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Sources of calcium and α_1-adrenoceptor-mediated contraction in rat tail artery

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1 The relative importance of intracellular and extracellular Ca^{2+} on α_1-adrenoceptor-mediated contraction by noradrenaline and St-587 has been studied and correlated with the binding characteristics in intact tail artery from Sprague-Dawley rats.

2 Noradrenaline and St-587 behaved as full agonists inducing a concentration-dependent vasoconstriction.

3 Nifedipine (1 μM and 10 μM) blocked by 50% ($P<0.001$) and 75% ($P<0.001$) respectively, the maximum contraction (E_{max}) induced by St-587. Nevertheless, to reach 40% inhibition of E_{max} on noradrenaline responses ($P<0.01$), 10 μM nifedipine was necessary.

4 Both agonists induced a concentration-dependent accumulation of inositol phosphates. Noradrenaline behaved as a full agonist and St-587 as a partial agonist for this response.

5 [^{3}H]-prazosin binding to intact tail artery rings was saturable and of high affinity ($K_D = 4.44 \pm 0.46$ nM; $B_{max} = 36.35 \pm 4.22$ fmol mg^{-1} tissue).

6 Competition curves for inhibition of specific [^{3}H]-prazosin binding by WB-4101 suggest that the rat tail artery contains two α_1-adrenoceptor subtypes in an approximate ratio of 60:40.

7 After irreversible alkylation of α_{1B}-adrenoceptors with 100 μM chloroethylclonidine (CEC), nifedipine (1 μM) influenced to a greater extent the St-587- than the noradrenaline-induced contraction.

8 Our results indicate that the degree of participation of intracellular and extracellular Ca^{2+} sources, on the α_1-adrenoceptor-mediated contraction, depends on the agonist used. The two α_1-adrenoceptor subtypes observed in binding experiments seem to be unrelated to the Ca^{2+} sources used for contraction.

Keywords: Tail artery; α_1-adrenoceptors; calcium antagonist; phosphoinositide hydrolysis; [^{3}H]-prazosin binding

Introduction

Vascular α_1-adrenoceptor-mediated contractile responses are linked to an increase of cytosolic-free calcium through two different mechanisms: influx from the external medium and release from intracellular pools (Cauvin & Malik, 1984). Stimulation of α_1-adrenoceptors activates a membrane phospholipase C-mediated breakdown of phosphatidylinositol bisphosphate to form two second messengers; inositol 1,4,5-trisphosphate (IP_3) and diacylglicerol. IP_3 has been shown to release calcium from sarcoplasmic reticulum (Michell, 1985; Chiu *et al.*, 1987). The translocation of calcium from the extracellular fluid to the cytoplasm is also mediated by α_1-adrenoceptors coupled, through a less clearly defined pathway, to the opening of membrane calcium channels (Godfraind, 1982; Nahorski *et al.*, 1994; Ruffolo & Hieble, 1994). In some blood vessels, like rat aorta, the extent to which these two processes are utilized varies with the different α_1-adrenoceptor agonists (Chiu *et al.*, 1986) but the expression of full agonism of the contraction needs the expression of a full agonism for both calcium processes. In addition, tissue differences in the degree of activation of these postreceptor mechanisms evoked by α_1-adrenoceptors have been observed (Cauvin & Malik, 1984). Stimulation of α_1-adrenoceptors in rat tail artery has been shown to induce the accumulation of inositol phosphates to a greater extent than in aorta (Labelle & Murray, 1990; Vila *et al.*, 1993). However, the relative roles of both mechanisms in the coupling of α_1-adrenoceptor activation are as yet, unclear.

The rat tail artery is a well-innervated tissue where the smooth muscle contraction is mediated predominantly through α_1-adrenoceptors. However, a small α_2-adrenoceptor population has also been found (Hicks *et al.*, 1984; Tsai *et al.*, 1993; Vila *et al.*, 1993; Redfern *et al.*, 1995). In this vessel, the stimulation of α_1-adrenoceptors by agonists leads to a rapid increase in the formation of inositol phosphates (Fox *et al.*, 1985; Vila *et al.*, 1993). In addition, an important pool of "spare" α_1-adrenoceptors seems to be present in this tissue (Tabernero *et al.*, 1996). This fact would explain why a partial agonist like St-587 with low intrinsic efficacy in rat aorta (Chiu *et al.*, 1986), could behave as a strong α_1-adrenoceptor agonist with a high efficacy for contraction in rat tail artery (Tabernero *et al.*, 1996).

