Br. J. Pharmacol. (1990), **101**, 961–967

Differential effects of calcium antagonists and Bay K 8644 on contractile responses to exogenous noradrenaline and adrenergic nerve stimulation in the rabbit ear artery

Tor V. Ch. Skärby & [1]Edward D. Högestätt

Department of Clinical Pharmacology, University Hospital, Lund University, S-221 85 Lund, Sweden

1 The effects of three calcium antagonists (nifedipine, verapamil, diltiazem) and the calcium agonist Bay K 8644 were compared on contractile responses of similar amplitude elicited by noradrenaline (NA) and electrical nerve stimulation (ENS) in the rabbit isolated ear artery.

2 Contractions induced by both NA (3×10^{-7} M) and ENS (10 Hz, 10 s) were almost exclusively mediated by α_1-adrenoceptors, since 10^{-7} M prazosin abolished (NA) or almost abolished (ENS) the responses, and prazosin was more than three orders of magnitude more potent than rauwolscine on both types of response.

3 ENS-induced contractions were considerably less inhibited by nifedipine, verapamil and diltiazem than were those elicited by NA. Bay K 8644 enhanced responses to NA more than those to ENS.

4 The inhibitory effect of nifedipine and Ca^{2+} deprivation on NA-induced contractions decreased with increasing NA concentration. Reduction of the NA response by prazosin or phenoxybenzamine increased the nifedipine inhibition.

5 Reduction of the ENS-induced contractions by prazosin or phenoxybenzamine, or by use of a lower stimulation frequency did not increase the inhibitory effect of nifedipine.

6 In conclusion, the differential effects of the calcium antagonists on NA- and ENS-induced contractions were not related to differences in α-adrenoceptor subtype (α_1/α_2), receptor reserve or response amplitude, but may rather reflect temporal and spatial differences in α-adrenoceptor activation between the responses.

Introduction

Organic calcium antagonists and α-adrenoceptor blockers (non-selective and α_1-subtype selective) are effective antihypertensive agents. Orthostatic side effects are, however, more frequently encountered with α-adrenoceptor blockers than with calcium antagonists. A conceivable explanation may be that reflex sympathetic vasoconstriction is better preserved during treatment with the latter than with the former group of drugs.

In line with this notion, experimental studies on pithed rats have shown that calcium antagonists more effectively antagonize vasopressor responses to exogenous noradrenaline (NA) than those to sympathetic nerve stimulation (Pedrinelli & Tarazi, 1984). However, in studies on perfused tissues, calcium antagonists seem to affect vasoconstrictor responses to NA and electrical nerve stimulation (ENS) to a similar degree (Armstead *et al.*, 1987; Lippton *et al.*, 1987; Kadowitz *et al.*, 1988). In the rabbit isolated ear artery (REA), supplied with a dense adrenergic innervation, contractions elicited by ENS were even more sensitive to calcium antagonists than were those induced by NA (Kajiwara & Casteels, 1983). However, inhibitory effects were observed only at high calcium antagonist concentrations, and the relative amplitudes of the responses to NA and ENS were not defined.

A body of information suggests that intracellular Ca^{2+} release, in contrast to Ca^{2+} influx, contributes comparatively more to α_1- than α_2-adrenoceptor-mediated vasoconstriction in arteries (see Van Meel, 1982). It is also known that the α-adrenoceptor subtype located intra- and extrajunctionally vary considerably between different vascular preparations (Langer *et al.*, 1980; 1981; Yamaguchi & Kopin, 1980; Starke & Docherty, 1982; McGrath, 1982; Gardiner & Peters, 1982; Elsner *et al.*, 1984; Hicks *et al.*, 1984).

In the present study, the effects of three calcium antagonists (nifedipine, verapamil, diltiazem) and the calcium agonist Bay K 8644 were examined on responses elicited by NA and ENS in the isolated REA. The α-adrenoceptors stimulated by exogenous NA and neuronally released NA were characterized pharmacologically by use of selective α_1- and α_2-adrenoceptor agonists and antagonists. Since the inhibitory effects of CAs generally decrease with increasing NA concentration and consequently with the contraction amplitude (cf. van Breemen *et al.*, 1982b), drug effects were evaluated on contractions of similar amplitude induced by NA and ENS in the present study.

Methods

Female albino rabbits of the Danish Land strain were killed by air injection into an auricular vein. The middle third of the central ear artery with an inner diameter of 0.4–0.5 mm was rapidly dissected out under microscope. All dissections were performed in cold (8–10°C) Krebs solution of the following composition (in mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, (+)-glucose 6.0. The arteries were divided into 2 mm long segments. Each segment was mounted on two L-shaped metal holders (0.2 mm diameter) in a temperature-controlled (37°C) organ bath containing 5 ml Krebs solution. The solution was continuously bubbled with 95% O_2 and 5% CO_2, resulting in a pH of approximately 7.4. One of the metal holders was fixed to a movable unit for adjustments of resting tension and the other was attached to a tension transducer (Grass FT03C). Detailed information on

[1] Author for correspondence.

the experimental method has been given previously (Högestätt *et al.*, 1983; Skärby *et al.*, 1983).

During an equilibration period of 1 h, the resting tension was gradually increased to approximately 4 mN. Each preparation was then contracted by 10^{-4} M NA (giving a maximum response, see Figure 3) and the response was used as an internal reference. Agonist concentration-response relationships were obtained by exposure to increasing drug concentrations in a cumulative manner (i.e. the next drug concentration was introduced when the effect of the former reached a plateau). The effects of α-adrenoceptor antagonists, calcium antagonists and Bay K 8644 on NA-induced contractions were evaluated according to the following protocol. The preparations were repeatedly contracted by 3×10^{-7} M or 10^{-5} M NA with 30 min intervals, each NA exposure lasting for 5–10 min. When two reproducible contractions were obtained (difference <10%), the lowest drug concentration was introduced 15–20 min prior to the next NA application. This sequence was repeated with increasing drug concentrations. As shown in parallel control experiments with NA alone, the NA-induced contractions remained reproducible throughout the experimental period.

