.5% (lateral saphenous vein, $n = 5$), 70.6 $\pm$ 6.9% (plantaris vein, $n = 5$) and 63.4 $\pm$ 6.1% (ear vein, $n = 4$) of the maximum response to NA. These responses to 55 mM KCl were abolished in 0.1 μ M Ca²⁺-buffered saline. Responses of the abdominal aorta (66.6 $\pm$ 4.1%, $n = 4$) and distal saphenous artery (89.7 $\pm$ 3.2%, $n = 5$) to 55 mM KCl in modified Krebs-Henseleit saline were markedly reduced to 4.2 $\pm$ 1.2% and 4.2 $\pm$ 1.3%, respectively, in 0.1 μ M Ca²⁺-buffered saline. In these two vessels the residual responses consisted of a slowly developing contraction.

Figure 1 shows representative trace recordings of responses in all vessels examined of the maximal responses to NA in modified Krebs-Henseleit saline and 0.1 μ M Ca²⁺-buffered saline. In arterial preparations an equilibrium response in modified Krebs-Henseleit saline was achieved 5–8 min after

Figure 1 Representative trace recordings of (–)noradrenaline-induced contractions in several isolated blood vessels from the rabbit. Responses were elicited in physiological salt solution (modified Krebs-Henseleit saline; upper responses) and then repeated after a 10 min exposure to a 'buffered' low ionized calcium ($0.1 \mu\text{M}$) physiological salt solution (Ca^{2+} -buffered saline; lower response). The blood vessels shown are as follows: (a) the abdominal aorta; (b) the distal saphenous artery; (c) the left renal vein; (d) the ear vein; (e) the lateral plantaris vein; (f) the lateral saphenous vein. Note the change in response calibration for (f) in the presence modified Krebs-Henseleit saline and Ca^{2+} -buffered saline, and the change in time base for (a). Values indicating the concentration of noradrenaline employed in each preparation are in μM units. Calibrations represent g wt.

addition of NA, while in the venous preparations the peak response was attained after 3–4 min, after which the contraction waned to 70–80% of the maximum. Responses to NA in all tissues were reduced in the presence of Ca^{2+} -buffered saline and consisted of an initial transient contraction (ITC), which peaked within 60 s of the initiation of the response, followed by relaxation to a residual response.

Both the equilibrium response (modified Krebs-Henseleit saline) and the ITC (Ca^{2+} -buffered saline) to NA in the two arterial preparations were significantly greater than those elicited in the venous preparations, but there was no obvious correlation between the magnitude of the responses in modified Krebs-Henseleit saline and the relative size of the ITC (Figure 2). For example, in the lateral saphenous vein NA produced the third largest equilibrium response (modified Krebs-Henseleit saline) but the smallest ITC. The ITC elicited by NA in Ca^{2+} -buffered saline varied from 30–70% of the maximum response in modified Krebs-Henseleit saline (Figure 2). In the ear vein, the ITCs to NA in 3 of 32 preparations (10 animals)

Figure 2 A comparison of the responses of several isolated blood vessels from the rabbit to a maximal concentration (30–100 fold log EC_{50}) of (–)noradrenaline (NA) in (a) physiological salt solution (modified Krebs-Henseleit saline) and (b) 'buffered' low ionized calcium ($0.1 \mu\text{M}$) physiological salt solution (Ca -buffered saline). The blood vessels shown are as follows: the abdominal aorta; distal saphenous artery (DSA); plantaris vein (PV); left renal vein (RV); ear vein (EV); lateral saphenous vein (LSV). Responses in (a) are the maximum equilibrium response (g wt.) and in (b) the peak response of the initial transient contraction in Ca^{2+} -buffered saline (relative to maximum responses to NA in modified Krebs-Henseleit (KH) saline). Values shown are the mean of 5–10 observations from different animals and the vertical bars represent the s.e.mean.

were less than 10% of the maximum response in modified Krebs-Henseleit saline and were excluded from the analysis. Contractile responses of the aorta to NA were more resistant to Ca -buffered saline; time to peak of the ITC was 90–120 s and the residual response was $32.8 \pm 3.9\%$ ($n = 10$) of the maximum in modified Krebs-Henseleit saline. In all other

Table 1 The effect of Ca^{2+} -buffered saline on responses to $3 \mu\text{M}$ noradrenaline in four isolated vessels from the rabbit

		A	B	C	D
		$0.01 \mu\text{M}$ Ca^{2+} buffered saline	$0.1 \mu\text{M}$ Ca^{2+} buffered saline	$1 \mu\text{M}$ Ca^{2+} buffered saline	$0.1 \mu\text{M}$ Ca^{2+} buffered saline (no prior exposure to modified Krebs-Henseleit saline)
Abdominal aorta	ITC	3.47 ± 0.34 *	3.3 ± 0.16 *	3.3 ± 0.39 *	—
	Residual response	1.2 ± 0.1 *	1.55 ± 0.19 *	1.83 ± 0.16 *	0.75 ± 0.17
Distal saphenous artery	ITC	3.3 ± 0.14 *	3.45 ± 0.21 *	3.9 ± 0.2 *	—
	Residual response	0.15 ± 0.05	0.25 ± 0.08	0.4 ± 0.1 *	0.16 ± 0.02
Plantaris vein	ITC	1.06 ± 0.06 *	1.05 ± 0.06 *	1.37 ± 0.21 *	—
	Residual response	0.05 ± 0.02	0.07 ± 0.03	0.3 ± 0.12 *	0.02 ± 0.02
Saphenous vein	ITC	0.61 ± 0.17 *	0.61 ± 0.12 *	0.81 ± 0.24 *	—
	Residual response	0.07 ± 0.02	0.11 ± 0.03	0.3 ± 0.19 *	0.07 ± 0.02