In some vascular and non-vascular tissues, binding experiments have presented evidence of two α_1-adrenoceptor binding sites (Han *et al.*, 1987; Minneman *et al.*, 1988) that were designated α_{1A} and α_{1B} by Morrow & Creese (1986). Recently, a third α_1-adrenoceptor subtype, α_{1D}, has been described in several tissues, among them the rat aorta (Saussy *et al.*, 1994; Hieble *et al.*, 1995). This receptor seems to be the previously cloned $\alpha_{1A/D}$-adrenoceptor subtype (Hieble *et al.*, 1995). The possibility that specific α_1-adrenoceptor subtypes may be linked to different second messenger systems was suggested early on (Han *et al.*, 1987). Studies on isolated muscles suggested that α_{1B} mobilizes intracellular Ca^{2+}, probably *via* inositol phosphate formation, while α_{1A} evokes cellular responses increasing the entry of extracellular Ca^{2+} (Minneman & Esbenshade, 1994). However, later studies have indicated exceptions to this generalization (Lepetre *et al.*, 1994).

The present study was designed to evaluate the relative importance of extracellular calcium influx and the intracellular calcium release for the vasoconstriction elicited by noradrenaline and St-587. The existence of different α_1-adrenoceptor subtypes and their role in the calcium mechanisms involved in contraction have been also evaluated.

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Methods

The experiments were performed on 3–4 month old male Sprague-Dawley rats. The animals were killed by a sharp blow to the head and the tail artery quickly removed, cleaned of adherent tissue and placed in gassed (95% O_2, 5% CO_2) physiological salt solution (PSS) of the following composition (in mM): NaCl 112.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. Desipramine (0.1 μM), normetanephrine (1 μM), Na_2EDTA (23 μM), propranolol (1 μM) and yohimbine (0.1 μM) were present throughout the experiment to block neuronal and extraneuronal uptake, to prevent noradrenaline oxidative degradation and stimulation of β- and α_2-adrenoceptors, respectively.

Contractile studies

Rings of proximal tail artery (2–3 mm) were set up in 5 ml organ baths containing PSS maintained at 37±0.5°C and continuously gassed with 95% O_2, 5% CO_2. A resting tension of 7.35 mN was applied and changes in tension recorded with a PIODEN (UF-1) isometric transducer attached to an Omniscribe pen recorder. The preparations were left to equilibrate for 30 min and tension was readjusted if necessary. The tissues were contracted 3 or 4 times with KCl 75 mM every 5 min until the amplitude of contractile response was of similar magnitude. After a 30 min equilibration period each ring was contracted with noradrenaline (0.03 μM) and relaxed with acetylcholine (1 μM) to verify the functional state of the endothelium. Only preparations that relaxed by more than 60% were used. Rings were washed with PSS and after a further 30 min equilibration, a cumulative agonist concentration-effect (E/[A]) curve to noradrenaline (3 nM–30 μM) or St-587 (0.01–100 μM) was constructed.

The effect of nifedipine was examined to see whether noradrenaline- or St-587-induced contractions were sensitive to blockade of extracellular Ca^{2+} entry through Ca^{2+}-channels. After the first cumulative E/[A] curve, tissues were exposed for 60 min to nifedipine 1 μM and the agonist E/[A] curve was repeated. Preparations were washed and subsequently exposed to nifedipine 10 μM for 60 min. At the end of this period, a third cumulative E/[A] curve was constructed. To prevent nifedipine degradation, experiments were carried out under sodium vapour lamps. Three agonist E/[A] curves, in the presence of the vehicle (ethanol) at the appropriate concentrations, were carried out in parallel.

In a set of experiments, the tissues were incubated for half an hour with 100 μM chloroethylclonidine (CEC) followed by a 30 min washing period before the first E/[A] curve to the agonist was constructed. After the agonist had been washed out, tissues were incubated with nifedipine (1 μM, 60 min) and a second E/[A] curve was constructed. Control experiments, in the presence of CEC and ethanol at the concentration used to dissolve nifedipine, were run in parallel to check the reproducibility over time between the two curves performed in the above mentioned conditions.

