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SELECTIVE α_1-AND α_2-ADRENOCEPTOR AGONIST-INDUCED CONTRACTIONS AND ^{45}Ca FLUXES IN THE RAT ISOLATED AORTA

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1 Contractile responses produced by the α_1-adrenoceptor selective agonist, phenylephrine, and the α_2-adrenoceptor selective agonists, oxymetazoline and clonidine, have been compared to those produced by noradrenaline (non selective) in the rat aorta.

2 The relative order of potency of the agonists was noradrenaline > phenylephrine > clonidine > oxymetazoline. Noradrenaline and phenylephrine produced similar maximal responses. The maximal responses produced by oxymetazoline and clonidine were about 59% and 24% respectively of those produced by noradrenaline.

3 Concentrations of agonists producing maximal contractions exhibited different response-time relationships. Responses to noradrenaline and phenylephrine were biphasic while responses induced by oxymetazoline and clonidine were monophasic.

4 In calcium-free solution, contractions stimulated by oxymetazoline and clonidine were almost abolished while those stimulated by noradrenaline and phenylephrine were reduced by about 60–70%. The calcium entry blocker, cinnarizine, almost completely inhibited responses to oxymetazoline and clonidine and reduced noradrenaline- and phenylephrine-stimulated responses by about 60%.

5 All the agonists stimulated the uptake of ^{45}Ca into the La^{3+}-resistant Ca^{2+} fraction of the artery but only noradrenaline and phenylephrine stimulated the efflux of ^{45}Ca into calcium-free solution. The ^{45}Ca uptake stimulated by oxymetazoline and clonidine was abolished by cinnarizine and that stimulated by noradrenaline and phenylephrine was reduced by about 85%.

6 It is concluded that clonidine and oxymetazoline stimulate contractions that are totally dependent on extracellular calcium. Noradrenaline and phenylephrine stimulate contractions that are partly dependent on extracellular calcium and partly dependent on intracellular calcium stores.

Introduction

Recently, evidence has been presented for the presence of both α_1- and α_2-adrenoceptor subtypes in the plasma membrane of vascular smooth muscle, stimulation of both types by noradrenaline mediating vasoconstriction (Docherty, MacDonald & McGrath, 1979; Timmermans, Kwa & van Zwieten, 1979; Yamaguchi & Kopin, 1980; De Mey & Vanhoutte, 1981). In the pithed rat, Van Meel, De Jonge, Wilffert, Kalkman, Timmermans & van Zwieten (1981) have shown that contractions of vascular smooth muscle by stimulation of α_1- but not α_2-adrenoceptors are resistant to the calcium entry blocking drugs verapamil, D600 and nifedipine, suggesting that α_1-adrenoceptor-stimulated contractions are mostly mediated by the release of intracellularly stored calcium while α_2-adrenoceptor-stimulated contractions are dependent on extracellular calcium. De Mey & Vanhoutte (1981) working with isolated arteries and veins of the dog have reached the opposite conclusion. In view of these conflicting reports we have investigated the effects of the relatively selective α-adrenoceptor agonists, phenylephrine (α_1) and oxymetazoline and clonidine (α_2) compared to noradrenaline (α_1 and α_2) in the rat isolated aorta preparation where the α-adrenoceptors have been classified as α_2 on the basis of their relative affinity for clonidine and yohimbine (Ruffolo, Yaden & Waddell, 1980 Ruffolo, Waddell & Yaden, 1981), and where the contraction stimulated by noradrenaline consists of phasic and tonic components which reflect a release of intracellularly stored calcium and an influx of extracellular calcium respectively (Godfraind & Kaba, 1972).

The results show that the α_2-adrenoceptor selective agonists, oxymetazoline and clonidine, stimulate contractions that are totally dependent on extracellu-

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lar calcium while contractions stimulated by noradrenaline and phenylephrine are partly dependent on extracellular calcium and partly dependent on intracellular calcium.

