Br. J. Pharmacol. (1991), **103**, 1493–1499

The effects of nifedipine on α_2-adrenoceptor-mediated contractions in several isolated blood vessels from the rabbit

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1 The effects of the dihydropyridine calcium channel blocker, nifedipine, on noradrenaline-induced contractile responses have been examined in several isolated blood vessels from the rabbit, with particular emphasis on responses mediated via postjunctional α_2-adrenoceptors.

2 In the isolated renal vein, ear vein, distal saphenous artery, saphenous vein and plantaris vein, 0.1 μM and 1 μM nifedipine reduced responses elicited by 54 mM KCl by more than 70%. The remaining responses were abolished by α-adrenoceptor blockade, suggesting the involvement of noradrenaline released from neurones activating a dihydropyridine-resistant mechanism.

3 In the renal vein (α_1-), ear vein (predominantly α_2-), distal saphenous artery (α_1- > α_2-), saphenous vein and plantaris vein (α_2- > α_1-), 0.01 μM and 0.1 μM nifedipine produced concentration-related reductions in the maximum response to noradrenaline. However, 1 μM nifedipine was no more effective than 0.1 μM nifedipine and the reduction in the maximum varied from 10–25% of the control response. Thus, a sizeable component of the α-adrenoceptor-medaited response in all blood vessels is resistant to dihydropyridine calcium channel blockers and this appears to be unrelated to the α-adrenoceptor subtype involved.

4 Following irreversible inactivation of α_1-adrenoceptors and isolation of functional α_2-adrenoceptors in the saphenous vein, plantaris vein and distal saphenous artery (the latter requiring the presence of angiotensin II), the effect of nifedipine on responses to noradrenaline was increased. However, a component of the α_2-adrenoceptor response in each preparation was present even after the concentration of nifedipine was increased to 1 μM.

5 In the saphenous vein, a preparation in which it has been demonstrated previously that α_2-adrenoceptor-mediated responses are highly dependent upon the presence of extracellular calcium ions, partial depolarization with 20 mM KCl failed to increase the inhibitory effect of 0.1 μM nifedipine. This suggests the involvement of dihydropyridine-resistant Ca^{2+} channels. The possible relationship between these dihydropyridine-resistant Ca^{2+} channels, α-adrenoceptor subtypes and 'receptor-operated' Ca^{2+} channels is discussed.

Keywords: Nifedipine; dihydropyridine-sensitive Ca^{2+} channels; dihydropyridine-resistant Ca^{2+} channels; α_1-adrenoceptors; α_2-adrenoceptors; vascular smooth muscle

Introduction

The introduction of dihydropyridine Ca^{2+} entry blockers, e.g. nifedipine, has provided a pharmacological basis for determining the involvement of L-type voltage-operated Ca^{2+} channels and receptor-operated, voltage-independent Ca^{2+} channels in contractile responses of vascular smooth muscle (Godfraind *et al.*, 1986). The α-adrenoceptor system in vascular smooth muscle and in particular responses mediated by the α_1- and α_2-adrenoceptor subtypes, has been a major focus of attention for the action of these agents because of their contribution to vascular tone in both normal and hypertensive states.

In the pithed rat, nifedipine and the dihydropyridine 'Ca^{2+} agonist', Bay K 8644, were found to inhibit and potentiate, respectively, pressor responses to the bolus injection of selective α_2-adrenoceptor agonists and 'low efficacy' selective α_1-adrenoceptor agonists (van Meel *et al.*, 1983; Wilffert *et al.*, 1984). Pressor responses to the bolus injection of 'high efficacy' selective α_1-adrenoceptor agonists were relatively unaffected (Timmermans & van Zwieten, 1982; Wilffert *et al.*, 1984). This was interpreted as evidence that while α_1-adrenoceptors mobilize intracellular Ca^{2+} and open dihydropyridine-sensitive Ca^{2+} channels, pressor responses to α_2-adrenoceptors are entirely dependent upon the entry of Ca^{2+} via dihydropyridine-sensitive Ca^{2+} channels (van Zwieten & Timmermans, 1987). However, in several other papers a different conclusion was reached. Calcium entry blockers failed to exhibit differential potency against responses arising from the infusion of various selective agonist in the spinal dog (Morita *et al.*, 1985), pithed rat (O'Brien & McGrath, 1987) and conscious unrestrained rat (Lefevre-Borg *et al.*, 1988). Thus, the manner in which α-adrenoceptor agonists are administered is a major factor in determining the sensitivity of the pressor responses to Ca^{2+} entry blockers and, therefore, the contribution of Ca^{2+} channels to responses via each subtype appears to be similar.