Inositol phosphates assay

The tail artery was rinsed with buffered Krebs solution (KRB, composition in mM: NaCl 118.3, KCl 4.7, $CaCl_2$ 1.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1) equilibrated with 95% O_2, 5% CO_2. Four to five rats were used in each individual experiment to obtain sufficient material. The arteries were cleaned of adherent tissue and then cut into rings (4 mm) and pooled. The rings were incubated at 37±0.5°C and aerated with 95% O_2, 5% CO_2 for 30 min in KRB. The Krebs solution was changed every 10 min. Subsequently, the vessels were labelled with 1 μM [^{3}H]-*myo*inositol (specific activity 18.8 Ci mmol^{-1}) in KRB buffer pH 7.4 equilibrated with 95% O_2, 5% CO_2 at 37±0.5°C for 2 h. After incubation, the rings were washed twice with KRB and a third time with KRB containing LiCl (10 mM), desipramine (0.1 μM), normetanephrine (1 μM) and propranolol (1 μM). Two pieces of tail artery were incubated for 30 min in a final volume of 550 μl of KRB buffer containing LiCl 10 mM with the above mentioned inhibitors. Samples were stimulated with increasing concentrations of noradrenaline (0.1 μM–1 mM) or St-587 (0.1–100 μM) for 30 min under an atmosphere of 95% O_2, 5% CO_2. Control tubes with ascorbic acid 0.1 mg ml^{-1} or distilled water were also assayed simultaneously. Experiments with noradrenaline and St-587 were performed in parallel. The reaction was stopped by the addition of 2 ml $CH_3OH/CHCl_3/HCl$ (40:20:1 v/v/v) mixture and the sample sonicated for 45 min at 2–3°C. After the addition of 0.63 ml of $CHCl_3$ and 1.26 ml distilled water, the samples were centrifugated at 2500 r.p.m. for 10 min to facilitate phase separation. The aqueous phase was neutralized and applied to columns containing Dowex AG 1X8 formate form ion exchange resin previously equilibrated with 30 ml 10 mM Tris-formate, pH 7.4. The columns were then washed with 15 ml of unlabelled *myo*inositol (5 mM) and 10 ml of 60 mM sodium formate/5 mM sodium borate. Total [^{3}H]-inositol phosphates were eluted by washing with 0.1 M formic acid in 1 M ammonium formate according to the method of Berridge *et al.*, (1982), and counted for radioactivity. The lipid layer remaining after removal of the aqueous phase was used for measurement of the [^{3}H]-phosphatidylinositols. Aliquots of the lipid phase (0.2 ml) were removed, left to evaporate overnight, and counted for radioactivity.

[^{3}H]-prazosin binding assay

Binding studies were performed by incubation of rat tail artery rings (1.5–2 mg wet weight) under the same conditions as those previously described for rat mesenteric artery (Morel & Godfraind, 1991).

For saturation experiments, rings (one per tube) of tail artery were incubated for 60 min with [^{3}H]-prazosin (0.05–8 nM) in 0.25 ml (final volume) buffer pH 7.4 (composition in mM: HEPES 8.3, NaCl 130, KCl 5.6, $CaCl_2$ 2, $MgCl_2$ 0.24 and glucose 11), maintained at 37±0.5°C and gassed with a mixture of 95% O_2, 5% CO_2. At the end of the incubation period each ring was washed in buffer solution (5 s), dried on filter paper, weighed and dissolved in 0.1 ml of a mixture of perchloric acid:H_2O_2 (1:1). Radioactivity of the tissue was determined by liquid scintillation spectrometry with a counting efficiency of 60%. Non-specific binding was defined as that insensitive to 100 μM phentolamine.

Competition studies were carried out employing 0.25 ml buffer with a single concentration of [^{3}H]-prazosin (2–3 nM) in the presence of increasing concentrations of WB-4101 (10 pM–30 μM). Incubation and assay conditions were the same as those described for saturation studies. The binding experiments were always performed in triplicate.

Data analysis

Pragmatic logistic curve fitting: Each individual set of E/[A] curve data was fitted to a logistic function of the form:

$$E = \frac{\alpha[A]^m}{[EC_{50}]^m + [A]^m} \quad (1)$$

in which E and [A] are the pharmacological effect and the concentration of agonist, respectively; α, EC_{50} and m are the asymptote, location and slope parameters, respectively. Location parameters were actually estimated as negative logarithms (pEC_{50}, i.e., the concentration required to cause 50% of the maximum response).