Electrical nerve stimulation was produced by a Grass S48 stimulator connected to two platinum wire electrodes placed immediately below and above each vessel segment. Square wave pulses with a duration of 0.3 ms were delivered with a frequency of 5 or 10 Hz in 10 s trains at 2.5 min intervals. The electrode polarity was changed after each pulse. The voltage was adjusted to give a maximum response in each experiment. When 6–10 reproducible contractions were obtained (difference <10%), the various drugs were added into the organ bath in a cumulative manner. Control experiments were always run in parallel. In a few experiments, preparations were stimulated continuously for several minutes with a pulse frequency of 10 Hz.

Drugs

(±)-Noradrenaline HCl, (−)-phenylephrine HCl (Sigma), rauwolscine HCl (Roth) and clonidine (Boehringer Ingelheim) were dissolved in 0.9% NaCl containing 1.0 mM ascorbic acid. Prazosin (Pfizer) was dissolved in 1% methanol and 1 mM HCl, whereas Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) (Bayer) and phenoxybenzamine were dissolved in ethanol, giving stock solutions of 1 mM. Verapamil HCl (Knoll) and diltiazem HCl (Tanabe) were dissolved in water. Stock solutions were stored at −70°C. Nifedipine (Bayer) was provided in ampoules containing 0.1 mg ml^{-1} nifedipine in 150 mg ml^{-1} ethanol, 150 mg ml^{-1} polyethylene glycol and water. Bay K 8644 and nifedipine were kept in the dark until use to avoid light-induced degradation. Drug dilutions were made with 0.9% NaCl containing 1 mM ascorbic acid.

Calculation and statistics

The maximum drug effect (E_{max}) and the drug concentration producing 50% of E_{max} (EC_{50}) were calculated for each drug when possible. pEC_{50} denotes the negative logarithm of EC_{50}. Results given in the text, tables and figures are expressed as mean ± s.e., followed by the number of experiments performed (n). Student's t test was used to determine differences between groups of data. Analysis of variance was performed when multiple comparisons were made. A probability level <0.05 was accepted as significant.

Results

Responses to noradrenaline

The NA-induced contraction was generally biphasic with an initial rapid component and an ensuing tonic component, reaching a maximum within 5–10 min. When related to the tonic component, the initial rapid response, where distinguishable, was considerably smaller at a concentration of 3×10^{-7} M NA than at 10^{-5} M or 10^{-4} M NA (Figure 1a). The maximum response to 3×10^{-7} M NA was 51 ± 3% (n = 38) of that to 10^{-4} M NA. Incubation in Ca^{2+}-depleted medium (with 10^{-4} M EGTA) for 10 min reduced the contractile responses to 3×10^{-7} M and 10^{-5} M NA by 87 ± 4% (n = 9) and 72 ± 6% (n = 8), respectively, leaving only a transient response. The difference in reduction between the two NA concentrations was statistically significant.

Responses to electrical nerve stimulation

Continuous ENS (10 Hz) also elicited a biphasic contraction similar to the exogenous NA response, although the second component gradually declined with time (Figure 1b). The initial rapid component was generally fully developed within 10 s. The second component reached a maximum after 1–3 min.

Electrical nerve stimulation (10 Hz) for 10 s periods every 2.5 min elicited reproducible phasic contractions (Figure 2). The contractions amounted to 37 ± 2% (n = 42) of the 10^{-4} M NA response. Tetrodotoxin (3×10^{-7} M) reduced the ENS-induced responses by 96 ± 1% (n = 27), and a few min incubation in Ca^{2+}-free medium (with 10^{-4} M EGTA) abolished them (n = 5).

Effects of α-adrenoceptor agonists

Noradrenaline, phenylephrine and clonidine elicited concentration-dependent contractions in the REA with pEC_{50} values of 6.26 ± 0.17 (n = 4), 6.54 ± 0.17 (n = 5) and

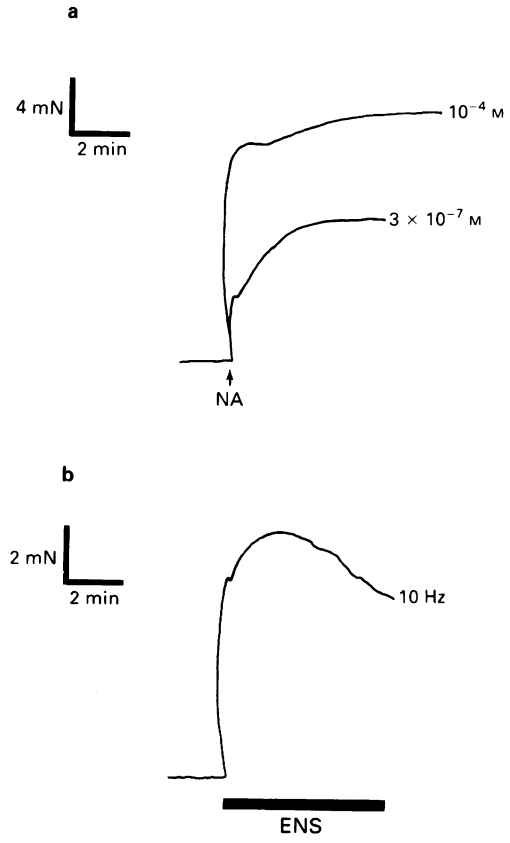

Figure 1 Contractile responses to noradrenaline (NA) and electrical nerve stimulation (ENS). (a) Superimposed tracings of contractions elicited by two NA concentrations in the same arterial segment; (b) shows the response to continuous ENS.