Methods

Twelve to fifteen week-old female Wistar rats (weighing about 240 g) were killed by decapitation and the thoracic aorta removed and cleaned of all loosely adherent tissue. Rings of aorta about 2 mm wide were cut from close to the aortic arch (Godfraind, 1979) and suspended in 50 ml organ baths under a tension of 2 g. The physiological solution (composition mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25 and glucose 11.5) was maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2. Calcium-free solution was prepared by omission of calcium when required. Contractile responses were measured with an isometric strain gauge coupled to a potentiometric pen recorder. After an equilibration period of 60 min the artery preparations were contracted maximally either in a depolarizing medium (composition mM: NaCl 17, KCl 100, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25 and glucose 11.5) or by noradrenaline 1 μM, washed and allowed a further 60 min period of equilibration. Cumulative concentration-effect curves to the α-adrenoceptor agonists noradrenaline, phenylephrine, oxymetazoline and clonidine were then obtained by increasing the bath concentration in approximately 3 fold steps, allowing equilibration at each concentration. After obtaining a maximal response the preparations were washed until baseline tension was regained and then incubated with the calcium entry blocking drug, cinnarizine (Godfraind, Kaba & Rojas, 1973; Godfraind, 1974) (0.1 to 10 μM) for 90 min before a second cumulative concentration-effect curve was obtained. Control experiments, omitting cinnarizine, were performed at the same time.

In other experiments contractions were obtained to single concentrations of α-adrenoceptor agonists or by depolarization in place of cumulative drug additions using the same protocol. To obtain contractions in a calcium-free medium, artery preparations were equilibrated for 60 min after exposure to noradrenaline 1 μM and then contracted by 1 μM of α-adrenoceptor agonist under test. After washing and a further 60 min equilibration period the preparations were washed 3 times over a 10 min period with a calcium-free solution (containing 0.2 mM EGTA) before an α-adrenoceptor agonist contraction was elicited. This treatment is sufficient to inhibit completely contractile responses to depolarization (Godfraind & Kaba, 1969).

In some experiments, these preparations were further washed in normal physiological solution for 60 min before being equilibrated with cinnarizine 3 μM for 90 min. At this time a second contraction in calcium-free solution was obtained.

Measurement of ^{45}Ca influx and efflux

The net rate of uptake of calcium into the smooth muscle cells of the rat aorta stimulated by the α-adrenoceptor agonists, noradrenaline, phenylephrine, oxymetazoline and clonidine or by depolarization was estimated by measuring the increase in ^{45}Ca content of the smooth muscle produced by these agonists during exposure of the tissue to a ^{45}Ca-containing physiological solution. Lanthanum, which has been shown to displace extracellular calcium while having little or no effect on the intracellular content of calcium under certain conditions (van Breemen, Farinas, Gerba & McNaughton, 1972; Godfraind, 1974; 1976) was used to remove the relatively large amount of ^{45}Ca in the extracellular space which would otherwise interfere with the determination of cellular ^{45}Ca content, using the method of Godfraind (1976).

The arteries were cut open longitudinally to form flat strips weighing about 6–11 mg and equilibrated for at least 60 min in physiological solution (composition mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 11) maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2. After a preincubation period of 90 min in physiological solution containing cinnarizine (0.1 to 10 μM) the artery strips were further incubated for 5 min in 50 ml of physiological solution containing ^{45}Ca (1 μCi/ml) as well as cinnarizine, then for another 2 min in the same solution with the addition of the α-adrenoceptor agonist under test or for 2 min or 35 min in a depolarizing solution (composition mM: NaCl 27, KCl 100, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 11) also containing cinnarizine. Thereafter, the preparations were washed for 5 min in 500 ml of a La^{3+} solution (composition mM: NaCl 122, KCl 5.9, $MgCl_2$ 1.25, $LaCl_3$ 50, glucose 11 and Tris maleate 15 (pH 6.8)) to remove extracellular Ca^{2+} from the tissue. Parallel control experiments, omitting cinnarizine, were always performed at the same time. After the La^{3+} wash, the artery strips were placed between two sheets of filter paper and pressed three times with a roller weighing 350 g. Each strip was weighed, dissolved in 0.1 ml of a solution composed of equal parts of perchloric acid (37% w/v) and H_2O_2 (30 vol) by heating for 15 min at 75°C. After cooling, 5 ml of Aqualuma (Lumac) was added and the radioactivity of the samples counted in a liquid scintillation counter as usual with appropriate controls. The results of each determina-

tion have been converted to the apparent tissue content of Ca (mol/kg wet wt), Godfraind (1976).