After 30 min in modified Krebs-Henseleit saline, preparations were exposed to the Ca -buffered saline for 10 min before the addition of noradrenaline (columns A, B and C). Noradrenaline was removed by repeated washing with modified Krebs-Henseleit saline. In some experiments (column D), removal of noradrenaline between consecutive responses was effected by repeated washing with $0.1 \mu\text{M}$ Ca^{2+} buffered saline, thereby ensuring that the calcium stores were not refilled before the next exposure to noradrenaline. The values (g wt.) shown are the mean $\pm$ s.e.mean of four observations. The initial transient contraction (ITC) is defined as the peak response of the contraction in Ca -buffered saline which attains a peak (usually within 40 s of agonist addition) followed by a relaxation (>50%) back towards the baseline. The residual response is the contraction in Ca -buffered saline remaining after relaxation.

* Responses significantly different from column D (Student's paired t test $P < 0.05$).

preparations, the residual responses in Ca^{2+} -buffered saline were <5% of the maximum response in modified Krebs-Henseleit saline.

Table 1 shows the effect of Ca^{2+} -buffered saline with different $[\text{Ca}^{2+}]$ on responses to $3\text{ }\mu\text{M}$ NA in several blood vessels. In all preparations examined, the magnitude of the ITC was independent of the concentration of buffered $[\text{Ca}^{2+}]$ ($0.01\text{ }\mu\text{M}$ – $1\text{ }\mu\text{M}$). However, an ITC to NA could not be repeated unless preparations were re-equilibrated in modified Krebs-Henseleit saline after exposure to Ca^{2+} -buffered saline and NA and removal of the agonist (column D).

The effects of single 'selective' concentrations of prazosin and rauwolscine on responses to NA in $0.1\text{ }\mu\text{M}$ Ca^{2+} -buffered saline

Figure 3 shows the effects of $0.1\text{ }\mu\text{M}$ prazosin, $1\text{ }\mu\text{M}$ rauwolscine and a combination of both antagonists on the ITC responses to NA in several isolated blood vessels in Ca^{2+} -buffered saline. In the aorta $0.1\text{ }\mu\text{M}$ prazosin practically abolished responses. In the renal vein and distal saphenous artery, prazosin reduced the ITC by more than 60% but the combination of $0.1\text{ }\mu\text{M}$ prazosin and $1\text{ }\mu\text{M}$ rauwolscine was no more effective than $1\text{ }\mu\text{M}$ prazosin alone (Figure 3b and c). In contrast to the aorta and renal vein, $1\text{ }\mu\text{M}$ rauwolscine produced a significant reduction in the ITC elicited by NA in the distal saphenous artery. Based upon the selectivity of these concentrations of the antagonists for α_1 - and α_2 -adrenoceptors (Daly *et al.*, 1988a,b), these observations indicate that the ITCs to

Figure 3 The effect of saline (Control), $0.1\text{ }\mu\text{M}$ prazosin (P), $1\text{ }\mu\text{M}$ rauwolscine (R) and both antagonists in combination (P + R) on the initial transient contraction to (-)-noradrenaline (NA) in several isolated blood vessels from the rabbit exposed to a $0.1\text{ }\mu\text{M}$ Ca^{2+} -buffered saline for 10 min. Responses shown are the mean of 4–6 observations and the vertical bars represents the s.e.mean. Comment: $0.1\text{ }\mu\text{M}$ prazosin produced a significant inhibition of responses to NA in all preparations, but $1\text{ }\mu\text{M}$ rauwolscine produced a significant inhibition only in the distal saphenous artery, ear vein and plantaris vein. * Indicates a significant reduction in the initial transient contraction compared to saline control. ** Indicates a significant difference ($P < 0.05$) between the effect of $0.1\text{ }\mu\text{M}$ prazosin alone and the effect of the combination of $0.1\text{ }\mu\text{M}$ prazosin and $1\text{ }\mu\text{M}$ rauwolscine. In the ear vein only 1 of 5 preparations responded to NA in the presence of both antagonists, the dashed lines indicate the limit of detection for a response in this preparation.

Figure 4 Representative trace recordings of the effects of $1\text{ }\mu\text{M}$ rauwolscine and $0.1\text{ }\mu\text{M}$ prazosin on the contraction to $3\text{ }\mu\text{M}$ noradrenaline (NA) in the lateral saphenous vein after a 10 min exposure to $[0.1\text{ }\mu\text{M} \text{Ca}^{2+}]_{\text{free}}$ buffered physiological salt solution. It is noteworthy that although rauwolscine did not affect the size of the initial transient contraction it did reduce the time course of the response.