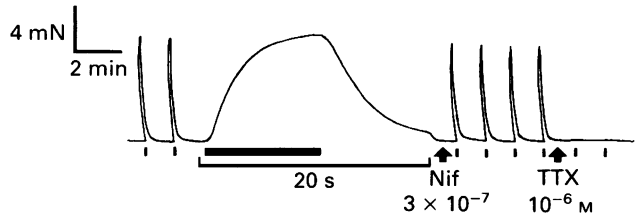

Figure 2 Tension tracing showing the effects of nifedipine (Nif) and tetrodotoxin (TTX) on responses induced by repeated electrical nerve stimulation (10 Hz, 10 s). Bars below tracings indicate the stimulation periods. The time scale was expanded during the third contraction to illustrate the time course of the response.

6.61 ± 0.11 (n = 6), respectively. The potencies of the agonists did not differ significantly. E_{max} for phenylephrine and clonidine were 108 ± 2.5% (n = 5) and 64 ± 10% (n = 6) of the 10^{-4} M NA-induced contraction, respectively (Figure 3).

Effects of α-adrenoceptor antagonists

Prazosin (10^{-6} M) reduced the contractile responses to repeated 10 s ENS (10 Hz) by 95 ± 1% (n = 6) and abolished those to 3×10^{-7} M NA. The ENS responses were enhanced by 10^{-8}–10^{-6} M rauwolscine, resulting in a biphasic concentration-response curve with an enhancement at low and inhibition at high ($\geq 10^{-5}$ M) concentrations (Figure 4). Prazosin was 3300 and 4500 times more potent than rauwolscine in inhibiting contractions elicited by NA and ENS, respectively (Figure 4, Table 1).

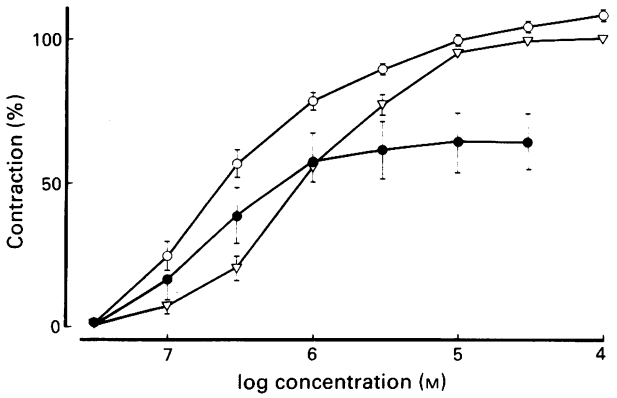

Figure 3 Concentration-response curves to noradrenaline (NA) (▽), clonidine (●) and phenylephrine (○). Contractions are expressed as a percentage of those induced by 10^{-4} M NA. Each point represents mean of 4–6 experiments; s.e.mean shown by vertical bars.

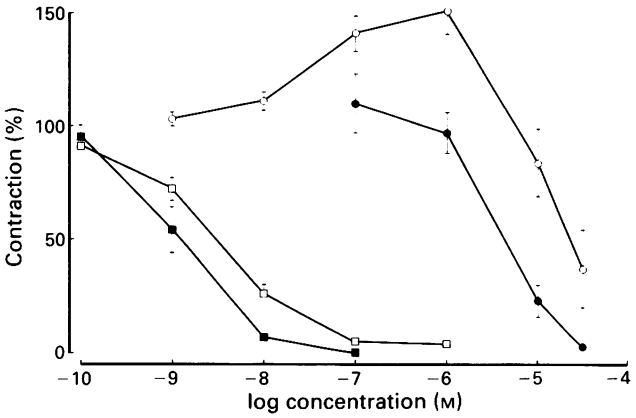

Figure 4 Concentration-inhibition curves showing the effects of prazosin (■, □) and rauwolscine (●, ○) on contractions elicited by 3×10^{-7} M noradrenaline (filled symbols) and 10 Hz electrical nerve stimulation (open symbols). Contractile responses are expressed as a percentage of those obtained before drug administration. Each point represents mean of 4–7 experiments; s.e.mean shown by vertical bars.

Effects of Bay K 8644 and calcium antagonists

None of the calcium antagonists nor Bay K 8644 *per se* affected the baseline tension of unstimulated preparations. However, during repeated 10 s ENS (10 Hz), Bay K 8644 induced a small but concentration-dependent tonic contraction, amounting to 13 ± 3% (n = 8) of the 10^{-4} M NA response. Bay K 8644 enhanced and nifedipine inhibited contractions induced by NA (3×10^{-7} M) significantly more than those induced by ENS (10 Hz, 10 s). The effects of nifedipine and Bay K 8644 on the ENS responses were too small to allow calculation of pEC_{50} (Figure 5a, Table 1).

Verapamil was 46 times and diltiazem 60 times more potent in inhibiting responses induced by NA than those elicited by ENS. At a concentration of 10^{-4} M, both verapamil and diltiazem abolished the ENS responses (Figure 5b and c, Table 1). Approximately 30% of the NA-induced contraction remained in the presence of 10^{-4} M diltiazem or 3×10^{-6} M nifedipine, whereas 10^{-4} M verapamil almost abolished the response.

Nifedipine (3×10^{-7} M) reduced the response to 3×10^{-7} M NA (64 ± 4%, n = 6) significantly more than that to 10^{-5} M NA (21 ± 4%, n = 5). The NA-induced contractions were sustained in the presence of nifedipine, whereas they were transient in Ca^{2+}-free medium (see above). Reduction of the

Table 1 Effects of α-adrenoceptor blockers, calcium antagonists and Bay K 8644 on contractile responses evoked by 3×10^{-7} M noradrenaline and electrical nerve stimulation (10 Hz, 10 s)

	Noradrenaline			*Electrical nerve stimulation*		
	pEC_{50}	E_{max}	n	pEC_{50}	E_{max}	n
Prazosin	8.96 ± 0.15	−100 ± 1	6	8.50 ± 0.09	−95 ± 1	6
Rauwolscine	5.44 ± 0.13	−97 ± 1	7	4.85 ± 0.11[a]		4
Verapamil	6.51 ± 0.25	−93 ± 1	6	4.85 ± 0.12	−100 ± 1	5
Diltiazem	6.45 ± 0.22	−72 ± 7[b]	5	4.67 ± 0.04	−99 ± 1	5
Nifedipine	8.23 ± 0.13	−67 ± 3	6		−10 ± 3	7
Bay K 8644	8.71 ± 0.28	+66 ± 24	6		+11 ± 4	8

The sign preceding each E_{max} value indicates inhibition (−) or enhancement (+) of contraction.