Noradrenaline- phenylephrine- and clonidine-dependent ^{45}Ca efflux was estimated in artery strips after they had been preincubated in ^{45}Ca (2 μCi/ml) containing physiological solution for 120 min. Tissues were then rinsed for 5 min in non-radioactive Ca^{2+}-free physiological solution containing 0.2 mM EGTA before being transferred to non-radioactive Ca^{2+}-free physiological solution also containing 0.2 mM EGTA and either noradrenaline, phenylephrine or clonidine (1 μM) for a further 2 min, after which they were placed in lanthanum containing solution and treated as described for the ^{45}Ca influx experiments. Control preparations were rinsed in non-radioactive solution for 7 min. Similar efflux experiments were carried out in the presence of cinnarizine 1 μM and 5 μM. Ca^{2+}-free solution was prepared by the omission of calcium. In all ^{45}Ca efflux experiments equal numbers of control and agonist-treated preparations were always processed at the same time.

Drugs

Noradrenaline bitartrate (Flucker) was dissolved in distilled water containing 7.9 mM Na_2SO_3 and 34 mM HCl as a stock solution of 10 mM. Phenylephrine HCl (Sterling-Winthrop) oxymetazoline HCl (Merck) and clonidine HCl (Boehringer) were prepared each day as 10 mM stock solutions dissolved in distilled water. Cinnarizine (Janssen Pharmaceutica) was dissolved in an aqueous solution of 100 mM tartaric acid (pH 3.1) to a concentration of 1 mM.

Ethyleneglycol-bis-(β-amino-ethyl ether) *N, N'*-tetraacetic acid (EGTA) was dissolved in distilled water to a concentration of 100 mM buffered with tris-(hydroxymethyl)-aminomethane to pH 7.4. ^{45}Ca (specific activity about 2 mCi/μmol) was obtained from the Radiochemical Centre, Amersham. Further drug dilutions were prepared in distilled water as required. All drug concentrations are expressed in terms of the base.

Statistical analysis

The data are expressed as means ± s.e.mean. In the case of experiments involving ^{45}Ca, the number of observations (n) applies to both control and non-control groups. Tests of significance have been made using Student's t test, or paired t test where possible, P values less than 0.05 being considered significant. A least squares linear regression analysis has been used to fit straight lines to data where appropriate. The concentration of an agonist producing 50% of the maximal response for that agonist (EC_{50} value) and the concentration of cinnarizine producing 50% of the maximal reduction of the contractile response (IC_{50}) or of the maximal agonist-dependent ^{45}Ca influx (I_{50}) and their s.e. were estimated from concentration-effect curves.

Results

Noradrenaline, phenylephrine, oxymetazoline and clonidine produced concentration-related increases in tension of the rat isolated aorta preparation when the drug concentration was increased cumulatively in the organ bath (Figure 1). Respective concentrations producing 50% of the individual maximal responses (EC_{50} values) were noradrenaline $2.9 \pm 0.4 \times 10^{-8}$ M ($n = 22$), phenylephrine $4.7 \pm 0.5 \times 10^{-8}$ M ($n = 17$), clonidine $8.8 \pm 0.8 \times 10^{-8}$ M ($n = 16$), oxymetazoline $7.7 \pm 1.4 \times 10^{-7}$ M ($n = 10$). The relative order of potency of these drugs is therefore noradrenaline > phenylephrine > clonidine > oxymetazoline.

Noradrenaline and phenylephrine produced similar maximal contractions; however, the maximal contractions produced by oxymetazoline and clonidine were significantly smaller, amounting to 58.5 ± 5.2% and 23.8 ± 4.6% respectively of the maximal contraction produced by noradrenaline (Figure 1).

When added to the organ bath at a single final concentration of 1 μM, noradrenaline, phenylephrine, oxymetazoline and clonidine all produced maximal contractions of the rat aorta which were not significantly different from the contractions achieved by the same concentrations when added to the organ bath by cumulative addition. Single concentrations of

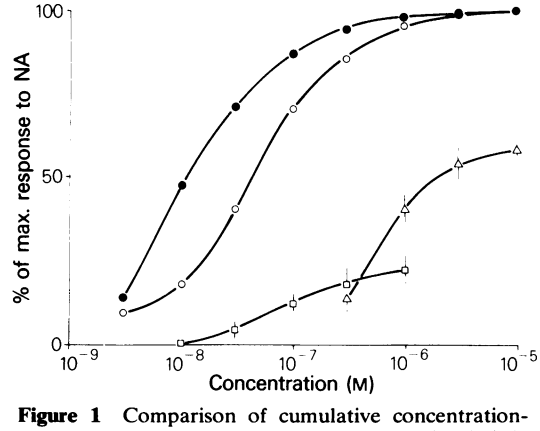

Figure 1 Comparison of cumulative concentration-effect curves stimulated by noradrenaline (NA) (●), phenylephrine (○), oxymetazoline (△) and clonidine (□) in the rat aorta. Responses are expressed as a percentage of the maximal response produced by noradrenaline which was 2.2 ± 0.2 g developed tension. Each curve is the mean of at least 6 observations. Vertical lines indicate standard errors where they exceed the size of the symbol.