A systematic examination of the effect of Ca^{2+} entry blockers on α_1- and α_2-adrenoceptor-mediated responses in isolated blood vessels would both circumvent the problems of non-equilibrium responses and permit an examination of the contribution of cellular Ca^{2+} to responses. However, this has been hampered by the paucity of blood vessels that possess a functional population of postjunctional α_2-adrenoceptors. In the majority of isolated blood vessels with postjunctional α_1-adrenoceptors, a sizeable component of contractile responses is either resistant to dihydropyridine Ca^{2+} entry blockers or is still observed following removal of extracellular Ca^{2+} ions (see: Godfraind *et al.*, 1986). This lends support to the general view that α_1-adrenoceptor-mediated contractions are maintained by Ca^{2+} derived from two sources. In the few preparations with postjunctional α_2-adrenoceptors that have been examined, the results with Ca^{2+} entry blockers have varied greatly. Responses have been found to be either very sensitive to Ca^{2+} entry blockade, e.g. in the cat middle cerebral artery (Skärby, 1984) and the rat tail artery (Abe *et al.*,

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1987), or to exhibit marked resistance to Ca^{2+} entry blockers, e.g. in the canine isolated saphenous vein (DeMey & Vanhoutte, 1981; Matthews et al., 1984) or rabbit isolated saphenous vein (Schümann & Lues, 1983). However, direct comparisons versus different receptors under similar conditions in a single species have not been made.

We have recently characterized the α-adrenoceptors mediating contractions in several isolated blood vessels from the rabbit and identified preparations with either a mixture or a practically homogeneous population of α_1- or α_2-adrenoceptors (Daly et al., 1988a,b,c; Dunn et al., 1989). In addition, the contribution of cellular-bound calcium to contractions elicited by noradrenaline has been examined in each of these preparations (Daly et al., 1990). This has provided preliminary evidence that both α_1- and α_2-adrenoceptors can mediate the release of cellular Ca^{2+}. We have, therefore, examined the effects of nifedipine on noradrenaline-induced contractions in several blood vessels from the rabbit, both before and after pharmacological isolation of postjunctional α_2-adrenoceptors, to determine the relative contribution of dihydropyridine-sensitive and dihydropyridine-resistant Ca^{2+} channels to contractile responses.

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 distal saphenous artery (DSA), the left renal vein (LV), the ear vein (EV), the lateral saphenous vein (SV) and the plantaris vein (PV) were cleaned of fat and connective tissue *in situ* and then placed in ice-cold modified Krebs-Henseleit saline (composition 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 taken as a 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., 1988b). 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 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% CO_2 in O_2. No attempt was made to remove the endothelium in any preparation.

After a 90 min equilibration period, during which a steady resting tension was achieved, each preparation was exposed to 3 μM noradrenaline (NA) and allowed to contract for 10 min. After a complete washout, an additional 1 h equilibration period was allowed before starting the experiment. 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; 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 6025 pen recorder.

A cumulative concentration-response curve (CRC) to NA was constructed in each preparation by increasing the concentration of NA by approximately a half log unit only after the preceding response had peaked or reached an equilibrium. Following attainment of the maximum response, preparations were washed repeatedly with Krebs-Henseleit saline to effect complete relaxation and then exposed to either 0.02% absolute ethanol or nifedipine (0.1 μM and 1 μM) for 40 min and the CRC repeated. Each preparation was exposed to only one concentration of nifedipine and all experiments were performed in subdued lighting. The effect of 0.1 μM nifedipine was also examined against responses to 54 mM KCl (no equimolar replacement of NaCl).

Isolation of postjunctional α_2-adrenoceptors

In one series of experiments, postjunctional α_2-adrenoceptors in the lateral saphenous vein, the plantaris vein and the distal saphenous artery were isolated by the receptor protection procedure described previously (Daly et al., 1988c). Briefly, preparations were exposed to 1 μM rauwolscine 10 min before the addition of 0.3 μM phenoxybenzamine. Both antagonists were applied to the tissue for either 30 min (veins) or 60 min (artery) and the irreversible antagonist was then washed out by replacing the bathing solution twice with modified Krebs-Henseleit saline containing 1 μM rauwolscine only. Finally, 1 μM rauwolscine was removed by replacing the bathing solution 5 times during a period of 60 min. A CRC to NA was constructed as described above, and repeated after 40 min exposure to either 0.02% absolute ethanol or nifedipine (0.01 μM to 1 μM). In the distal saphenous artery (only) α_2-adrenoceptor-mediated responses were observed only if the preparation was exposed to 0.05 μM angiotensin II (Dunn et al., 1991; see Figure 3). Thus, all responses to NA were elicited in the presence of angiotensin II. In a further series of experiments on the saphenous vein, preparations were exposed to 20 mM KCl (without replacement of equimolar NaCl) for 35 min of the equilibration period with 0.1 μM nifedipine. At the end of this period the CRC to NA was repeated.

The effects of 0.1 μM prazosin and 1 μM rauwolscine, concentrations selective for α_1- and α_2-adrenoceptors, respectively (Daly et al., 1988a,b), versus responses to NA in the distal saphenous vein and the plantaris vein were examined before and after pharmacological isolation of postjunctional α_2-adrenoceptors.

Calculation of results

Unless otherwise stated, responses to agonists are expressed as a percentage (mean ± s.e.mean) of the maximum response to noradrenaline in the first concentration-response curve. The negative logarithm of the concentration of the agonist required to produce 50% of the maximum contractile response ($-\log EC_{50}$ or pD_2) was also determined in the presence and absence of nifedipine. The maximum response to NA in the presence of nifedipine (E_{max}, relative to the 1st CRC) has also been determined after correction for time-related changes in parallel control preparations exposed to 0.02% absolute ethanol. The significance of differences between mean values was determined with either a paired or unpaired Student's t test as appropriate. Unless otherwise stated, $P < 0.05$ is considered significant.