[a] Calculations based on a presumed E_{max} of 100%.

[b] The inhibition produced by 10^{-4} M diltiazem was accepted as E_{max}, although the concentration-response curve did not reach a clear plateau.

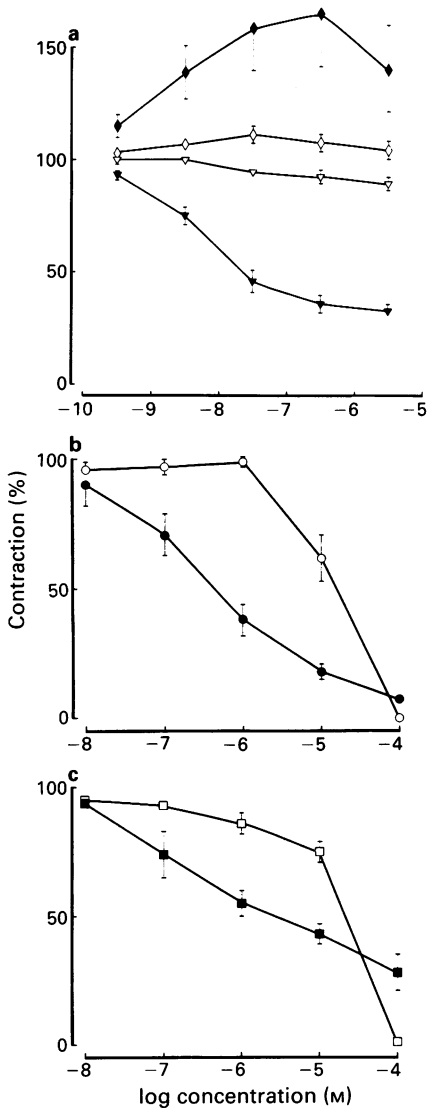

Figure 5 Effects of (a) nifedipine (▼, ▽) and Bay K 8644 (◆, ◇), (b) verapamil (●, ○) and (c) diltiazem (■, □) on contractions elicited by 3×10^{-7} M noradrenaline (filled symbols) and 10 Hz electrical nerve stimulation (open symbols). Contractile responses are expressed as a percentage of those obtained prior to drug administration. Each point represents mean of 4–8 experiments; s.e.mean shown by vertical bars.

10^{-5} M NA response by prazosin (1×10^{-7}–3×10^{-7} M) or phenoxybenzamine (Pbz) (1×10^{-8}–3×10^{-8} M, 5 min pretreatment) to a level similar to the 3×10^{-7} M NA response, significantly augmented the inhibitory effect of nifedipine (Figure 6). Furthermore, the inhibition produced by 3×10^{-7} M nifedipine did not differ between contractions induced by 3×10^{-7} M NA and those elicited by 10^{-5} M NA after α-adrenoceptor blockade by prazosin or Pbz. The contraction elicited by 5 Hz ENS was, however, not more affected by nifedipine than was that induced by 10 Hz, despite the fact that the response to 5 Hz was only 38 ± 4% ($n = 5$) of that to 10 Hz. Reduction of the 10 Hz ENS response to a level similar to the 5 Hz ENS response by prazosin (3×10^{-8}–10^{-7} M) or phenoxybenzamine (3×10^{-8}–10^{-7} M, 5 min pretreatment) did not enhance the effect of nifedipine. The results from these experiments are summarized in Figure 6.

Discussion

The REA is endowed with a dense adrenergic innervation and promptly constricts in response to perivascular nerve stimulation (Waterson & Smale, 1967; Steinsland *et al.*, 1973; 1985).

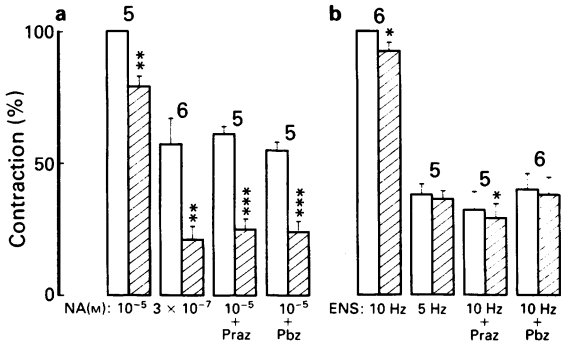

Figure 6 Effects of 3×10^{-7} M nifedipine (hatched columns) on contractions elicited by noradrenaline (NA) or repeated electrical nerve stimulation (ENS) in the presence and absence of partial α-adrenoceptor blockade by prazosin (Praz) or phenoxybenzamine (Pbz). Contractile responses are expressed as a percentage of those obtained by 10^{-5} M noradrenaline (a) or 10 Hz ENS (b) in the absence of inhibitors. Open columns, controls. Each column represents mean with s.e. shown by vertical bars. The numbers of experiments are given above each pair of columns. Asterisks indicate significant inhibition by nifedipine (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). For further explanation, see Results.

The contractile response was markedly reduced or abolished by superior cervical ganglionectomy or guanethidine (De la Lande & Rand, 1965). A significant phentolamine-resistant neurogenic contraction has, however, been demonstrated in small branches of the REA (Owen *et al.*, 1985). The ENS-induced contractions in the present study were Ca^{2+}-dependent and almost abolished by tetrodotoxin or α-adrenoceptor blockade, indicating their adrenergic origin.