10 μM of noradrenaline, phenylephrine and clonidine produced maximal contractions which were not significantly different from those produced by single concentrations of 1 μM. In the case of oxymetazoline, a final concentration of 10 μM achieved as a single concentration increase, produced a significantly smaller maximal contraction ($0.02 < P < 0.05$, unpaired t test) than did 1 μM oxymetazoline. Cumulative addition of 10 μM oxymetazoline final concentration to the organ bath produced a maximal contraction of $58.5 \pm 5.2\%$ ($n = 12$) of that produced by noradrenaline and significantly increased the degree of contraction over that produced by cumulative additions to 1 μM oxymetazoline ($P < 0.001$, paired t test) (Figure 1). Prominent spontaneous contractile activity was frequently superimposed on contractions produced by 1 μM oxymetazoline but was never observed associated with contractions produced by clonidine.

The contraction produced by noradrenaline 1 μM was characterized by 2 phases. An initial phasic response consisting of a rapid rise in tension and amounting to $39.1 \pm 3.2\%$ ($n = 14$) of the maximal tension attained was followed by a secondary tonic phase when tension increased more slowly. Maximal tension was reached in about 20–25 min. Phenylephrine 1 μM produced a maximal contraction which was also biphasic and of similar magnitude to that produced by noradrenaline 1 μM ($100.3 \pm 4.8\%$ $n = 29$), the initial phasic contraction being $44.0 \pm 3.4\%$ ($n = 14$), of the total contraction (Figure 2). Maximal contractile tension was achieved in about 10 min. Single 1 μM concentrations of oxymetazoline and clonidine produced maximal con-

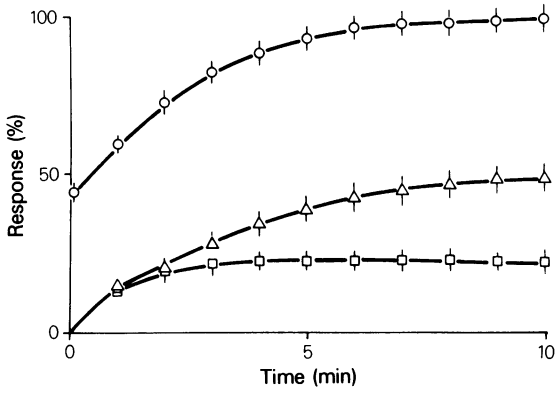

Figure 2 Response time curves stimulated by 1 μM phenylephrine (○), oxymetazoline (△) and clonidine (□) in rat aorta. Responses are expressed as a percentage of the maximal response produced by 1 μM of noradrenaline. Each curve is the mean of at least 14 observations. Vertical lines indicate standard errors.