Drugs and solutions

The composition of the modified Krebs-Henseleit saline was (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2 and glucose 11.1. Na_2EDTA (23 μM) was included in all experiments to prevent oxidative degradation of NA. Propranolol 1 μM and cocaine, 10 μM, were also included to inhibit β-adrenoceptors and uptake$_1$, respectively. The following drugs were used: (−)-noradrenaline bitartrate (Sigma), prazosin HCl (Pfizer), rauwolscine HCl (Roth), phenoxybenzamine HCl (SK&F), nifedipine (Bayer), (±)-propranolol HCl (Sigma), angiotensin II amide (Hypertensin, Ciba) and cocaine HCl (Macarthys). Nifedipine and phenoxybenzamine were dissolved in 20% absolute ethanol and NA was dissolved in 23 μM Na_2EDTA. All other drugs were dissolved in distilled water and added to the organ baths in volumes of 0.1 ml.

Results

The effects of 0.1 μM nifedipine on responses to 54 mM KCl

The response to 54 mM KCl in control preparations of the renal vein, distal saphenous artery, saphenous vein, plantaris vein and ear vein varied between 20–80% of the maximum response to NA (Figure 1). Nifedipine, 0.1 μM reduced responses to KCl in all preparations by more than 70%. In the ear vein and plantaris vein a 10 fold increase in the concentration of nifedipine failed to produce a significantly greater reduction in responses. The nifedipine-resistant response appears to be due to neuronal release of noradrenaline, since these responses in the saphenous vein, distal saphenous artery and renal vein were abolished by 0.1 μM prazosin, while those in the ear vein and plantaris veins were blocked by 1 μM phentolamine. These observations indicate that 0.1 μM nifedipine effectively blocks all voltage-operated Ca^{2+} channels (activated by KCl) that participate in contractile events in rabbit isolated blood vessels.

The effects of nifedipine on noradrenaline-induced contractions

The vehicle used in these experiments (maximum concentration of 0.02% ethanol) exerted no effect on either the sensitivity or the maximum response to noradrenaline in any preparation, over that observed with time controls.

Figure 2 shows the effects of 0.1 μM and 1 μM nifedipine on noradrenaline-induced contractions. In the saphenous vein and ear vein (Figure 2c,e), both concentrations of nifedipine produced between 15–30% reduction in the maximum response to noradrenaline (relative to the time control, but there was no significant difference between the effects of the two concentrations, see also Table 1). This was not markedly different from the effect of 0.1 μM nifedipine on NA responses in the renal vein and plantaris vein (Figure 2a,d. Table 1). Furthermore, with the exception of the plantaris vein, the sensitivity of the venous preparations to NA was unaltered by 0.1 μM nifedipine (Table 1). Thus, the overall effect of nifedipine on noradrenaline-induced contraction in venous smooth muscle is essentially the same, irrespective of whether the receptors mediating the response belong to the α_1-subtype (renal vein, Daly *et al.*, 1988b), the α_2-subtype (ear vein, Daly *et al.*, 1988a), or a mixture of both (saphenous vein and plantaris vein, Daly *et al.*, 1988b).

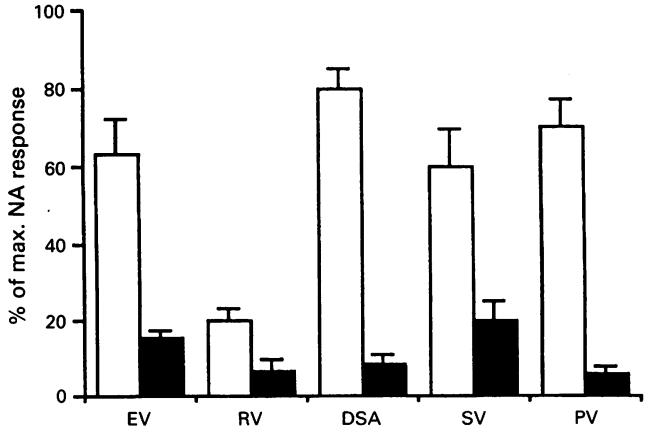

Figure 1 Contractile responses to 54 mM KCl in the ear vein (EV), renal vein (RV), distal saphenous artery (DSA), saphenous vein (SV) and plantaris vein (PV) isolated from the rabbit in the absence (open columns) and in the presence (closed columns) of 0.1 μM nifedipine. Responses have been expressed as a percentage of the maximum response to noradrenaline in each preparation and are shown as the mean of 4–6 observations; vertical bars show s.e.mean.