Whether activated by exogenous NA or neuronally released NA, the α-adrenoceptor mediating the contractile response appeared to be mainly of the α_1-type. This conclusion, which agrees well with the results reported by others (Manzini *et al.*, 1983; Hieble & Woodward, 1984), was based on the following findings. Prazosin was more than three orders of magnitude more potent than rauwolscine in inhibiting contractions elicited by both NA and ENS. Prazosin at a concentration of 10^{-7} M, which in radioligand binding studies has virtually no effect on α_2-adrenoceptors (Hoffman & Lefkowitz, 1980; Andersson *et al.*, 1984), abolished (NA) or almost abolished (ENS) the responses. Inhibitory effects of rauwolscine were observed only at high concentrations ($>10^{-6}$ M) lacking α_2-adrenoceptor selectivity. The enhancement of the ENS-induced contractions by 10^{-8}–10^{-6} M rauwolscine rather reflects antagonism of prejunctional α_2-adrenoceptors (see Skärby & Larsson, 1987). The relative potencies of phenylephrine and clonidine are also consistent with an α_1-adrenoceptor (see Starke, 1981).

As clearly shown in the present study, contractile responses to exogenous NA were significantly more sensitive to nifedipine, verapamil and diltiazem than were those induced by ENS. Bay K 8644, which promotes Ca^{2+} influx by interacting with dihydropyridine receptors on the Ca^{2+} channels (Spedding, 1985), substantially augmented the NA-induced contraction, whereas those elicited by ENS were less affected. Obviously, these differential effects cannot be attributed to a heterogeneous distribution of α_1- and α_2-adrenoceptors on the vascular smooth muscle cells. It has, however, been suggested that the receptor reserve, rather than the α-adrenoceptor subtype, is a major determinant of intracellular Ca^{2+} release (see Ruffolo & Nichols, 1988). This concept is gaining wide support, and results obtained in several studies have shown that responses to α_1-adrenoceptor stimulation become sensitive to calcium antagonists only after reduction of the receptor reserve or by using partial agonists (Ruffolo *et al.*, 1984; Pedrinelli & Tarazi, 1985; Jim *et al.*, 1986; Wanstall & O'Donnell, 1988; Bognar & Enero, 1988). The REA contains a large α-adrenoceptor reserve, and half-maximum contraction is achieved at 1% receptor occupancy (Purdy *et al.*, 1983). As shown in the present study, reduction of the α-adrenoceptor

number by the irreversible receptor blocker Pbz did not increase the inhibitory effect of nifedipine on the ENS-induced contraction, whereas this pretreatment enhanced the inhibition of the 10^{-5} M NA-induced response. Thus, the differential effects of the calcium antagonists on contractions induced by exogenous and endogenous NA do not appear to be related to differences in receptor reserve. Interestingly, the response to 10^{-5} M NA after Pbz pretreatment was not more inhibited by nifedipine than was that obtained in the presence of prazosin, although the two α-adrenoceptor blockers reduced the NA response to the same extent (see Figure 6). This finding does not favour the view that the α-adrenoceptor reserve is of crucial importance for the calcium antagonist sensitivity in this preparation.

Nifedipine and Ca^{2+} depletion reduced the contractile response to 3×10^{-7} M NA significantly more than that to 10^{-5} M NA. This correlates well with previous studies showing that contractions induced by high NA concentrations are more dependent on intracellular Ca^{2+} release than are those elicited by low NA concentrations (Casteels *et al.*, 1977) and consequently less inhibited by calcium antagonists (van Breemen *et al.*, 1982b). Indeed, NA-induced intracellular Ca^{2+} release was shown to have a 10 fold higher threshold than stimulation of Ca^{2+} influx in the rabbit aorta (van Breemen *et al.*, 1982a). In the present study, reduction of the 10^{-5} M NA response by 50%, with prazosin or Pbz, considerably increased the inhibitory effect of nifedipine. Thus, it appears that the larger the NA-induced contraction, the smaller the calcium antagonist sensitivity. The ENS-induced contraction, however, turned out to be slightly smaller than the response to 3×10^{-7} M NA; still it was considerably less affected by the calcium antagonists.

Prazosin was approximately three times less potent in inhibiting contractions induced by ENS than those elicited by 3×10^{-7} M NA. Assuming equilibrium at the receptor level, this would predict an intrajunctional NA concentration of about 10^{-6} M according to receptor theory. However, since equilibrium conditions for NA are probably not obtained during a short period of ENS, leading to an overestimation of the prazosin-induced inhibition, the intrajunctional NA concentration may be even higher than 10^{-6} M (cf. Neild & Zelcer, 1982; Bevan, 1984). In contrast to the response to exogenous NA, a 70% reduction of the ENS-induced contraction by prazosin or Pbz, or by use of a lower stimulation frequency (5 Hz) did not enhance the inhibitory effect of nifedipine. This may indicate that contractions evoked by endogenous NA rely almost entirely on intracellular Ca^{2+} release irrespective of the response magnitude, although Ca^{2+} influx through calcium antagonist-insensitive membrane channels cannot be excluded.