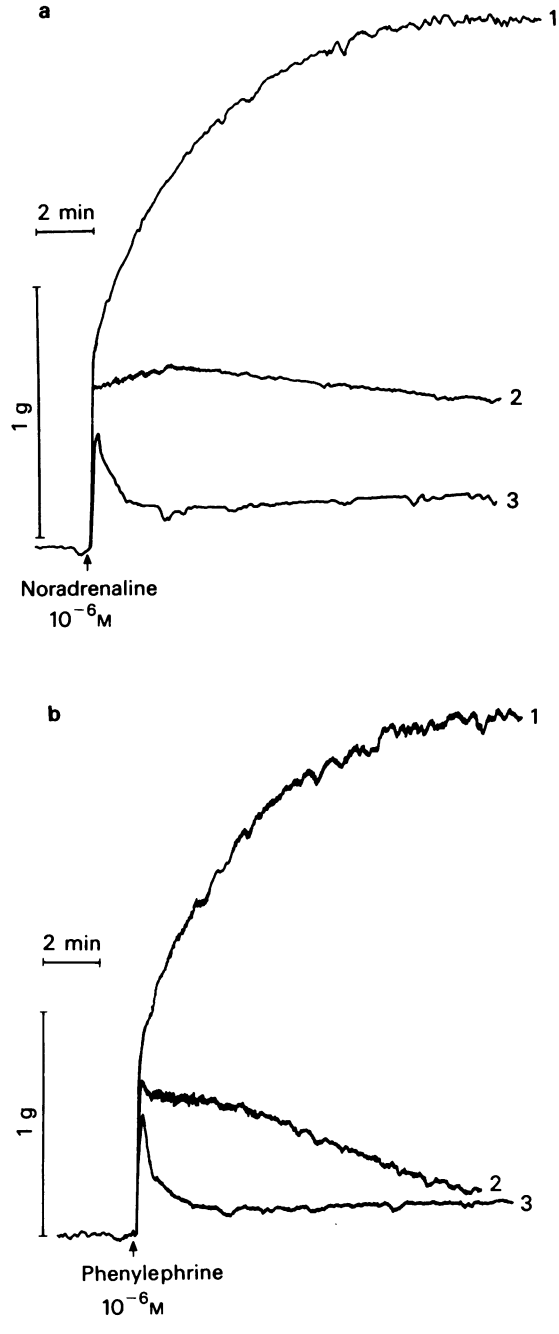

Figure 3 Representative traces showing responses induced by 1 μM noradrenaline (a) and 1 μM phenylephrine (b) in rat aorta. Response (1) was produced in normal physiological solution, response (2) was produced in calcium-free solution, and response (3) was produced in calcium-free solution after 90 min incubation with 3 μM cinnarizine.

tractions of markedly variable magnitude, being 48.6 ± 5.2% ($n = 14$, range 16.4 to 84.5%) and 24.1 ± 2.7% ($n = 47$, range 5.0 to 46.1%) of that produced by noradrenaline 1 μM. These contractions were monophasic and consisted of a tonic increase in tension which attained its maximum in about 10 min (Figure 2).

In Ca^{2+}-free physiological solution, contractions produced by oxymetazoline 1 μM and clonidine 1 μM were respectively reduced to 6.0 ± 1.4% ($n = 5$) and 9.0 ± 3.6% ($n = 6$) of their maximal response in normal physiological solution. That is, they were reduced to about 3% and 2% of the maximal contraction produced by noradrenaline 1 μM in normal physiological solution. Noradrenaline 1 μM and phenylephrine 1 μM produced phasic contractions in Ca^{2+}-free physiological solution of 28.8 ± 5.4% ($n = 5$) and 39.7 ± 4.3% ($n = 5$) respectively of their maximal contractions in normal physiological solution. The contractions produced by noradrenaline (Figure 3a, trace 2) were sustained over at least the next 25 min (data not shown) while the phenylephrine-induced contractions relaxed rapidly over 10 min to about 30% of their initial phasic response (Figure 3b, trace 2). In the presence of the calcium entry blocking drug, cinnarizine (3 μM), contractions produced by 1 μM noradrenaline and phenylephrine were similar and consisted of a rapid phasic contraction which quickly relaxed to a stable plateau tension of about 28% of the initial phasic response over about 3 min (Figure 3a and b, trace 3).

In normal physiological solution cinnarizine (0.01 to 10 μM) depressed contractions produced by 1 μM noradrenaline, phenylephrine, oxymetazoline and clonidine in a concentration-dependent manner (Table 1). Maximal inhibition of contraction was produced by cinnarizine 3 μM. The concentrations of cinnarizine producing 50% of the maximal inhibitory effect (IC_{50} values) were about 5×10^{-7} M (noradrenaline), 0.63×10^{-7} M (oxymetazoline), 2.4×10^{-7} M (phenylephrine) and 5.5×10^{-7} M clonidine).

K^+-depolarization of arterial preparations produced a maximal contraction of 97.1 ± 9.0% ($n = 11$) of that produced by 1 μM noradrenaline. The depolarization induced contractions were depressed by cinnarizine (0.01 to 10 μM) in a concentration-dependent manner and there was an increase in the degree of inhibition of contraction with time. Maximal inhibition of contraction (expressed as a percentage of the maximal contraction produced in the absence of cinnarizine) was produced by cinnarizine 10 μM which completely abolished depolarization-induced responses. The IC_{50} values (Figure 4) were $1.9 \pm 0.9 \times 10^{-7}$ M when measured 2 min after initial depolarization and $2.5 \pm 1.1 \times 10^{-8}$ M ($n = 3$) when measured 35 min after initial depolarization.