Experimental points and results from pragmatic logistic curve fitting are expressed as mean±s.e.mean; *n* is the number of rings for contractile studies and the number of experiments performed in triplicate in the inositol phosphates and binding assays and is indicated in the legends of the figures. Contractile

responses are expressed either as a percentage of the E_{max} (maximum contraction expressed in mN) of the control curve or as absolute value (mN).

Accumulation of [^{3}H]-inositol phosphates was calculated as the percentage of [^{3}H]-inositol labelled lipids in each individual sample to correct for interexperimental variations in labelling and sample size. The statistical significance of the observed differences was assessed by the Student's two-tailed t test for paired or unpaired (contractile responses of noradrenaline *vs* St-587) observations. In all cases significance was set at a P value of less than 0.05.

Ligand binding data were analyzed with computerized curve-fitting programs as described by McPherson (1983). The dissociation constant (K_D) and the total number of binding sites (B_{max}) were estimated from saturation experiments. The measured K_D of [^{3}H]-prazosin for α_1-adrenoceptor was subsequently used for analysis of the competition data. The affinity constants for WB-4101 were estimated from the results of displacement experiments. Data from competition curves were analysed with both one-site and two-sites models, and the F-test analysis was used to decide whether a model of one or two binding sites was more appropriate ($P<0.05$). K_i values for the high-affinity (K_{high}) and low-affinity (K_{low}) binding sites are expressed as negative logarithms (pK_i).

Drugs and isotopes

(−)-Noradrenaline bitartrate, acetylcholine HCl, yohimbine HCl, (±)-propranolol HCl, lithium chloride, *myo*inositol, desipramine HCl and normetanephrine HCl were purchased from Sigma Chemical Co; [^{3}H]-*myo*inositol and [^{3}H]-prazosin from Amersham International; St-587 (2-(2-chloro-5-trifluormethyl-phenylimino)-imidazoline) from Boehringher Ingelheim; nifedipine from Bayer, phentolamine mesylate, chloroethylclonidine HCl and WB-4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane) from Research Biochemical Incorporated (RBI). All drugs were prepared in physiological salt solution except noradrenaline which was prepared in 23 μM Na_2EDTA (contractile studies) or 0.1% ascorbic acid (inositol phosphate assay); nifedipine was diluted in ethanol, the final ethanol concentration was always lower than 0.01%. All other chemicals used were of analytical grade.

Results

Contractile studies

Noradrenaline and St-587 contracted in a concentration-dependent manner the tail artery rings (Figure 1). St-587 showed a potency (pEC_{50}: 6.16±0.08) and a maximum contraction (E_{max}: 17.82±1.39 mN, $n=20$) similar to those observed with noradrenaline (pEC_{50}: 6.43±0.14; E_{max}: 21.02±1.78 mN; $n=20$). However, the slope of the E/[A] curve was different between agonists (St-587: 1.07±0.18; noradrenaline: 0.62±0.10; $n=20$, $P<0.05$).

Nifedipine (1 and 10 μM) produced a concentration-related inhibition of noradrenaline- and St-587-induced contractions (Figure 2). With the concentrations used, nifedipine shifted to the right the E/[A] curves to noradrenaline (pEC_{50}: 6.13±0.10 (control), 5.46±0.13 (nifedipine 1 μM) and 5.28±0.41 (nifedipine 10 μM; $P<0.01$), $n=6$). The E_{max} of the noradrenaline-induced contraction was slightly modified by the lowest concentration of nifedipine. To reach a 40% inhibition of the maximum contraction ($P<0.01$), a concentration of 10 μM nifedipine was necessary (Figure 2a). However, when the agonist used was St-587, nifedipine depressed by 50% (1 μM; $P<0.001$) and by 75% (10 μM; $P<0.001$) the E_{max} (Figure 2b). The St-587 E/[A] curve was also displaced to the right (pEC_{50} values: 6.38±0.08 (control), 6.03±0.14 (nifedipine 1 μM), 5.81±0.29 (nifedipine 10 μM; $P<0.05$), $n=5$), by the calcium antagonist. No difference was observed in agonist-induced E/[A] curves in the presence of the vehicle (results not shown).