As shown in a previous study on the perfused REA (Steinsland *et al.*, 1973), the contractile responses to NA and continuous ENS were found to be distinctly biphasic, composed of an initial rapid component, reaching a maximum within 10–15 s, and a second tonic component developing over several minutes. This largely agrees with the results obtained in the present study. Furthermore, the initial component of the NA response was rendered unaffected by several procedures interfering with Ca^{2+} influx in contrast to the tonic component (Steinsland *et al.*, 1973). Similar findings have been reported for several other arteries, suggesting that the initial rapid and the ensuing tonic component are due to intracellular Ca^{2+} release and Ca^{2+} influx, respectively (Bolton 1979; van Breemen *et al.*, 1982b; Skärby *et al.*, 1984). In a more recent study, the initial rapid response to ENS (3 Hz) was shown to be relatively resistant to verapamil and especially nitrendipine, whereas the tonic component was highly sensitive to these agents (Steinsland *et al.*, 1985). Since in the present study the preparations were stimulated for only 10 s, the effects of the calcium antagonists on the tonic ENS response were not evaluated. It would thus appear that the initial rapid component of the ENS response was compared with the tonic component of the NA response in the present study. The relevance of these components for α-adrenoceptor activation *in vivo* is, however, unclear. Based on microelectrode recordings from human sympathetic nerves *in vivo*, it was suggested that the sympathetic outflow to muscles is important for buffering temporary changes in blood pressure. The sympathetic impulses mostly appeared in high frequency bursts during 1–3 s with silent intervals of several seconds (see Wallin, 1981). In an attempt to reproduce this discharge pattern, the REA was stimulated with trains (2 s long) of pulses (25 Hz) every 5 s. This resulted in a sustained contraction, which was not appreciably affected by nifedipine (unpublished observations). It is conceivable that this type of intermittent ENS as well as short period ENS lead to a rapid and intense α-adrenoceptor stimulation restricted to membrane patches adjacent to the nerve terminals, and hence intracellular Ca^{2+} release, whereas exposure to exogenous NA activates α-adrenoceptors over almost the entire cell surface during a longer period of time, thus favouring Ca^{2+} influx. However, other possibilities such as, e.g., α_1-adrenoceptor subtype heterogeneity and release of other neurotransmitters along with NA (e.g. adenosine triphosphate, neuropeptide Y), influencing calcium antagonist sensitivity, cannot be ruled out at present.

The sensitivity of the 3×10^{-7} M NA-induced response to the calcium antagonists, Bay K 8644 and Ca^{2+} deprivation indicates that it was highly dependent on Ca^{2+} influx. In conjunction with the present study, we also examined the inhibitory effects of the calcium antagonists on the tonic component of the 124 mM K^+-induced contraction (unpublished observations). The mean amplitude of the tonic K^+ component and the 3×10^{-7} M NA response were almost identical (52% versus 51% of the 10^{-4} M NA response). Interestingly, neither pEC_{50} for nifedipine nor those for diltiazem and verapamil differed significantly between the two types of contraction. Although early electrophysiological studies on the REA were unable to record membrane potential changes in response to NA application (Droogmans *et al.*, 1977), later investigations have shown a close correlation between NA-induced contraction and depolarization in this artery (Trapani *et al.*, 1981; Suzuki & Kou, 1983). In the light of these findings it is tempting to suggest that NA, in concentrations that release only small amounts of intracellular Ca^{2+}, can promote Ca^{2+} influx through potential-operated channels.

In agreement with the present study, contractions induced by endogenous NA were fairly insensitive to calcium antagonists in the canine saphenous vein (Takata & Kato, 1984; 1987), rabbit pulmonary artery (Zelis *et al.*, 1985), rabbit ear artery (Kajiwara & Casteels, 1983) and rat vas deferens (Brown *et al.*, 1983; Amobi & Smith, 1985). Exposure to verapamil and diltiazem concentrations below 10^{-6} M had virtually no effect on the response to endogenous NA (present study; Kajiwara & Casteels, 1983; Takata & Kato, 1984; Amobi & Smith, 1985; Zelis *et al.*, 1985). When diltiazem concentrations above 10^{-6} M were used, a parallel inhibition of contraction and transmitter release was observed (Takata & Kato, 1984; Zelis *et al.*, 1985). Thus, a prejunctional action may well explain the complete inhibition of the ENS response produced by 10^{-4} M diltiazem in the present study. The ENS-induced contraction was also abolished by 10^{-4} M verapamil. Several studies have shown an enhancement of adrenergic transmitter release by verapamil concentrations above 10^{-6} M (Larsson *et al.*, 1984; Takata & Kato, 1984). In the nerve-stimulated canine saphenous vein, 10^{-5} M verapamil produced a 50% increase of NA release but still reduced the contractile responses by 30% (Takata & Kato, 1984). These findings agree with a simultaneous pre- and postjunctional α-adrenoceptor blocking effect of verapamil. Indeed, in radioligand binding studies, verapamil has been shown to interact with both α_1- and α_2-adrenoceptors in smooth muscle with dissociation constants ranging between 0.1 and 6.8 μM (Motulsky *et al.*, 1983; Larsson *et al.*, 1984; Descombes & Stoclet, 1985; Nishimura *et al.*, 1986). Bay K 8644, on the other hand, affected neither contraction nor NA release

evoked by ENS in the canine saphenous vein (Takata & Kato, 1987). Nifedipine appears also to have only minimal effects on adrenergic transmitter release (Högestätt *et al.*, 1982; Larsson *et al.*, 1984).

It is concluded that nifedipine, verapamil and diltiazem in concentrations $\leq 10^{-6}$ M affected only marginally ENS-induced contractions in the REA, whereas those elicited by exogenous NA were effectively reduced. In contrast, prazosin was an effective inhibitor of contractions elicited by both exogenous and endogenous NA. The differential effects of the calcium antagonists were not related to differences in α-adrenoceptor subtype (α_1/α_2), receptor reserve or response amplitude, but may rather reflect temporal and spatial differences in α-adrenoceptor activation between the two types of response.

References

AMOBI, N.I.B. & SMITH, I.B.H. (1986). Differential effects of calcium channel blockers on the responses of the rat vas deferens to intramural nerve stimulation and exogenous drugs. *Experientia*, **42**, 410–412.

ANDERSSON, K.-E., LARSSON, B. & SJÖGREN, C. (1984). Characterization of the α-adrenoceptors in the female rabbit urethra. *Br. J. Pharmacol.*, **81**, 293–300.

ARMSTEAD, W.M., LIPPTON, H.L., HYMAN, A.L. & KADOWITZ, P.J. (1987). Influence of nisoldipine on vascular resistance and vasoconstrictor responses in cats. *Am. J. Physiol.*, **252**, H816–H825.

BEVAN, J.A. (1984). The γ-connection: are we ready to throw out the α-adrenoceptor in sympathetic vasoconstriction? *Trends Pharmacol. Sci.*, **5**, 53–55.