^{45}Ca uptake

The four agonists noradrenaline, phenylephrine, oxymetazoline and clonidine at a concentration of 1 μM stimulated the uptake of ^{45}Ca into the lanthanum-resistant fraction of the rat aorta. ^{45}Ca uptake stimulated by phenylephrine was about equal to that produced by noradrenaline. ^{45}Ca uptake stimulated by oxymetazoline and clonidine was approximately 41.8% ($n = 12$) and 30.9% ($n = 6$) of that produced by noradrenaline (Table 2). Cinnarizine 10 μM had a small but significant depressant effect on this resting calcium exchange, reducing it from 75.5 ± 3.7 to 67.7 ± 1.0 μmol Ca^{2+}/kg wet weight but lower concentrations were ineffective. Cinnarizine (0.1 to 10 μM) produced a concentration-dependent inhibition of ^{45}Ca uptake into the lanthanum-resistant Ca^{2+} fraction stimulated by noradrenaline 1 μM and phenylephrine 1 μM. Maximal inhibition was obtained with 3 μM cinnarizine which reduced noradrenaline-stimulated ^{45}Ca uptake by about 85% ($n = 12$) and phenylephrine-stimulated ^{45}Ca uptake by about 88% ($n = 6$). ^{45}Ca uptake stimulated by oxymetazoline 1 μM and clonidine 1 μM was completely abolished by cinnarizine 3 μM.

The concentration of cinnarizine producing a 50% inhibition of ^{45}Ca uptake into the lanthanum-resistant Ca^{2+} fraction (I_{50} value) was about

Table 1 Effect of the calcium entry blocking drug, cinnarizine, on the maximal responses produced by 1 μM noradrenaline, phenylephrine, oxymetazoline or clonidine in the rat aorta

Cinnarizine (M)	*Response* (% of control ± s.e.mean) *Noradrenaline*	*Phenylephrine*	*Oxymetazoline*	*Clonidine*
0	100	100	100	100
3×10^{-8}			78.6 ± 8.0 (6)	66.6 ± 6.9 (5)
10^{-7}	84.6 ± 3.4 (7)	78.0 ± 9.3 (3)	37.3 ± 6.8 (5)	
3×10^{-7}		54.9 ± 5.5 (4)		47.5 ± 0.8 (2)
10^{-6}	56.3 ± 3.3 (6)			35.2 ± 2.0 (3)
3×10^{-6}	40.5 ± 4.5 (9)	36.4 ± 4.9 (7)	6.9 ± 1.4 (4)	8.1 ± 1.1 (3)

Values in parentheses represent number of observations.