NA in these preparations are mediated exclusively via α_1 -adrenoceptors.

In the plantaris vein and lateral saphenous vein, $0.1\text{ }\mu\text{M}$ prazosin produced a 60% reduction in the ITC to NA. Rauwolscine $1\text{ }\mu\text{M}$ effected a significant inhibition of the peak response of the ITC to NA in the plantaris vein. In the lateral saphenous vein the peak height of the response was unchanged but the duration of the response was reduced (Figure 4). In both preparations, the combination of the antagonists produced a greater effect than did prazosin alone (Figure 3d and e). In the isolated ear vein, $1\text{ }\mu\text{M}$ rauwolscine produced a greater inhibition of the NA ITC than $0.1\text{ }\mu\text{M}$ prazosin, and the combination of both antagonists abolished responses in 4 out of 5 of preparations (Fig. 3f). Thus, although α_1 -adrenoceptors participate in the ITC to NA in these three venous preparations, α_2 -adrenoceptors play a dominant and subsidiary role, respectively, in the isolated ear vein and plantaris vein. In the lateral saphenous vein, the precise role of α_2 -adrenoceptors in the ITC elicited by NA is unclear.

The effects of $0.1\text{ }\mu\text{M}$ Ca^{2+} buffered saline on isolated α_2 -adrenoceptor-mediated responses in the lateral saphenous vein and plantaris vein

After exposure of the lateral saphenous vein and plantaris vein to NA and to $0.3\text{ }\mu\text{M}$ phenoxybenzamine/ $1\text{ }\mu\text{M}$ rauwolscine to inactivate the α_1 -adrenoceptors, the maximum equilibrium responses of the vessels in modified Krebs-Henseleit saline were reduced to $65.3 \pm 5.4\%$ ($n = 7$) and $59.1 \pm 4.3\%$ ($n = 8$), respectively, of the maximum response in modified Krebs-Henseleit saline. In the lateral saphenous vein, a response to NA in the presence of $0.1\text{ }\mu\text{M}$ Ca^{2+} -buffered saline was observed in just 4 of 7 preparations. Furthermore, these were slow in onset without a well defined 'peak' and less than 10% of the maximum response (Figure 5). The effects of the antagonists were not examined. In contrast, in phenoxybenzamine/rauwolscine-treated preparations of the plantaris vein NA produced a small ITC ($11.0 \pm 1.2\%$ ($n = 8$)) of the response in modified Krebs-Henseleit saline. This response was abolished by $1\text{ }\mu\text{M}$ rauwolscine in 3 out of 4 preparations but was unaffected by $0.1\text{ }\mu\text{M}$ prazosin ($n = 4$).

Discussion

Contractile responses in vascular smooth muscle depend upon an elevation in intracellular $[\text{Ca}^{2+}]$ via (i) release of cellular-

Figure 5 Representative trace recording of noradrenaline (NA)-induced contractions of (a) the lateral saphenous vein and (b) the plantaris vein after 60 min exposure to a combination of $1 \mu\text{M}$ rauwolscine (35 min) and $0.3 \mu\text{M}$ phenoxybenzamine (30 min). Responses were elicited in modified Krebs-Henseleit saline (upper responses) and then repeated after a 10 min exposure to $0.1 \mu\text{M}$ Ca^{2+} -buffered saline (lower response). The values indicating the concentration of noradrenaline employed in each preparation are in μM units. Note that compared with the control responses in Figures 3d and e, the responses in $0.1 \mu\text{M}$ Ca^{2+} -buffered saline are considerably reduced.

bound or sequestered Ca^{2+} , (ii) entry of Ca^{2+} via voltage-operated calcium channel (VOCs) or (iii) entry of Ca^{2+} via receptor-operated calcium channels (ROCs) (Bolton, 1979). The present study concentrates on the contribution of the release of intracellular Ca^{2+} to responses mediated by postjunctional α_1 - and α_2 -adrenoceptors in isolated vascular smooth muscle.

Three observations indicate that the ITC to NA in $0.1 \mu\text{M}$ Ca^{2+} -buffered saline is a consequence of the release of intracellular Ca^{2+} from a store. Firstly, $0.1 \mu\text{M}$ Ca^{2+} -buffered saline virtually abolished contractions to KCl in all preparations—indicating that VOCs require a higher concentration of 'free' Ca^{2+} to contribute to responses. Secondly, the magnitude of the ITC to NA in four preparations examined was independent of the buffered $[\text{Ca}^{2+}]$ in the bathing medium from $1 \mu\text{M}$ down to $0.01 \mu\text{M}$. Thirdly, the ITC to NA in Ca -buffered saline could not be repeated unless the preparation was re-exposed to modified Krebs-Henseleit saline (2.5 mM Ca^{2+}) between successive responses. Furthermore, the choice of $0.1 \mu\text{M}$ for the $[\text{Ca}^{2+}]_{\text{free}}$ of the Ca^{2+} -buffered saline was made with the aim of reducing to a minimum the magnitude of the outwardly-directed diffusion gradient for Ca^{2+} , thereby preserving the integrity of the intracellular Ca^{2+} stores. It should be noted, however, that confirmation that an ITC is associated with the liberation of cellular-bound Ca^{2+} requires demonstration of an increase in intracellular $[\text{Ca}^{2+}]$ such as increased $^{45}\text{Ca}^{2+}$ efflux (e.g. Chui *et al.*, 1986), or direct measurement of changes in intracellular $[\text{Ca}^{2+}]_{\text{free}}$ with indicators such as fluorescent Ca^{2+} -sensitive dyes (e.g. Reynolds & Dubyak, 1985).