BOGNAR, I.T. & ENERO, M. (1988). Influence of receptor reserve on the inhibition by calcium channel blockers of alpha adrenoceptor-mediated responses in rats isolated vascular tissues. *J. Pharmacol. Exp. Ther.*, **245**, 673–681.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Pharmacol. Rev.*, **59**, 606–718.

BROWN, D.A., DOCHERTY, J.R., FRENCH, A.M., MACDONALD, A., MCGRATH, J.C. & SCOTT, N.C. (1983). Separation of adrenergic and non-adrenergic contractions to field stimulation in the rat vas deferens. *Br. J. Pharmacol.*, **79**, 379–393.

VAN BREEMEN, C., HWANG, O. & CAUVIN, C. (1982a). Ca antagonist inhibition of norepinephrine stimulated Ca influx in vascular smooth muscle. In *Calcium Modulators*. ed. Godfraind, T., Albertini, A. & Paoletti, R. pp. 185–198. Amsterdam: Elsevier Biomedical Press.

VAN BREEMEN, C., MANGEL, A., FAHIM, M. & MEISHERI, K. (1982b). Selectivity of calcium antagonistic action in vascular smooth muscle. *Am. J. Cardiol.*, **49**, 507–510.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977). Excitation-contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.*, **271**, 63–79.

DE LA LANDE, I.S. & RAND, M.J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. Exp. Biol. Med. Sci.*, **43**, 639–656.

DESCOMBES, J.-J. & STOCLET, J.-C. (1985). Diphenylalkylamine calcium antagonists interact with α-adrenoceptor binding sites in aortic membranes. *Eur. J. Pharmacol.*, **115**, 313–316.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. Gen. Physiol.*, **70**, 129–148.

ELSNER, D., SAEED, M., SOMMER, O., HOLZ, J.& BASSENGE, E. (1984). Sympathetic vasoconstrictions sensitive to alpha$_2$-adrenergic receptor blockade. No evidence for preferential innervation of alpha$_1$-adrenergic receptors in the canine femoral bed. *Hypertension*, **6**, 915–925.

GARDINER, J.C. & PETERS, C.J. (1982). Postsynaptic alpha$_1$- and alpha$_2$-adrenoceptor involvement in the vascular responses to neuronally released and exogenous noradrenaline in the hindlimb of the dog and cat. *Eur. J. Pharmacol.*, **84**, 189–198.

HIEBLE, J.P. & WOODWARD, D.F. (1984). Different characteristics of postjunctional alpha-adrenoceptors on arterial and venous smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 44–50.

HICKS, P.E., MEDGETT, I.C. & LANGER, S.Z. (1984). Postsynaptic alpha$_2$-adrenergic receptor-mediated vasoconstriction in SH rat tail arteries *in vitro*. *Hypertension*, **6** (suppl. 1), 1–12.

HOFFMAN, B.B. & LEFKOWITZ, R.J. (1980). An assay for alpha-adrenergic receptor subtypes using [^{3}H] dihydroergocryptine. *Biochem. Pharmacol.*, **29**, 452–454.

HÖGESTÄTT, E.D., ANDERSSON, K.-E. & EDVINSSON, L. (1982). Effects of nifedipine on potassium-induced contraction and noradrenaline release in cerebral and extracranial arteries from rabbit. *Acta Physiol. Scand.*, **114**, 283–296.

HÖGESTÄTT, E.D., ANDERSSON, K.-E. & EDVINSSON, L. (1983). Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol. Scand.*, **117**, 49–61.

JIM, K.F., MARCIA, R.A. & MATTHEWS, W.D. (1986). Role of receptor reserve in the inhibition of alpha-1 adrenoceptor-mediated pressor responses by calcium antagonists in the pithed rat. *J. Pharmacol. Exp. Ther.*, **238**, 89–94.

KADOWITZ, P.J., ARMSTEAD, W.M., ROLAND, P.Y., HYMAN, A.L. & LIPPTON, H.L. (1988). Inhibitory effects of diltiazem on vasoconstrictor responses in the cat. *J. Pharmacol. Exp. Ther.*, **244**, 84–90.

KAJIWARA, M. & CASTEELS, R. (1983). Effects of Ca-antagonists on neuromuscular transmission in the rabbit ear artery. *Pflügers Arch.*, **396**, 1–7.

LANGER, S.Z., MASSINGHAM, R. & SHEPPERSON, N.B. (1980). Presence of postsynaptic α_2-adrenoceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hindlimb. *Clin. Sci.*, **59**, 225–228.

LANGER, S.Z., SHEPPERSON, N.B. & MASSINGHAM, R. (1981). Preferential noradrenergic innervation of alpha-adrenergic receptors in vascular smooth muscle. *Hypertension*, **3** (suppl 1), 112–118.

LARSSON, B., HÖGESTÄTT, E.D., MATTIASSON, A. & ANDERSSON, K.-E. (1984). Differential effects of nifedipine, verapamil, and diltiazem on noradrenaline-induced contractions, adrenergic transmitter release, and alpha-adrenoceptor binding in the female rabbit urethra. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 14–21.

LIPPTON, H.L., ARMSTEAD, W.M., HYMAN, A.L. & KADOWITZ, P.J. (1987). Influence of calcium-entry blockade on vasoconstrictor responses in feline mesenteric vascular bed. *Circ. Res.*, **61**, 570–580.

MANZINI, S., MAGGI, C.A. & MELI, A. (1983). α-Adrenoceptor subtypes and Ca^{2+} mobilization of the rabbit ear artery. *J. Pharm. Pharmacol.*, **35**, 584–589.

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

VAN MEEL, J.C.A. (1982). The vascular postjunctional alpha$_2$- adrenoceptor. *Thesis, University of Amsterdam.*

MOTULSKY, H.J., SNAVELY, M., HUGHES, R.J. & INSEL, P.A. (1983). Interaction of verapamil and other calcium channel blockers with α_1- and α_2-adrenergic receptors. *Circ. Res.*, **52**, 226–231.