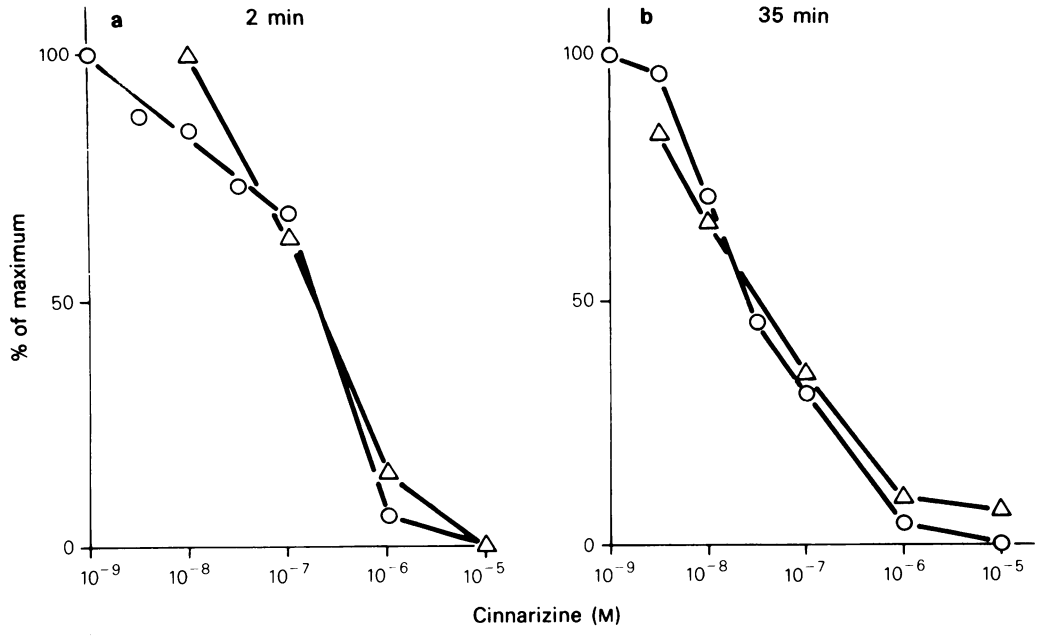

Figure 4 The depressant effect of cinnarizine on KCl depolarization-induced contractions (○) and KCl-dependent uptake of ^{45}Ca (△) in the rat aorta. Responses were measured after 2 min (a) and after 35 min (b) exposure to the depolarizing solution. The preparations were preincubated for 90 min in the presence of cinnarizine. Each point is the mean of at least 5 observations. The standard error at any point did no exceed 10% of the calculated mean.

7.0×10^{-7} M for noradrenaline and 1.7×10^{-7} M for phenylephrine.

K^+-depolarization of the arteries stimulated an enhanced uptake of ^{45}Ca which was depressed in a concentration-dependent manner by cinnarizine (0.01 to 10 μM). Maximal inhibition was obtained with cinnarizine 3 μM which reduced the ^{45}Ca uptake by about 100% after 2 min and by about 93% after 35 min. The I_{50} values (Figure 4) were $1.8 \pm 1.0 \times 10^{-7}$ M (2 min) and $3.8 \pm 1.4 \times 10^{-8}$ M (35 min) ($n = 6$). These I_{50} values for cinnarizine measured 2 min and 35 min after depolarization are similar to the IC_{50} values for cinnarizine measured at the same time intervals (Figure 4).

^{45}Ca efflux

The agonist-stimulated efflux of ^{45}Ca from the smooth muscle of the aorta was measured in Ca^{2+}-free EGTA containing solution to ensure that no agonist-stimulated influx of Ca^{2+} could displace intracellular ^{45}Ca and result in an overestimation of agonist-stimulated release of intracellular calcium. The effect of cinnarizine 1 and 5 μM on the Ca^{2+} efflux stimulated by noradrenaline 10 μM was also determined by including it in the incubating and washing solutions. Noradrenaline 1 μM and phenylephrine 1 μM stimulated the efflux of ^{45}Ca to about the same extent, reducing tissue content of

Table 2 Action of 1 μM noradrenaline, phenylephrine, oxymetazoline and clonidine on calcium influx in rat aorta, measured as changes in ^{45}Ca resistant to displacement by lanthanum

Tissue content of Ca^{2+} (μmol/kg wet wt. ± s.e.mean)

Control	73.2 ± 3.1	Noradrenaline	133.0 ± 8.1 (6)
Control	73.9 ± 2.3	Phenylephrine	133.4 ± 5.0 (10)
Control	75.5 ± 4.5	Oxymetazoline	101.1 ± 9.1 (10)
Control	82.2 ± 2.4	Clonidine	100.7 ± 4.7 (6)

Control calcium influx was measured after 7 min incubation in ^{45}Ca containing physiological solution. Stimulated calcium uptake was measured by including an agonist in the incubating solution during the last 2 min. Numbers in parentheses represent number of arteries.

exchangeable calcium from 169.4 ± 4.6 μmol Ca^{2+}/kg wet weight to 134.4 ± 6.0 and 142.9 ± 3.1 μmol Ca^{2+}/kg wet weight respectively. Clonidine 1 μM did not stimulate the efflux of ^{45}Ca at all. Noradrenaline 10 μM stimulated ^{45}Ca efflux to the same extent as noradrenaline 1 μM. Cinnarizine at 1 μM or 5 μM had no significant effect on the efflux of ^{45}Ca induced by noradrenaline 10 μM in Ca^{2+}-free solution.

Discussion

If only α_1-adrenoceptors were present in the rat aorta then the expected order of potency of the α-adrenoceptor agonists used in this study should be phenylephrine > noradrenaline > oxymetazoline > clonidine (Langer, Shepperson & Massingham, 1981). The reverse order might be expected if only α_2-adrenoceptors were present. The order of potency observed, noradrenaline > phenylephrine > clonidine > oxymetazoline is mixed and might indicate that the agonists interact, in this artery, with more than one subtype of α-adrenoceptor which could be described as α_1-like and α_2-like.