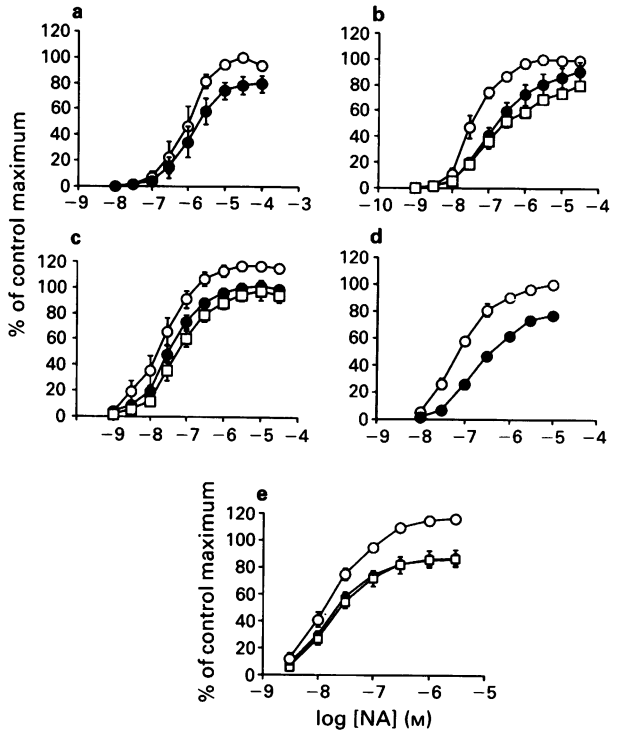

Figure 2 The effects of 0.1 μM (●) and 1 μM (□) nifedipine on contractile responses elicited by noradrenaline in (a) the renal vein, (b) the distal saphenous vein, (c) the saphenous vein, (d) the plantaris vein and (e) the ear vein isolated from the rabbit. All responses have been expressed as a percentage of the maximum response to noradrenaline in the first concentration-response curve, and are shown as the mean of a minimum of 5 observations: vertical bars show s.e.mean. Responses to noradrenaline in the presence of 0.02% absolute ethanol are shown by (○).

In the distal saphenous artery, 0.1 μM nifedipine significantly reduced the sensitivity of the preparation to NA (an effect also seen with 0.01 μM nifedipine) but failed to alter the maximum response (Table 1). However, 1 μM nifedipine significantly reduced both parameters (Figure 2b).

Table 1 Mean pD_2 values and E_{max} values (with s.e. of the mean) for noradrenaline (NA) in the presence of 0.1 μM nifedipine as compared to control values corrected for time-related changes in responses in the left renal vein (LRV), the distal saphenous artery (DSA), the saphenous vein (SV), the plantaris vein (PV) and ear vein (EV)

	pD_2 values		E_{max}
Preparation	*Control*	*Nifedipine*	*Nifedipine*
LRV (n = 5)	6.07 ± 0.20	5.90 ± 0.16	0.80 ± 0.07*
DSA (n = 5)	7.45 ± 0.15	6.88 ± 0.05*	0.91 ± 0.07
SV (n = 5)	7.47 ± 0.07	7.46 ± 0.09	0.86 ± 0.04**
PV (n = 6)	7.13 ± 0.09	6.72 ± 0.10*	0.75 ± 0.05**
EV (n = 6)	7.76 ± 0.07	7.77 ± 0.06	0.76 ± 0.06**
DSA–RP (n = 4)	6.66 ± 0.29	6.95 ± 0.16	0.30 ± 0.11**
PV–RP (n = 6)	6.93 ± 0.15	6.77 ± 0.13	0.42 ± 0.09**
SV–RP (n = 4)	6.66 ± 0.17	6.54 ± 0.24	0.63 ± 0.05**

Preparations exposed to 1 μM rauwolscine and 0.3 μM phenoxybenzamine to isolate the α_2-adrenoceptors are indicated by the abbreviation (–RP).

Differences between control and treated preparations were considered statistically significant if $P < 0.05$ for either paired or unpaired observations (Student's t test) and are denoted by: * $0.05 > P > 0.01$, ** $0.01 > P > 0.001$.