The ability of Ca^{2+} -buffered saline to reduce the maintained contraction to NA in all preparations (with the exception of the aorta) to less than 5% of the modified Krebs-Henseleit saline maximum indicates that responses mediated by both α_1 - and α_2 -adrenoceptors are highly dependent upon the presence of extracellular Ca^{2+} ($> 1 \mu\text{M}$) to support contractions. Although the relative contributions of VOCs and ROCs to these responses were not examined in the present study, preliminary experiments with $1 \mu\text{M}$ nifedipine in the lateral saphenous vein revealed that responses to KCl were abolished but those to NA were relatively unaffected (unpublished observations). Similarly, Schumann & Lues (1983) have shown that nitrendipine failed to reduce responses to phenylephrine in the lateral saphenous vein. These observations also highlight the danger inherent in making assumptions about receptor-response coupling based solely upon the effect of Ca^{2+} -entry blockers, which is necessitated *in vivo* (see van Zweiten & Timmermans, 1987). Clearly, responses to NA require extracellular free Ca^{2+} , which presumably moves into

the cells, but not via dihydropyridine-sensitive channels: this is similar to the non-vascular smooth muscle in the rat anococcygeus (McGrath, 1985).

The relatively large residual response of the aorta to NA in the presence of Ca^{2+} -buffered saline is similar to that described by Hester (1988) who employed 'zero' calcium, EGTA and the calcium-entry blocker D-600. This indicates that the relative contribution of cell-derived Ca^{2+} to α -adrenoceptor-mediated contractions in the aorta is considerably greater than that for most other blood vessels, and is in accord with data suggesting an inverse relationship between vessel calibre and the significance of extracellular Ca^{2+} in these responses (Cauvin *et al.*, 1985; Owen *et al.*, 1987).

Initial transient contractions, release of cellular-bound Ca^{2+} and postjunctional α_1 - and α_2 -adrenoceptors

The ITC in each of the different vessels was between 30 and 70% of the maximum response in modified Krebs-Henseleit saline but was consistent within each. Thus, the relative contribution of cell-derived Ca^{2+} to contractile responses varies considerably between anatomically distinct vessels. In general, it is greater in arteries than in veins. It should be emphasized that a comparison based upon the height of the ITC alone probably underestimates the differences between preparations; no attempt has been made to compare the area under the ITC response (cf. measurement of $^{45}\text{Ca}^{2+}$ efflux from pre-labelled preparations. Chui *et al.*, 1986).

As judged by the effect of $0.1 \mu\text{M}$ prazosin on ITC responses to NA, α_1 -adrenoceptors contributed to the release of intracellular Ca^{2+} in all preparations even when the α_2 -adrenoceptor was the dominant subtype responsible for the equilibrium response (Table 2). In the abdominal aorta, the ITC response was practically abolished by $0.1 \mu\text{M}$ prazosin, while $1 \mu\text{M}$ rauwolscine was without effect. This is similar to observations in the renal vein, where the combination of both antagonists produced no greater inhibition of the ITC than that observed with prazosin alone. Since α_1 -adrenoceptors mediate the equilibrium response to NA in these two preparations (Daly *et al.*, 1988a), the failure of $1 \mu\text{M}$ rauwolscine to reduce responses is further evidence that this concentration of the antagonist is devoid of activity at α_1 -adrenoceptors. In addition, the pA_2 value for rauwolscine at postjunctional α_1 -adrenoceptors in both the rabbit isolated pulmonary artery and ear artery has been found to be less than 6 (Weitzell *et al.*, 1979; Hieble & Woodward, 1984). It should be noted, however, that the

Table 2 A comparison of the relative roles of α -adrenoceptor subtypes in the equilibrium response (modified Krebs-Henseleit saline) and the initial transient contraction (Ca^{2+} -buffered saline) to noradrenaline (NA) in several isolated blood vessels from the rabbit.

Preparation	Equilibrium response (modified Krebs-Henseleit saline)*	ITC (Ca^{2+} -buffered saline)
Abdominal aorta	α_1	α_1
Left renal vein	α_1	α_1
Distal saphenous artery	α_1^{**}	α_1
Lateral saphenous vein	$\alpha_2 > \alpha_1$	$\alpha_1 \gg \alpha_2$
Lateral saphenous vein (SV.RP)	α_2	[NP]
Plantaris vein	$\alpha_2 > \alpha_1$	$\alpha_1 > \alpha_2$
Plantaris vein (PV.RP)	α_2	α_2
Ear vein	$\alpha_2 > \alpha_1$	$\alpha_2 > \alpha_1$

* Data taken from Daly *et al.* (1988a,b,c).