The monophasic type of contraction produced by the α_2-selective agonists clonidine and oxymetazoline (Figure 2), is markedly different from the biphasic type of contraction produced by noradrenaline and phenylephrine. The contractions activated by clonidine and oxymetazoline were almost totally dependent on extracellular calcium, as seen by the diminished responses produced in Ca^{2+}-free solution and in the presence of the calcium entry blocker, cinnarizine, whereas responses to phenylephrine and noradrenaline are only partly inhibited in these circumstances. These observations suggest a variable ability to make intracellularly stored calcium available to the contractile proteins (Van Breemen *et al.*, 1972; Godfraind, 1978a) and the calcium efflux experiments confirm that both noradrenaline and phenylephrine were able to release intracellular calcium which was not releasable by clonidine. These results are compatible with the presence of two subtypes of α-adrenoceptor. There is a similarity between the IC_{50} values for cinnarizine as an inhibitor of contractions induced by noradrenaline, phenylephrine and clonidine. In the case of noradrenaline (as reported by Godfraind, 1978b) and phenylephrine the respective I_{50} values are also similar. The results indicate that these drugs activate the same or similar calcium channels in the smooth muscle cell membrane to allow the entry of extracellular calcium necessary for all (clonidine) or part (noradrenaline and phenylephrine) of the induced contraction. Oxymetazoline-induced contractions occasionally displayed rhythmic activity and were more sensitive to inhibition by cinnarizine. This might indicate that it also has effects other than those due to stimulation of α-adrenoceptors.

The time course of the inhibitory effect of cinnarizine was markedly different when contractions were induced by depolarization or by agonists. In the former case the degree of inhibition of the response increased with the duration of depolarization to reach a plateau, although the inhibitory effect of cinnarizine was not changed by the duration of contractions induced by agonists. Measurement of inhibition of ^{45}Ca influx shows a similar time course. These observations confirm the existence of a use dependency for the action of calcium blockers in depolarized preparations (Godfraind & Dieu, 1981; Godfraind & Miller, 1982) and suggest that either different calcium gating mechanisms might be activated in each case, having differing sensitivities to cinnarizine, or that separate calcium channels having different characteristics are activated (Bolton, 1979).

To try and differentiate a possible selective effect of phenylephrine over that of noradrenaline on α receptors of this tissue the experiments depicted in Figure 3 were performed.

In calcium-free solution, phenylephrine-induced contractions were markedly phasic, relaxing quickly to a residual plateau contraction but noradrenaline-induced contractions were well maintained. If phenylephrine is a more selective agonist for α_1-like adrenoceptors than noradrenaline and if receptor-operated calcium channels are mostly associated with the stimulation of α_2-like-adrenoceptors, then these results might be explained if noradrenaline, by opening receptor-operated calcium channels in the membrane allows a recycling of calcium, which is inaccessible to EGTA, through the membrane. Phenylephrine being a more selective agonist for α_1-like-adrenoceptors perhaps opens the channels coupled to the α_2-like-adrenoceptors to a lesser extent than does noradrenaline and would not stimulate this recycling, calcium extruded from the cell being therefore unable to re-enter. Such a site for calcium, extracellular but inaccessible to EGTA might be situated in the membranal caveoli (Keatinge, 1972). The receptor-operated calcium channels associated with this calcium site are however accessible to the lipid-soluble calcium entry blocker, cinnarizine since in the presence of cinnarizine 3 μM, noradrenaline-induced contractions in Ca^{2+}-free solution are no longer maintained, but resemble more those produced by phenylephrine (Figure 3b). The phenylephrine-induced contractions are affected to a much lesser extent. This concentration of cinnarizine was sufficient to block almost completely α-adrenoceptor-operated calcium channels stimulated by noradrenaline and phenylephrine without affect-

ing the efflux of calcium from the cell stimulated by these agents.

Data in Ca-free solution are therefore also consistent with the concept of two different post junctional α-adrenoceptors mediating release of intracellular Ca (α_1-like) and entry of extracellular calcium (α_2-like), only the latter being sensitive to calcium entry blockers.

However, this hypothesis should now be analyzed by the use of specific α-adrenoceptor antagonists since, as discussed by McGrath (1982), various α_1 and α_2-adrenoceptor agonists could interact differently with a single population of postjunctional α-adrenoceptors to produce a contraction.

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