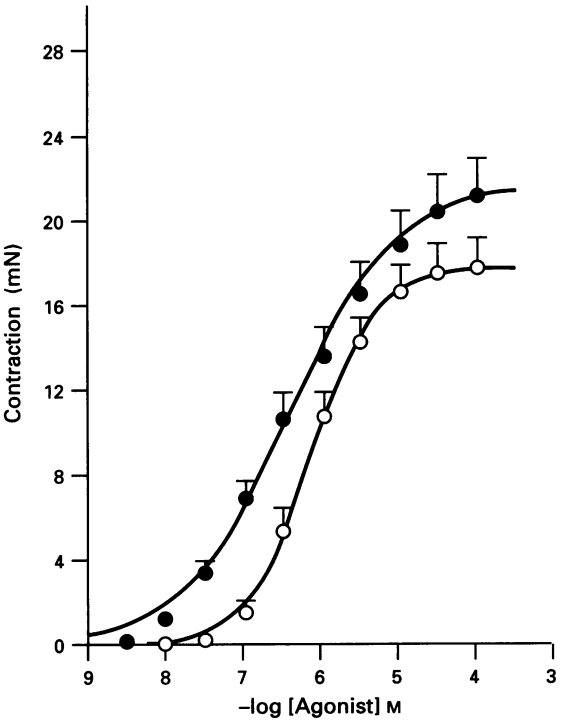

Figure 1 Concentration-response curves for noradrenaline (●) and St-587 (○). The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Each point represents the mean of 20 experiments; s.e.mean is shown by the vertical lines.

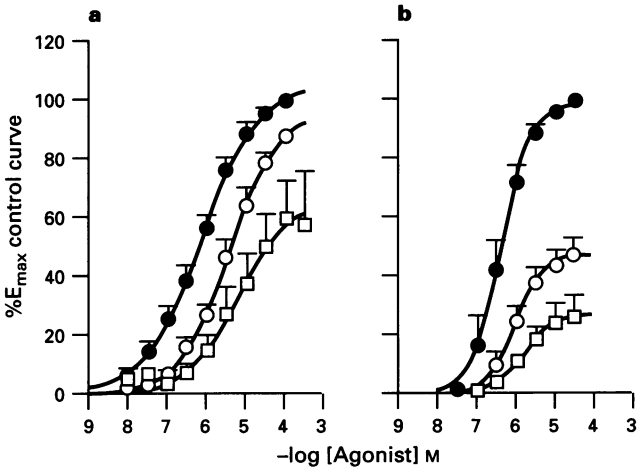

Figure 2 Concentration-response curves for noradrenaline- (a) and St-587- (b) induced contraction in tail artery before (●) and after incubation with 1 μM (○) and 10 μM (□) nifedipine. The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Each point represents the mean of 5–6 experiments; s.e.mean is shown by the vertical lines.

Inositol phosphates assay

To test the role of intracellullar calcium release in noradrenaline- and St-587-mediated contractile responses, the ability of the agonists to induce the accumulation of inositol phosphates was studied. St-587 and noradrenaline induced inositol phosphates accumulation in a concentration-related manner (Figure 3). Unstimulated inositol phosphates accumulation did not differ between agonists (see legend of Figure 3) but the maximal effect obtained with St-587 (42.25±2.21%

[^{3}H]-inositol lipids, $n=4$) was significantly lower ($P<0.05$) than that of noradrenaline (79.32±0.36% [^{3}H]-inositol lipids, $n=4$). The potencies exhibited by the two α_1-adrenoceptor agonists (noradrenaline: 6.42±0.07, $n=4$; St-587: 6.47±0.31, $n=4$) were similar.

[^{3}H]-prazosin binding assay

To evaluate if the differences observed in contraction between the agonists could be explained by the presence of α_1-adrenoceptor subtypes, binding experiments were performed. First of all, saturation studies were carried out to determine the K_D and the B_{max} values in our experimental conditions. In this way, we observed that [^{3}H]-prazosin labelled a homogeneous population of binding sites in rings of tail artery. [^{3}H]-prazosin binding was specific, saturable and showed high affinity. The specific binding represented approximately 50–60% of the total binding at a concentration of [^{3}H]-prazosin close to its K_D. Nonlinear regression analysis of the saturation data was consistent with the presence of a single population of sites. The derived K_D and B_{max} values were 4.44±0.46 nM and 36.35±4.22 fmol mg^{-1} tissue ($n=10$), respectively.