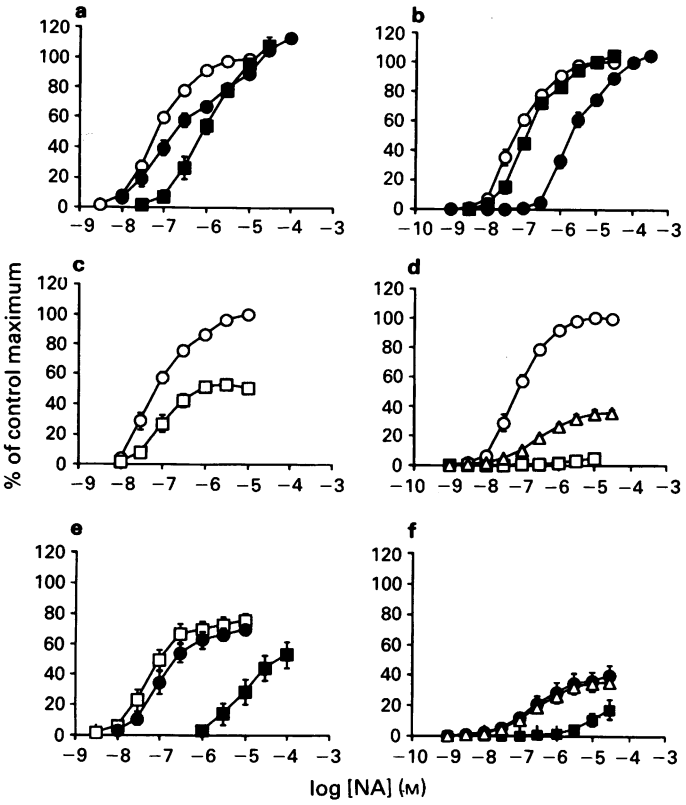

Figure 3 The effect of the receptor protection procedure with 1 μM rauwolscine and 0.3 μM phenoxybenzamine on the pharmacological characteristics of responses to noradrenaline in the isolated plantaris vein and isolated distal saphenous artery from the rabbit. (a and b) The effects of (●) 0.1 μM prazosin and (■) 1 μM rauwolscine on responses to noradrenaline in (a) the isolated plantaris vein and (b) the isolated distal saphenous artery. (c and d) Concentration-response curves to noradrenaline (○) before and (□) after the receptor protection procedure in (c) the isolated plantaris vein and (d) the isolated distal saphenous vein. The effect of 0.05 μM angiotensin II (△) on post-receptor protection responses to noradrenaline in the distal saphenous vein is also shown. (e and f) The effects of (●) 0.1 μM prazosin and (■) 1 μM rauwolscine on responses to noradrenaline following receptor protection in the (e) isolated plantaris vein and (f) isolated distal saphenous vein (in the presence of 0.05 μM angiotensin II) (cf. a and b). In (a–d), the control concentration-response curve to noradrenaline before receptor protection is represented (○) and after receptor protection by either (□ or △ if AII is present). Responses have been expressed as a percentage of the maximum response to noradrenaline in control preparations and are shown as the mean of a minimum of 5 observations; vertical bars show s.e.mean.

Isolation of α_2-adrenoceptor-mediated responses by irreversible inactivation of postjunctional α_1-adrenoceptors

Unlike in the renal and ear veins, the α-adrenoceptors in the distal saphenous artery, saphenous vein and plantaris vein consist of a mixture of α-adrenoceptor subtypes: $\alpha_1 > \alpha_2$–distal saphenous artery (Dunn *et al.*, 1989); $\alpha_2 > \alpha_1$–saphenous vein and plantaris vein (Daly *et al.*, 1988b). Thus, to investigate further the effect of nifedipine on α_2-adrenoceptor-mediated responses, we have isolated postjunctional α_2-adrenoceptors in these three preparations by use of the receptor protection experiment developed earlier (Daly *et al.*, 1988c).

Figure 3a and b shows the effects of 0.1 μM prazosin and 1 μM rauwolscine on NA-induced contractions in the plantaris vein and the distal saphenous artery. In both preparations, the inhibitory activity of 0.1 μM prazosin indicates the presence of α_1-adrenoceptors (distal saphenous artery > plantaris vein), while the limited effect of 1 μM rauwolscine is not consistent with a homogeneous population of postjunctional α_2-adrenoceptors. Following receptor protection with 1 μM rauwolscine and exposure to 0.3 μM phenoxybenzamine (RP) the maximum response to noradrenaline was reduced by approximately 45% in the plantaris vein (PV–RP) and 95% in distal saphenous artery (DSA–RP) (Figure 3c and d). However, concentration-dependent responses of DSA–RP were observed in the presence of 0.05 μM angiotensin II (Figure 3d) and the maximum responses to NA increased from approximately 5% to 30% of the maximum pre- RP control response. This peptide produced a transient contractile response (22.7 ± 4.9% of the pre-RP

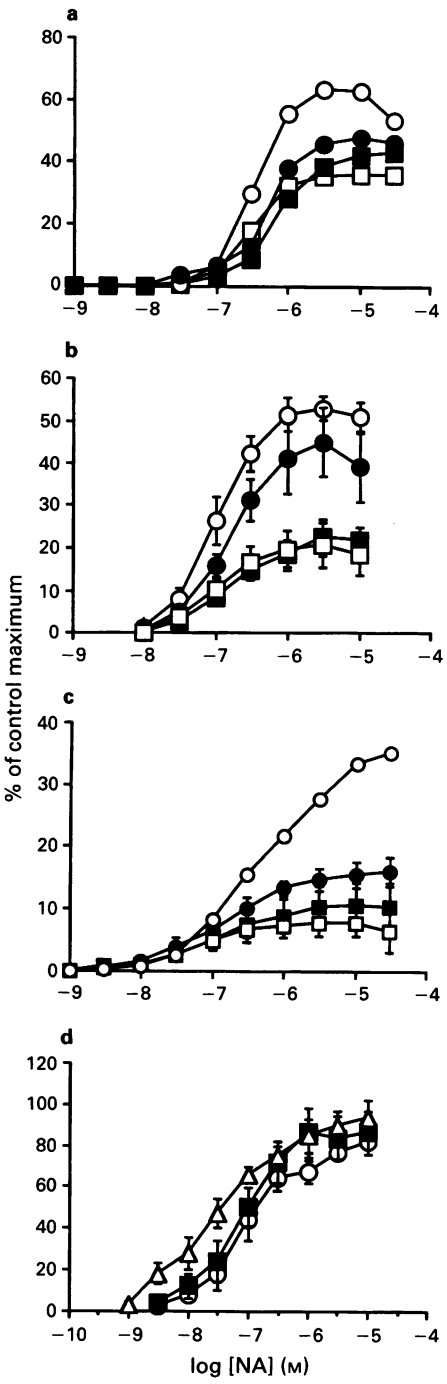

Figure 4 The effects of (●) 0.01 μM, (■) 0.1 μM and (□) 1 μM nifedipine on α_2-adrenoceptor-mediated responses in (a) the isolated saphenous vein, (b) the isolated plantaris vein and (c) the isolated distal saphenous artery. The effect of (■) 0.1 μM nifedipine on α_2-adrenoceptor responses of the rabbit isolated saphenous vein to noradrenaline (NA) in the presence of 20 mM KCl is shown in (d). The control concentration-response curve to noradrenaline following receptor protection is represented by (○) and that in the presence of 20 mM KCl by (△). All responses have been expressed as a percentage of the maximum response to noradrenaline prior to the receptor protection procedure, and are given as the mean of a minimum of 5 observations; s.e.mean shown by vertical bars. On graphs (a) and (c) the standard error bars (< 8%) have been omitted to increase clarity.