NEILD, T.O. & ZELCER, E. (1982). Noradrenergic neuromuscular transmission with special reference to arterial smooth muscle. *Prog. Neurobiol.*, **19**, 141–158.

NISHIMURA, J., KANAIDE, H. & NAKAMURA, M. (1986). Binding of [^{3}H] prazosin to porcine aortic membranes: interaction of calcium antagonists with vascular alpha-1 adrenoceptors. *J. Pharmacol. Exp. Ther.*, **236**, 789–793.

OWEN, M.P., QUINN, C. & BEVAN, J.A. (1985). Phentolamine-resistant neurogenic constriction occurs in small arteries at higher frequencies. *Am. J. Physiol.*, **249** (Heart Circ. Physiol. 18), H404–H414.

PEDRINELLI, R. & TARAZI, R.C. (1984). *In vivo* interference of calcium entry blockade with pressor responses to α-adrenergic stimulation: Effects of two unrelated blockers on responses to both exogenous and endogenously released norepinephrine. *Circulation*, **69**, 1171–1176.

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. Pharmacol. Exp. Ther.*, **233**, 636–642.

PURDY, R.E., ASHBROOK, D.W., STUPECKY, G.L. & WATANABE, M.Y. (1983). Qualitative and quantitative differences between the postsynaptic alpha-adrenoceptors of rabbit ear artery and thoracic aorta. *J. Pharmacol. Exp. Ther.*, **224**, 3543–3551.

RUFFOLO, R.R. Jr., 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. Ther.*, **230**, 587–594.

RUFFOLO, R.R. Jr. & NICHOLS, A.J. (1988). The relationship of receptor reserve and agonist efficacy to the sensitivity of α-adrenoceptor-mediated vasopressor responses to inhibition by calcium channel antagonists. *Ann. N.Y. Acad. Sci.*, **522**, 361–376.

SKÄRBY, T.V.CH., ANDERSSON, K.-E. & EDVINSSON, L. (1983). Pharmacological characterization of postjunctional α-adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol. Scand.*, **117**, 63–73.

SKÄRBY, T., HÖGESTÄTT, E.D. & ANDERSSON, K.-E. (1984). Influence of extracellular calcium and nifedipine on α_1- and α_2-adrenoceptor mediated contractile responses in isolated rat and cat cerebral and mesenteric arteries. *Acta Physiol. Scand.*, **123**, 445–456.

SKÄRBY, T.V.CH. & LARSSON, B. (1987). Theoretical and functional studies on α_1- and α_2-adrenoceptors: an examination using the Schild plot. *J. Auton. Pharmacol.*, **7**, 184–198.

SPEDDING, M. (1985). Calcium antagonist subgroups. *Trends Pharmacol. Sci.*, **6**, 101–114.

STARKE, K. (1981). α-Adrenoceptor subclassification. *Rev. Physiol. Biochem. Pharmacol.*, **88**, 199–236.

STARKE, K. & DOCHERTY, J.R. (1982). Types and functions of peripheral alpha-adrenoceptors. *J. Cardiovasc. Pharmacol.*, **4**, S3–S7.

STEINSLAND, O.S., FURCHGOTT, R.F. & KIRPEKA, S.M. (1973). Biphasic vasoconstriction of the rabbit ear artery, *Circ. Res.*, **32**, 49–58.

STEINSLAND, O.S., JONSON, E.C. & SCRIABINE, A. (1985). Comparative effects of nitrendipine and verapamil on neuroeffector transmission in the rabbit ear artery. *Gen. Cardiovasc. Pharmacol.*, **7**, 990–995.

SUZUKI, H. & KOU, K. (1983). Electrical components contributing to the nerve-mediated contractions in the smooth muscles of the rabbit ear artery. *Jpn. J. Physiol.*, **33**, 743–756.

TAKATA, Y. & KATO, H. (1984). Effects of Ca antagonists on the norepinephrine release and contractile responses of isolated canine saphenous veins to transmural nerve stimulation. *Jpn. J. Pharmacol.*, **34**, 397–409.

TAKATA, Y. & KATO, H. (1987). Effects of Bay K 8644 on the electrically evoked and KCl-evoked norepinephrine release and the corresponding contraction in isolated canine saphenous veins. *Jpn J. Pharmacol.*, **43**, 458–461.

TRAPANI, A., MATSUKI, N., ABEL, P.W. & HERMSMEYER, K. (1981). Norepinephrine produces tension through electromechanical coupling in rabbit ear artery. *Eur. J. Pharmacol.*, **72**, 87–91.

WANSTALL, J.C. & O'DONNELL, S.R. (1988). Inhibition of norepinephrine contractions by diltiazem on aorta and pulmonary artery from young and aged rats: influence of alpha-adrenoceptor reserve. *J. Pharmacol. Exp. Ther.*, **245**, 1016–1020.

WALLIN, B.G. (1981). New aspects of sympathetic function in man. In *Butterworths International Medical Reviews.* Neurology 1, Clinical Neurophysiology, pp. 145–167. London: Butterworths.

WATERSON, J.G. & SMALE, D.E. (1967). Location of adrenergic structures in the central artery of the rabbit ear. *Aust. J. Exp. Biol. Med. Sci.*, **45**, 301–308.

YAMAGUCHI, I. & KOPIN, I.J. (1980). Differential inhibition of $alpha_1$ and $alpha_2$ adrenoceptor-mediated pressor responses in pithed rats. *J. Pharmacol. Exp. Ther.*, **214**, 275–281.

ZELIS, R., WICHMANN, T. & STARKE, K. (1985). Inhibition by diltiazem of norepinephrine release from sympathetic nerves in the rabbit pulmonary artery. *Pharmacology*, **31**, 268–277.

(Received January 31, 1990
Revised August 3, 1990
Accepted August 11, 1990)