** In the absence of angiotensin II all responses are prazosin-sensitive and the rank order of potency of agonists is NA > phenylephrine > UK-14304. α_2 -Adrenoceptors are apparent only in the presence of angiotensin II (Dunn *et al.*, 1989). NP – not possible.

ability of 1 μM rauwolscine to inhibit the ITC to NA in the distal saphenous artery, without significantly enhancing the effect of prazosin, indicates that in this blood vessel the situation is more complex (see: Dunn *et al.*, 1989).

Even in the isolated ear vein, for which there is little evidence for postjunctional α_1 -adrenoceptors making a significant contribution to the equilibrium response to NA, 0.1 μM prazosin produced a significant inhibition of the NA ITC (Table 2). Similarly in the lateral plantaris vein and the saphenous vein, despite the apparently greater contribution of postjunctional α_2 -adrenoceptors to the equilibrium response (Daly *et al.*, 1988a,b), prazosin-sensitive (and thus α_1) adrenoceptors are chiefly responsible for the ITC to NA (Table 2). These observations agree with the well documented associations between postjunctional α_1 -adrenoceptors, liberation of cellular-bound Ca^{2+} and inositol phosphate metabolism (Reynolds & Dubyak, 1985; Abdel-Latif, 1986).

However, the novel finding of this study is that postjunctional α_2 -adrenoceptors can mediate ITCs to NA in vascular smooth muscle. In both the ear vein and lateral plantaris vein, the combination of both antagonists produced greater inhibition of the ITC to NA than observed with 0.1 μM prazosin alone (see Figure 3). Indeed, in the ear vein 1 μM rauwolscine produced greater inhibition of the ITC than did 0.1 μM prazosin. Further support for α_2 -adrenoceptor-mediated release of cell-derived Ca^{2+} is provided by prazosin-resistant, rauwolscine-sensitive ITCs to NA in preparations of the plantaris vein previously exposed to 1 μM rauwolscine/0.3 μM phenoxybenzamine to effect functional inactivation of the α_1 -adrenoceptors.

These observations contrast sharply with those obtained in other isolated veins and some in the pithed rat. In the canine saphenous vein (Jim & Matthews, 1985), the rat femoral vein (Stubbs *et al.*, 1988) and the cat middle cerebral artery (Skärby, 1984), the use of either the inorganic calcium channel blocker, La^{3+} , or exposure to Ca^{2+} -free modified Krebs-Henseleit solution (including EGTA) resulted in abolition of α_2 -adrenoceptor-mediated contractions. Moreover, in the present study an ITC to NA was not observed in 3 of 7 preparations of the saphenous vein following irreversible removal of α_1 -adrenoceptors. Nevertheless, it is clear that like α_1 -adrenoceptors, the α_2 -subtype in some preparations can be coupled to two transduction mechanisms (Ca^{2+} release and entry). It would, therefore, be fruitless to base any therapeutic or experimental strategy on an exclusive correlation between α_2 -adrenoceptor activation and Ca^{2+} entry to cells.

Of the two putative second messenger systems linked to activation of postjunctional α_2 -adrenoceptors, inhibition of adenylate cyclase activity (Fain & Garcia-Sainz, 1980) and acceleration of Na^+/H^+ exchange (Isom *et al.*, 1987), only the former could conceivably directly regulate the content of cellular-bound Ca^{2+} stores (see: Meisheri & van Breemen, 1982). Since there are no major differences in the time course of α_1 - and α_2 -adrenoceptor-mediated ITCs (compare ear vein and renal vein; Figure 1), the change in [cyclic AMP] (produced by a reduction in adenylate cyclase activity: α_2) would have to proceed at a rate similar to that of the rise of inositol triphosphate production (α_1). An alternative possibility is that α_2 -adrenoceptor-mediated release of cellular-bound Ca^{2+} is the product of a novel transduction process or inositol triphosphate.

Demonstration of postjunctional α_2 -adrenoceptors in vitro and possible relationship to calcium-coupling mechanisms

Negative coupling to a physiological process is a characteristic feature of many α_2 -adrenoceptors, e.g., neurotransmitter release (Starke, 1987), insulin secretion (Morantes *et al.*, 1988), lipolysis in adipocytes (Berlan *et al.*, 1982), growth hormone secretion or calcitonin secretion (Brown *et al.*, 1985). Thus, demonstration of the functional consequence of α_2 -adrenoceptor stimulation requires either basal or drug-induced elevation of a positively-coupled response. This is analogous to

the conditions required for demonstrating β -adrenoceptor-mediated relaxation of smooth muscle, i.e. a pre-existing contractile response. In contrast, α_2 -adrenoceptors linked to contraction of smooth muscle and the aggregation of platelets are examples of positively-coupled processes. A comparison of these two functional responses provides further insights into the difficulties associated with demonstrating prazosin-resistant, rauwolscine-sensitive smooth muscle contractions *in vitro*.