To determine the possible existence of α_1-adrenoceptor subtypes, competition binding studies with the reversible discriminating drug, WB-4101, were performed. This compound displaced [^{3}H]-prazosin binding in a concentration-dependent manner. The WB-4101 competition curves generated were shallow with a pseudo-Hill coefficient that deviated significantly from unity, and could be better described by assuming the presence of two [^{3}H]-prazosin binding sites (%R_{high}: 58.3±2.4) for which this drug displayed different affinities (pK_{high}: 8.95±0.37 and pK_{low}: 6.07±0.37; $n=13$). These sites could indicate the existence of a heterogeneous population of α_1-adrenoceptors.

CEC alkylation studies

Since competition binding studies indicate the existence of two different α_1-adrenoceptor binding sites, we were interested in testing to what extent nifedipine would affect the E/[A] curve after irreversible inactivation of α_{1B}-adrenoceptors with CEC. The effect of nifedipine on contraction by either agonist in the presence of CEC was studied (Figure 4). Nifedipine (1 μM) influenced to a greater extent the St-587-(Figure 4b) than the noradrenaline- (Figure 4a) induced contraction. The potencies exhibited by the two compounds in the presence of CEC were similar (noradrenaline: 5.60±0.05, $n=5$; St-587: 5.69±0.03, $n=4$). The presence of vehicle (ethanol) did not modify the second agonist E/[A] curve.

Discussion

Noradrenaline is a full α_1- and α_2-adrenoceptor agonist but St-587 has been described as an α_1-adrenoceptor agonist with different activity depending on the tissue studied. In the epididymal portion of the rat vas deferens (Badia & Sallés, 1989) and in the guinea-pig and rat aorta (Beckeringh *et al.*, 1984; Tabernero *et al.*, 1996) St-587 acts as a partial agonist but in the rat anococcygeus muscle (Vila *et al.*, 1984) and tail artery (Tabernero *et al.*, 1996) it behaved as a full agonist. The different behaviour of St-587 in tail artery and aorta observed previously in our laboratory was ascribed to the existence of a greater α_1-adrenoceptor reserve for contraction in tail artery (Tabernero *et al.*, 1996). In the present study, both noradrenaline and St-587 contracted the tail artery in a concentration-related manner. Confirming our previous results, St-587 behaved as a full agonist showing a maximum contractile effect close to that of noradrenaline. However, in each single experiment we observed that the development of contraction was faster when induced by noradrenaline than by St-587 (results not published) and that the slope of the E/[A] curve between agonists was significantly different. Since some (Weiss *et al.*, 1983; Tsai *et al.*, 1993; Vila *et al.*, 1993) but not all (Atkinson *et al.*, 1988) studies have suggested that α_1 and α_2-adrenoceptors are present in rat tail artery one could speculate that the differences observed between agonists could be due to

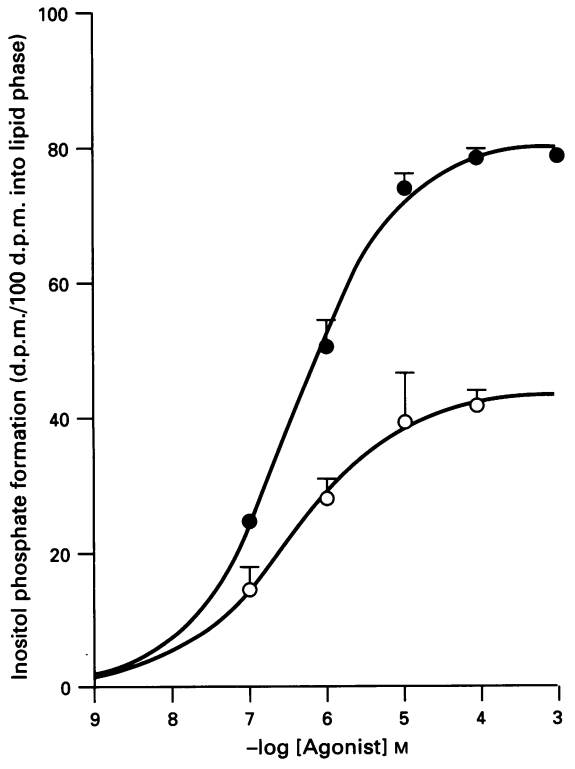

Figure 3 Concentration-response curves for noradrenaline- (●) and St-587- (○) induced accumulation of [^{3}H]-inositol phosphate formation in tail artery. The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Results are the mean of 4 experiments performed in triplicate; s.e.mean is shown by the vertical lines. Basal [^{3}H]-inositol phosphates average 3.90±0.63% [^{3}H]-lipids (noradrenaline) and 5.30±0.80% [^{3}H]-lipids (St-587).