maximum responses to NA, $n = 5$) which returned to baseline after 15 min, and has previously been used to uncover α_2-adrenoceptor-mediated responses in this preparation (Dunn *et al.*, 1989).

After the receptor protection procedure (and addition of 0.05 μM angiotensin II to DSA–RP), the pharmacological profile of the response to NA in both the plantaris vein and the distal saphenous artery was consistent with that of an α_2-adrenoceptor, the concentration-response curves were resistant to 0.1 μM prazosin and displaced approximately 100 fold by 1 μM rauwolscine (Figure 3e,f). The effect of this protocol on α-adrenoceptors is essentially similar to that previously observed in the saphenous vein (Daly *et al.*, 1988c).

The effects of nifedipine on α_2-adrenoceptor-mediated responses after irreversible inactivation of postjunctional α_1-adrenoceptors

Nifedipine, 0.01 μM and 0.1 μM, produced a concentration-dependent reduction of responses to NA in the saphenous vein (Figure 4a), the plantaris vein (Figure 4b) and the distal saphenous artery (Figure 4c) following isolation of the postjunctional α_2-adrenoceptors. This effect was observed in the absence of a change in the potency of NA (Table 1). In the distal saphenous artery the transient contractile response to 0.05 μM angiotensin II in the presence of 0.1 μM nifedipine (18.8 ± 4.3% of the pre-RP maximum to NA) was not significantly different from control responses (see above). Hence the inhibitory effect of nifedipine in this preparation is not the consequence of an indirect effect on the angiotensin II contraction required to uncover α_2-adrenoceptor-mediated responses. Based upon the reduction in the maximum responses to NA produced by 0.1 μM nifedipine, the rank order of susceptibility to Ca^{2+} entry blockade was distal saphenous artery > plantaris vein > saphenous vein (Table 1). However, 1 μM nifedipine failed to produce a greater effect than 0.1 μM nifedipine (see Figure 4a,b,c). Thus a significant component of the α_2-adrenoceptor response in each of these preparations is resistant to nifedipine.

Figure 4d shows the effect of 35 min equilibration of the saphenous vein (RV–RP) with 0.1 μM nifedipine in partially depolarized Krebs-Henseleit solution (20 mM KCl excess) on responses to NA. In this particular series of experiments the reduction in the maximum responses following exposure to phenoxybenzamine and rauwolscine was less than that previously observed (approximately 20% in this series and approximately 40% for the results in Figure 4a). However, the residual response to NA was sensitive to rauwolscine and there was no evidence of a resistant component (indicative of the presence of postjunctional α_1-adrenoceptors). Under these conditions, the presence of 20 mM KCl failed to alter the effect of 0.1 μM nifedipine against α_2-adrenoceptor-mediated responses.

Discussion

The increase in intracellular calcium that supports α-adrenoceptor-mediated contractions of vascular smooth muscle can arise from three different sources: the release of cellular-bound calcium; entry via voltage-dependent Ca^{2+} channels (VOCs); entry via receptor-operated, voltage-independent Ca^{2+} channels (ROCs) (Bolton, 1979; Godfraind *et al.*, 1986). In a previous paper, we examined the relative contributions of cellular-bound Ca^{2+} ions and of the influx of extracellular Ca^{2+} ions to contractions mediated by α_1- and α_2-adrenoceptors in several isolated blood vessels from the rabbit (Daly *et al.*, 1989a; 1990). The basis of these experiments was that a buffered low concentration of extracellular Ca^{2+} (0.1 μM) abolished contractile responses to KCl, yet noradrenaline elicited initial transient contractions reflecting the release of cellular-bound Ca^{2+} ions. Pharmacological examination of the initial transient contraction with selective antagonists suggested that both α_1- and α_2-adrenoceptors can mediate the release of cellular-bound Ca^{2+} ions and, as such, represented a major departure with the prevailing view that α_2-adrenoceptors are entirely dependent upon the entry of extracellular Ca^{2+} ions (Timmermans & Zwieten, 1987; Nichols & Ruffolo, 1988). Our results are further supported by the observations of Michel and coworkers (1989), who demonstrated that activation of α_2-adrenoceptors in human erythroleukaemia cells results in the elevation of intracellular Ca^{2+} ion concentration in the absence of extracellular Ca^{2+} ions.