In rat and rabbit platelets, demonstration of a pro-aggregatory response to α_2 -adrenoceptor stimulation requires the presence of a submaximal concentration of an aggregatory agent, e.g. thrombin or ADP- α -S (Kerry *et al.*, 1984). Although both processes are of the same functional polarity, this is analogous to the negatively-coupled α_2 -adrenoceptors described above. However, in human platelets adrenaline can elicit a prazosin-resistant aggregatory response in the absence of a secondary agent (Kerry *et al.*, 1984; Clare *et al.*, 1984). Since the densities of α_2 -adrenoceptors in human and rabbit platelets are similar (Kerry *et al.*, 1984), the observed functional differences can only be attributed to qualitative differences in the stimulus coupling mechanism for aggregation in these two species. This could be explained as follows. α_2 -Adrenoceptors in human platelets are linked to two activation processes, one of which is responsible for initiation of the aggregatory response, while α_2 -adrenoceptors on rabbit platelets are linked to a process dependent upon pre-existing aggregatory response. Thus, although α_1 -adrenoceptors are not present on rabbit platelets (Grant & Scrutton, 1980), if they were present and able to initiate aggregation (e.g. like thrombin), it would be predicted that α_2 -adrenoceptor mediated pro-aggregatory responses would appear to be sensitive to both rauwolscine and prazosin.

We have noted that the α -adrenoceptor population in the isolated lateral saphenous vein is unique among veins from the rabbit (Daly *et al.*, 1988a,b,c). In spite of the overwhelming evidence for postjunctional α_2 -adrenoceptors (agonist potency; UK-14304 > NA > phenylephrine, and antagonist potency; rauwolscine > corynanthine), responses to NA and UK-14304 were sensitive to low concentrations of prazosin (Daly *et al.*, 1988c) with no evidence for the 'resistant' component observed *in vivo* (McGrath, 1982). In contrast, α -adrenoceptors in the isolated ear vein and the isolated lateral plantaris vein possess the same agonist and antagonist profile yet contractile responses to NA are resistant to prazosin (Daly *et al.*, 1988a). This basic difference in the α -adrenoceptor-mediated equilibrium contractile response between these preparations is also apparent for the ITC to NA (Table 2). In particular, although rauwolscine reduced the duration of the ITC to NA in the saphenous vein (further evidence for an interaction between subtypes?) no part of this response could be described as resistant to prazosin.

Assuming that α_2 -adrenoceptors are not efficiently coupled to the process responsible for the ITC, the greater facility of α_1 -adrenoceptors for eliciting an ITC response will result in potentiation of α_2 -adrenoceptor-mediated equilibrium responses, thereby rendering them 'sensitive' to prazosin. However, this does not exclude the possibility that in the absence of α_1 -adrenoceptors, α_2 -adrenoceptors are still able to elicit a contractile response (e.g. saphenous vein after removal of α_1 -adrenoceptors). Thus, prazosin-resistant α -adrenoceptor-mediated responses in the ear vein and plantaris vein could arise from either a differential distribution of the two subtypes within the blood vessel wall, or from an α_2 -adrenoceptor-mediated ITC. For the preparations examined in the present study, the latter possibility is supported by the observations that in the saphenous vein and distal saphenous artery, preparations for which there is no evidence for an appreciable α_2 -adrenoceptor-mediated ITC (see Table 2), α_2 -adrenoceptor-mediated equilibrium responses are facilitated and uncovered, respectively, by the presence of angiotensin II (Schümann & Lues, 1983; Daly *et al.*, 1988d; Dunn *et al.*, 1989). Interestingly, angiotensin II produced little facili-

tation of α_2 -adrenoceptor-mediated responses to NA in the ear vein (unpublished observations). In the context of an interaction between α_2 -adrenoceptors and other receptor systems, it is noteworthy that angiotensin II (like α_1 -adrenoceptors) has long been associated with activation of the phosphatidylinositol cycle (Adbel-Latif, 1986).

In conclusion, we have presented evidence that both α_1 - and α_2 -adrenoceptors that mediate contraction in vascular smooth muscle can utilise Ca^{2+} from cellular and extracellular sources to support contraction. In general, the role for cellular-bound Ca^{2+} in α -adrenoceptor-mediated responses is greater for the α_1 -subtype and more pronounced in arteries. However, for the α_2 -subtype there appears to be a relationship between the release of intracellular Ca^{2+} and the ease of demonstrating this subtype in isolated veins.