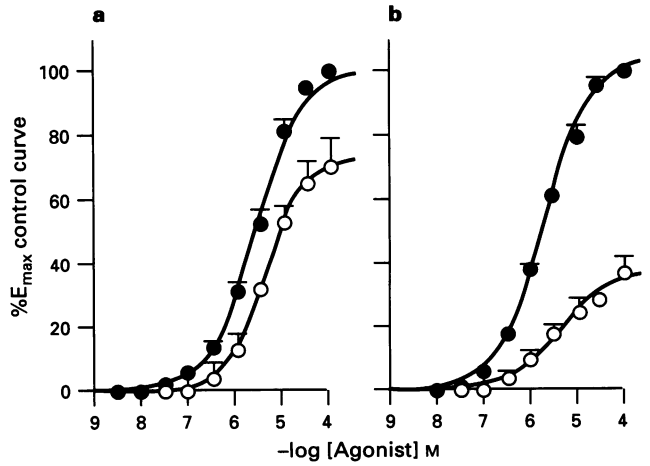

Figure 4 Effect of 1 μM nifedipine (○) on (a) noradrenaline- (●) and (b) St-587- (●) induced contraction after chloroethylclonidine pretreatment. The lines drawn through the data are the results of pragmatic logistic fitting (see Methods). Each point represents the mean of 4–5 experiments; s.e.mean is shown by the vertical lines.

a different interaction with either α_1 or α_2-adrenoceptor subtypes. This possibility should be excluded because all our experiments were carried out in the presence of yohimbine (0.1 μM) to prevent the stimulation of α_2-adrenoceptors by the agonists. Furthermore, we have also seen that E/[A] curves to St-587 and noradrenaline were inhibited by prazosin (3, 10 and 30 nM, results not published). Thus we can assume that the contractile responses obtained by both agonists are mediated by α_1-adrenoceptors.

Sulpizio & Hieble (1991) showed that the contractile responses induced by field stimulation in the perfused caudal artery occurred in two phases: an initial rapidly developing contraction (phasic) and a subsequent slowly developing contraction (tonic). These authors concluded that both phases were mediated by a single α_1-adrenoceptor population linked to different sources of calcium. We would like to point out that in tail artery rings we have been unable to differentiate between phasic and tonic contractile phases in response to α_1-adrenoceptor agonists. A study carried out in single isolated tail artery cells (Alexander & Cheung, 1994) showed that noradrenaline as well as caffeine induced a biphasic increase in the cytosolic free Ca^{2+} level similar to that obtained in other smooth muscle cells (Pacaud *et al.*, 1992). The transient increase is due to release of intracellular Ca^{2+} stores while the tonic increase is due to influx of extracellular Ca^{2+}. Alexander & Cheung (1994) observed a positive correlation between the transient cytosolic Ca^{2+} increase and the shortening of the cells. All of this led us to speculate that the differences in slope observed between the noradrenaline and St-587 E/[A] curves could be due to a different degree of participation of intra- and extracellular calcium mechanisms in the contractile responses either *via* a single or two different α_1-adrenoceptor subtypes.

Nifedipine, which blocks specifically voltage-dependent L-type Ca^{2+} channels on the surface cells, was used to analyse the participation of extracellular calcium in the agonist-induced contraction. The calcium antagonist exhibited a different effect on contraction induced by noradrenaline and St-587. In presence of nifedipine (1 μM), the maximal contraction by St-587 was decreased by 50%. When the concentration of the antagonist was 10 times higher, St-587 reached less than 30% of the maximum contractile effect obtained in the absence of the antagonist. In contrast, 10 μM nifedipine was necessary to obtain a 40% decrease in the contraction to noradrenaline. The results obtained with noradrenaline correlate with those observed in single cells (Alexander & Cheung, 1994) where nifedipine (1 μM) was unable to modify either the contraction or the Ca^{2+} levels induced by caffeine, a substance that uses the same calcium sources as noradrenaline. However, in the perfused artery rings nifedipine (0.1 μM) blocked the tonic (48%) but not the phasic phase of the contraction induced by field stimulation (Sulpizio & Hieble, 1991). In addition, we have previously observed that the contractile response to 10 μM noradrenaline does not seem to depend heavily on extracellular calcium (Vila *et al.*, 1993) results that concur with those from the present study.