In the present study we have adopted a similar approach with the Ca^{2+} entry blocker, nifedipine. This has allowed us to determine the contribution of dihydropyridine-sensitive and dihydropyridine-resistant Ca^{2+} channels to the component of α_1- and α_2-adrenoceptor-mediated contractions dependent upon the entry of extracellular Ca^{2+} ions. In each of the blood vessels examined, KCl-induced contractions were markedly reduced by 0.1 μM nifedipine and a 10 fold increase in the concentration of the Ca^{2+} entry blocker failed to produce a greater effect. Furthermore, the residual response in each preparation was abolished by α-adrenoceptor blockade (and was practically absent in low Ca^{2+}) suggesting that NA, released from neurones by depolarization (hence Ca^{2+} influx and exocytosis), causes contraction through α-adrenoceptors linked to nifedipine-resistant Ca^{2+} entry.

Thus, responses observed in the presence of 0.1 μM nifedipine can be attributed either to the release of cellular-bound Ca^{2+} ions, to dihydropyridine-resistant ROCs and/or to dihydropyridine-resistant VOCs. The possibility of the latter is unlikely because T and N-type VOCs are inactivated too quickly to make a significant contribution to the elevation of intracellular Ca^{2+} ions required to support vascular contractions (Yatani *et al.*, 1987). Furthermore, we know of no example of an L-type VOC resistant to the dihydropyridine Ca^{2+} entry blockers. Nonetheless, while we readily concede that dihydropyridine-resistant responses to noradrenaline may reflect the activation of receptor operated, voltage-independent Ca^{2+} channels, this term has been avoided in relation to our own observations because: (i) the electrophysiological changes that accompany the contractile events in each tissue were not determined; (ii) there are examples of blood vessels in which Ca^{2+} entry blockers display potency against α-adrenoceptor-mediated contractions that occur in the absence of measurable changes in membrane potential (e.g. Häusler 1985; Abe *et al.*, 1987).

Contribution of dihydropyridine-sensitive Ca^{2+}-channels to α_1- and α_2-adrenoceptor-mediated contractions

The results in the present study, in agreement with several other reports with *in vivo* models (Morita *et al.*, 1985; O'Brien & McGrath, 1987; Lefevre-Borg *et al.*, 1989), indicate that α_1- and α_2-adrenoceptor-mediated contractions of vascular smooth muscle are similarly dependent upon the entry of Ca^{2+} ions through dihydropyridine-sensitive Ca^{2+} channels. For example, the reduction in the maximum response to noradrenaline in the left renal vein (α_1-) and the ear vein (almost exclusively α_2-) produced by 0.1 μM nifedipine were of similar magnitude (see Table 1). Furthermore, since 1 μM rauwolscine did not mimic the effect of nifedipine in the distal saphenous artery (compare Figures 3b and 2b), the reduction in potency of noradrenaline can be partly attributed to an effect on the α_1-adrenoceptor component. This is consistent with observations in two other preparations with predominantly α_1-adrenoceptors, the rabbit isolated femoral artery and isolated renal artery, where nifedipine also significantly reduced the potency of noradrenaline (Purdy & Weber, 1988; Hashimoto *et al.*, 1989).

Even after irreversible inactivation of postjunctional α_1-adrenoceptors and isolation of α_2-adrenoceptors, when the effect of nifedipine was more pronounced (see Table 1), a significant component of the contractile response to noradrenaline in the distal saphenous vein, the plantaris vein and the

saphenous vein was resistant to nifedipine. This is in agreement with other reports in which α_2-adrenoceptor-mediated responses, in the rabbit saphenous vein (Schümann & Lues, 1983) and canine saphenous vein (De Mey & Vanhoutte, 1981; Cooke et al., 1985), displayed considerable resistance to Ca^{2+} entry blockade. These observations contrast sharply with α_1-adrenoceptor-mediated contractions in the rat isolated longitudinal portal vein (Jetley & Weston, 1979), canine coronary artery (Rimele et al., 1983) and rabbit mesenteric resistance arteries (Cauvin et al., 1984), in which practically the whole contractile response to NA is sensitive to Ca^{2+} entry blockade.

Clearly, the prevailing view that α_2-adrenoceptor-mediated responses are invariably more sensitive to Ca^{2+} entry blockade than those elicited via α_1-adrenoceptors (Timmermans & van Zwieten, 1987; Nichols & Ruffolo, 1988), developed primarily from data derived from the bolus injection of synthetic α-adrenoceptor agonists in *in vivo* models, is no longer tenable. In the present study we identified suitable isolated blood vessels with functional α_1- and α_2-adrenoceptors and elicited responses with the endogenous agonist, noradrenaline. Furthermore, evidence from studies on human isolated digital arteries and hand veins with noradrenaline indicate that both α_1- and α_2-adrenoceptor-mediated responses exhibit similar sensitivity to Ca^{2+} entry blockade (Stevens & Moulds, 1986; Arner et al., 1988). Thus, there is no exclusive contract between subtypes of α-adrenoceptors and particular Ca^{2+} activation mechanisms.

Nifedipine-resistant contractions: evidence for the release of intracellular Ca^{2+} ions or dihydropyridine-resistant Ca^{2+} channels?