References

- ABDEL-LATIF, A.A. (1986). Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers. *Pharmacol. Rev.*, **38**, 227–272.
- BERLAN, M., CARPENE, C., LAFONTAN, M. & DANG-TAN, L. (1982). α_2 -adrenergic antilipolytic effect in dog fat cells: incidence of obesity and adipose tissue localization. *Horm. Metab. Res.*, **14**, 257–260.
- BOLTON, T.B. (1979). Mechanism of action of transmitters and substances in smooth muscle. *Physiol. Rev.*, **59**, 606–718.
- BROWN, M.J., STRUTHER, A.D., DI SILVIO, L., YEO, T. GHATEI, M. & BURRIN, J.M. (1985). Metabolic and haemodynamic effects of α_2 -adrenoceptor stimulation and antagonism in man. *Clin. Sci.*, **68**, 137s–139s.
- CAUVIN, C.A., LUKEMAN, S., CAMERON, J., HWANG, O. & VAN BREEMEN, C. (1985). Differences in norepinephrine activation and diltiazem inhibition of cellular channels in isolated rabbit aorta and mesenteric resistance vessels. *Circ. Res.*, **56**, 822–828.
- CHUI, A.T., MCCALL, D.E., THOOLEN, M.J.M.C. & TIMMERMANS, P.B.M.W.M. (1986). Ca^{2+} utilization in the constriction of rat aorta to full and partial α_1 -adrenoceptor agonists. *J. Pharmacol. Exp. Ther.*, **238**, 224–231.
- CLARE, K.A., SCRUTTON, M.C. & THOMPSON, N.T. (1984). Effects of α_2 -adrenoceptor agonists and of related compounds on aggregation of, and on adenylate cyclase activity in, human platelets. *Br. J. Pharmacol.*, **82**, 467–485.
- DALY, C.J., McGRATH, J.C. & WILSON, V.G. (1988a). An examination of the post-junctional α -adrenoceptor subtypes for (–)-noradrenaline in several isolated blood vessels from the rabbit. *Br. J. Pharmacol.*, **95**, 473–484.
- DALY, C.J., DUNN, W.R., McGRATH, J.C. & WILSON, V.G. (1988b). An attempt at selective protection from phenoxybenzamine of post-junctional α -adrenoceptor subtypes mediating contraction to (–)-noradrenaline in the rabbit isolated saphenous vein. *Br. J. Pharmacol.*, **95**, 501–512.
- DALY, C.J., McGRATH, J.C. & WILSON, V.G. (1988c). Pharmacological analysis of post-junctional α -adrenoceptors mediating contractions to (–)-noradrenaline in the rabbit isolated saphenous vein can be explained by interacting responses to simultaneous activation of α_1 - and α_2 -adrenoceptors. *Br. J. Pharmacol.*, **95**, 485–500.
- DALY, C.J., DUNN, W.R., McGRATH, J.C. & WILSON, V.G. (1988d). The effect of angiotensin II on responses mediated via post-junctional α_1 - and α_2 -adrenoceptor in rabbit isolated blood vessels. *Br. J. Pharmacol. Proc. Suppl.*, **95**, 699P.
- DETH, R.C. & VAN BREEMEN, C. (1974). Relative contribution of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta. *Pflügers Arch.*, **348**, 13–22.
- DUNN, W.R., McGRATH, J.C. & WILSON, V.G. (1989). Post-junctional α_2 -adrenoceptors in the rabbit isolated saphenous artery – a permissive role for angiotensin II? *Br. J. Pharmacol.*, **96**, 259–261.
- FAIN, J.N. & GARCIA-SAINZ, A. (1980). Role of phosphatidylinositol in α_1 - and of adenylate cyclase in α_2 -effects of catecholamines. *Life Sci.*, **26**, 1183–1195.
- GRANT, J.A. & SCRUTTON, M.C. (1980). Interaction of selective α -adrenoceptor agonists and antagonists with human and rabbit blood platelets. *Br. J. Pharmacol.*, **71**, 121–134.
- HESTER, K.R. (1988). Functional importance of α -adrenoceptor-mediated, D-600-insensitive Ca^{2+} entry in rabbit aorta. *J. Pharmacol. Exp. Ther.*, **247**, 223–234.
- HIEBLE, J.P., DeMARINIS, R.M. & MATTHEWS, W.D. (1986). Evidence for and against heterogeneity of α_1 -adrenoceptors. *Life Sci.*, **38**, 1339–1350.
- Note added in proof
Michel *et al.* (1989) have recently demonstrated that α_2 -adrenoceptors on a human erythroleukaemia cell line can mobilise intracellular calcium, an effect apparently not linked to inhibition of adenylate cyclase, Na^+/H^+ exchange or PI turnover.
- This work was supported by the SERC and Roche Products UK as part of a Cooperative Research Grant. V.G.W. would like to express his personal gratitude to M.E.J. Hingley & Co (Birmingham) for generous financial support during part of this work. The financial support of the University of Glasgow Medical Research Funds is also gratefully acknowledged. W.R.D. held an SERC CASE studentship in conjunction with Dr A.F. Lever, M.R.C. Blood Pressure Unit.
- HIEBLE, J.P. & WOODWARD, D.F. (1984). Different characteristics of post-junctional alpha-adrenoceptors on arterial and venous smooth muscle. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **328**, 44–50.
- HILEY, C.R. & THOMAS, G.R. (1987). Effects of α -adrenoceptor agonists on cardiac output and its regional distribution in the pithed rat. *Br. J. Pharmacol.*, **90**, 61–71.
- ISOM, L.L., CRAGOE, E.J. & LIMBIRD, L.E. (1987). α_2 -adrenergic receptors accelerate Na^+/H^+ exchange in neuroblastoma x glioma cells. *J. Biol. Chem.*, **262**, 6750–6757.
- JIM, K. & MATTHEWS, W.D. (1985). Role of extracellular calcium in contractions produced by activation of postsynaptic α_2 -adrenoceptors in the canine saphenous vein. *J. Pharmacol. Exp. Ther.*, **234**, 161–164.
- KERRY, R., SCRUTTON, M.C. & WALLIS, R.B. (1984). Mammalian platelet adrenoceptors. *Br. J. Pharmacol.*, **81**, 91–103.
- LEFEVRE-BORG, F., MATHIAS, O. & CAVERO, I. (1988). Role of sympathetic nervous system on the maintenance of blood pressure and the antihypertensive effects of calcium entry blockers, in conscious SHR. *Hypertension*, **11**, 360–370.
- McGRATH, J.C. (1982). Evidence for more than one type of post-junctional α -adrenoceptor. *Biochem. Pharmacol.*, **31**, 467–484.
- McGRATH, J.C. (1985). α -adrenoceptor agonists and the Ca^{2+} -dependence of smooth muscle contraction: evidence for subtypes of receptors or for agonist-dependent differences in the agonist-receptor interactions. *Clin. Sci.*, **68**, Suppl. 10, 55s–64s.
- McGRATH, J.C. & WILSON, V.G. (1988). α -adrenoceptor subclassification by classical and response related methods: same question, different answers. *Trend. Pharmacol. Sci.*, **9**, 162–165.
- McGRATH, J.C., MILLER, D.J. & SMITH, G.L. (1984). Contractile responses of rat anococcygeus smooth muscle to noradrenaline at buffered low levels of ionized calcium. *J. Physiol.*, **353**, 66P.
- MEISHERI, K.D. & VAN BREEMEN, C. (1982). Effects of calcium movements in rabbit aortic smooth muscle: relationship with cyclic AMP. *J. Physiol.*, **331**, 429–441.
- MICHEL, M.C., BRASS, L.F., WILLIAMS, A., BOKOCH, G.M., LAMORTE, V.J. & MOTULSKY, H.J. (1989). α_2 -Adrenergic receptor stimulation mobilizes intra-cellular Ca^{2+} in human erythroleukemia cells. *J. Biol. Chem.*, **264**, 4986–4991.
- MILLER, D.J. & SMITH, G.L. (1984). EGTA purity and the buffering of calcium ions in physiological solutions. *Am. J. Physiol.*, **246**, C160–C166.
- MORANTES, J., CARPENE, C., DePABLOS, I. & REVERTE, M. (1988). Role of α_1 - and α_2 -adrenoceptors in catecholamine-induced hyperglycaemia, lipolysis and insulin secretion in conscious fasted rabbits. *Br. J. Pharmacol.*, **94**, 299–311.
- MORITA, T., MANIWA, T., SATOH, K. & TAIRA, N. (1985). Undifferentiated effects of calcium antagonists on pressor responses to selective α_1 - and α_2 -adrenoceptor agonists in anaesthetized, spinal dogs. *J. Pharmacol. Exp. Ther.*, **234**, 728–734.
- O'BRIEN, J.W. & McGRATH, J.C. (1987). Blockade by nifedipine of responses to intravenous bolus injection or infusion of α_1 - and α_2 -adrenoceptor agonists in pithed rat. *Br. J. Pharmacol.*, **91**, 355–367.
- OWEN, M.P., JOYCE, E.H. & BEVAN, J.A. (1987). Sources of activator calcium for extrinsic vascular tone and nimodipine inhibition of that tone in proximal vs. distal rabbit ear arteries. *J. Pharmacol. Exp. Ther.*, **243**, 27–34.
- REYNOLDS, E.E. & DUBYAK, G.R. (1985). Activation of calcium mobilization and calcium influx by α_1 -adrenergic receptors in a smooth muscle cell line. *Biochem. Biophys. Res. Commun.*, **130**, 627–632.