To evaluate the role of intracellular calcium in the contraction to both α_1-adrenoceptor agonists, the ability to accumulate inositol phosphates was studied. It is generally accepted that agonists have a smaller potency for inositol phosphates formation than for contraction. However, in our study the sensitivity for noradrenaline- and St-587-induced contraction was very close to the potency exhibited for inositol phosphates accumulation. This result confirms and extends our previous observations (Tabernero *et al.*, 1996) that α_1-adrenoceptors in rat tail artery rings are very well coupled to phosphatydylinositol turnover. Nevertheless, St-587 showed a similar potency but smaller efficacy for inositol phosphates formation than noradrenaline. This result indicates that St-587 behaves as a full α_1-adrenoceptor agonist for contraction but as a partial α_1-adrenoceptor agonist for inositol phosphates formation.

The results obtained with nifedipine together with those of inositol phosphates accumulation indicate that the two agonists depend on both intra- and extracellular Ca^{2+} for contraction but the participation of each mechanism varies depending on the agonist used. Thus, the contractile effects of St-587 rely more on the influx of extracellular calcium and in contrast noradrenaline-induced contraction utilizes predominantly intracellular calcium *via* inositol phosphates turnover.

Binding studies have repeatedly demonstrated the existence of two α_1-adrenoceptor binding sites (Morrow & Creese, 1986; Han *et al.*, 1987; Minneman *et al.*, 1988). In addition a third subtype of α_1-adrenoceptors (α_{1D}) has been pharmacologically described (Saussy *et al.*, 1994). The two binding sites designated as α_{1A} and α_{1B} by Morrow & Creese (1986), were based on the binding characteristics of two different α_1-adrenoceptor ligands. Several authors have proposed that both sites are linked to different second messenger systems (Han *et al.*, 1987; Michel *et al.*, 1990). Thus, the α_{1A} site would be linked to the opening of calcium channels which allows the influx of extracellular calcium and the α_{1B} site would be coupled to the formation of inositol trisphosphate which promotes the release of calcium from the sacroplasmic reticulum. However, to our knowledgement, the signal transduction mechanisms linked to stimulation of α_{1D}-adrenoceptors remains unknown.

Our binding studies with intact rings have demonstrated two α_1-adrenoceptor recognition sites with a similar density to that previously observed in membranes of the same tissue (Minneman *et al.*, 1988). Nevertheless, the difference in the pK_i values between the two binding sites observed in our study is greater than the difference obtained for WB-4101 (Michel *et al.*, 1995). We do not have a clear explanation for these results but it could be partly due to the use of intact tissue. The existence of two different binding sites, α_{1B} and α_{1A} or α_{1D}, together with the observed differences in calcium utilization for contraction by St-587 and noradrenaline could indicate that each agonist acts predominantly on one of the α_1-adrenoceptor subtypes. In this case, noradrenaline could act predominantly on α_{1B}-adrenoceptors and St-587 could have a greater affinity for α_{1A} or α_{1D}-adrenoceptors. Nevertheless, contraction induced by either agonist in the presence of CEC does not support this hypothesis. Once the population of α_{1B}-adrenoceptors has been substantially decreased by CEC, a selective irreversible α_{1B}-adrenoceptor antagonist, one would expect that the contraction induced by noradrenaline and St-587 would be blocked similarly by nifedipine. However, inhibition of the St-587 contractile response was again more affected than the contraction induced by noradrenaline indicating that the sources of calcium are not directly linked to the two α_1-adrenoceptor subtypes. Our results seem to correlate with those from previous studies on continuous cell lines showing that the α_{1B}, $\alpha_{1A/D}$, and α_{1A}-adrenoceptors can couple to inositol phosphates formation and to voltage-gated Ca^{2+} influx (Esbenshade & Minneman, 1995).

In summary, we have presented evidence that the contraction induced by α_1-adrenoceptor agonists can utilize Ca^{2+} from intracellular (via inositol phosphate turnover) and extracellular sources. However, the participation of each Ca^{2+} mechanism varies depending on the agonist used. In addition, we have also demonstrated the presence of two α_1-adrenoceptor binding sites that seem to be unrelated to the calcium sources used for contraction.

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