Triggle and coworkers (1988) have shown that the interaction of dihydropyridine Ca^{2+} entry blockers with their specific binding sites is a voltage-dependent phenomenon. Thus, the possibility exists that the resistance of α_1- and α_2-adrenoceptor-mediated contractions to nifedipine is attributable to the inability of noradrenaline to depolarize the tissue sufficiently to ensure nifedipine binding to essentially nifedipine-sensitive Ca^{2+} channels. However, the potency of nifedipine against α_2-adrenoceptor-mediated contractions in the saphenous vein (SV–RP) was not enhanced by equilibration in a partially depolarized Krebs-Henseleit solution (Figure 4d). Thus, the nifedipine-resistant contractions are due either to the release of intracellular Ca^{2+} ions or to the entry of Ca^{2+} ions via dihydropyridine-resistant channels.

Putney (1986) proposed a simple model for a link between the entry of extracellular Ca^{2+} ions via receptor-operated, voltage-independent channels and the release of cellular-bound Ca^{2+} ions, which has been refined further by Berridge & Irvine (1989). Basically, entry of Ca^{2+} ions is dependent upon prior emptying of the bound stores of Ca^{2+} ions by a receptor-linked intracellular messenger, e.g. inositol trisphosphate. Thus the superficial stores of bound Ca^{2+} ions act as a transit point for the entry of extracellular Ca^{2+} ions into the cell. Such a capacitive model for cellular Ca^{2+} movements is attractive because it obviates the need for an independent receptor-linked Ca^{2+} channel, unequivocal evidence for which has proved difficult to obtain (see: Benham & Tsien, 1987), and manages to accommodate several experimental findings.

First, contractile responses to noradrenaline in the rabbit isolated renal vein and rat isolated anococcygeus (α_1-adrenoceptors) and rabbit isolated ear vein (α_2-adrenoceptors) are not sustained in low ($<0.1\,\mu$M) concentrations of extracellular Ca^{2+} ions (McGrath, 1985; Daly et al., 1990), but are resistant to nifedipine in 2.5 mM Ca^{2+} (present study; Vila et al., 1985). Secondly, the effects of organic and inorganic (e.g. Ni^{2+} or La^{3+} ions) Ca^{2+} entry blockers on individual contractile responses show a characteristic difference: the latter inhibits all Ca^{2+} entry and, therefore, cellular stores are not refilled and the response is not sustained (Deth & van Breeman, 1974). Thirdly, α_1-adrenoceptor-mediated responses in the rat isolated longitudinal portal vein, canine coronary artery and the rabbit isolated mesenteric resistance vessels show marked sensitivity to Ca^{2+} entry blockers (Jetley & Weston, 1980; Rimele et al., 1983; Cauvin et al., 1984) and none of these preparations appear to have appreciable cellular-bound Ca^{2+} stores.

We have previously demonstrated that α_2-adreno- ceptor-mediated contractions of the rabbit isolated saphenous vein (SV–RP) are abolished following prolonged equilibration in nominally Ca^{2+}-free Krebs-Henseleit solution (Daly et al., 1989b) and are not associated with an initial transient contraction after a 10 min exposure to buffered low Ca^{2+} ($0.1\,\mu$M) Krebs-Henseleit solution (Daly et al., 1990). Thus, responses in this preparation appear to be entirely dependent upon the entry of extracellular Ca^{2+} ions and distinct from that proposed by Putney (1986), i.e. no passage via superficially located stores, but direct entry into the cytosol. Qualitatively similar observations have been reported in the arterial circulation of the rabbit isolated ear (Owen et al., 1987). The dependence of α-adrenoceptor-mediated contractions upon the presence of extracellular Ca^{2+} ions in this vascular bed was inversely related to the calibre of the arterial segment, yet this was not associated with a parallel increase in the sensitivity of responses to the dihydropyridine derivative, nimodipine. The possibility that these nifedipine-resistant Ca^{2+} channels are examples of receptor-operated, voltage-independent Ca^{2+} channels (Bolton, 1979; Godfraind et al., 1986) requires detailed electrophysiological examination, particularly in view of the recent report of a voltage-independent non-selective cation channel linked to α-adrenoceptor activation in the rabbit ear artery (Amedee et al., 1990).

In conclusion, we have provided evidence that α_1- and α_2-adrenoceptor-mediated contractions to noradrenaline in isolated blood vessels from the rabbit both utilize nifedipine-sensitive Ca^{2+} channels. Although no direct electrophysiological evidence has been provided, it seems likely that these channels are voltage-operated channels. These observations also complement our earlier findings that α_2-adrenoceptors, like those mediated by the α_1-subtype, can involve the mobilization of Ca^{2+} ions from cellular-bound stores. Furthermore, α_2-adrenoceptor-mediated contractions in the saphenous vein displayed considerable resistance to nifedipine, yet we found no evidence that this response was associated with the mobilization of intracellular Ca^{2+} ions. Could this be another example of receptor-operated, voltage-independent Ca^{2+} channels coupled to postjunctional α_2-adrenoceptors (see: Matthews et al., 1984)?

This work was supported by the Science and Engineering Research Council, through a Collaborative Project Grant in association with Roche Products, U.K. (C.J.D. & V.G.W.), and a CASE studentship in association with Dr A.F. Lever of the MRC Blood Pressure Unit (W.R.D.). We are grateful for support also to the Medical Research Funds of the University of Glasgow.

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(Received October 5, 1990
Revised January 14, 1991
Accepted January 28, 1991)