- SCHÜMANN, H.-J. & LUES, I. (1983). Post-junctional α -adrenoceptors in the isolated saphenous vein of the rabbit. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **323**, 328–334.
- SIGURDSSON, S., UVELIUS, B. & JOHANSSON, B. (1975). Relative contribution of superficially bound and extracellular calcium to activation of contraction in portal vein. *Acta. Scand. Physiol.*, **95**, 263–269.
- SKÄRBY, T.V.C. (1984). α -Adrenoceptors and calcium dependent contractions in isolated cerebral and peripheral arteries. *Ph.D. Thesis, Lund University, Lund, Sweden*. V3–V35.
- STARKE, K. (1987). Presynaptic α -autoreceptors. *Rev. Physiol. Biochem. Pharmacol.*, **107**, 74–146.
- STUBBS, D., SMITH, J.W., DOWNING, O.A. & WILSON, K.A. (1988). Sources of calcium for contraction of rat femoral vein to α_1 - and α_2 -adrenoceptor stimulation. *Br. J. Pharmacol. Proc. Suppl.*, **94**, 424P.
- VAN BREEMEN, C., LEITJEN, P., YAMAMOTO, H., AARONSON, P. & CAUVIN, C. (1986). Calcium activation of vascular smooth muscle. *Hypertension*, **8**, (Suppl. II), II89–II95.
- VAN ZWIETEN, P.A. & TIMMERMAN, P.B.M.W.M. (1987). α -Adrenoceptor stimulation and calcium movements. *Blood Vessels*, **24**, 271–280.
- WILSON, V.G., DOWNING, O.A. & WILSON, K.A. (1987). α -adrenoceptor mediated release of cellular-bound calcium by different agonists in the isolated rat aorta and the effect of prazosin. *J. Auton. Pharmacol.*, **7**, 162–169.
- WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and post-synaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **308**, 127–136.

(Received May 31, 1989
Revised September 29, 1989
Accepted October 5, 1989)