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CALCIUM EXCHANGE IN VASCULAR SMOOTH MUSCLE, ACTION OF NORADRENALINE AND LANTHANUM

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SUMMARY

1. The Na, K, Ca and Mg content and the ^{45}Ca uptake and loss were determined in rat aortae incubated in physiological solution or in solution containing $LaCl_3$ instead of $CaCl_2$.

2. Aortae washed in La-solution contained less Ca and Na than controls in physiological solution, the K content was not modified and the Mg content was slightly decreased.

3. In 50 mM-La solution the ^{45}Ca diffusion space was intermediate between the values found for the [^{14}C]sorbitol space and the [^{14}C]inulin space, indicating that there was no Ca entry within the cell nor Ca binding at superficial sites. ^{45}Ca loss from the tissue was directly related to the La concentration.

4. Noradrenaline increased the rate of uptake of ^{45}Ca into the Ca fraction resistant to displacement by La. This increase was dose dependent, a response of 50% of the maximum being produced by 2×10^{-8} noradrenaline as for the contraction. In the presence of phentolamine, the dose–effect curves for the action of noradrenaline on ^{45}Ca uptake were displaced in a manner characteristic of competitive antagonism. The pA_2 for phentolamine was 7·8.

5. In physiological solution, the rate of loss of ^{45}Ca, from the Ca fraction resistant to displacement by La, was increased by noradrenaline the ED_{50} was 2×10^{-8} M, and the effect was abolished by phentolamine.

6. In view of the similarity of phentolamine pA_2 estimated by measuring noradrenaline sensitive ^{45}Ca uptake or noradrenaline evoked contraction, it is likely that the activation of α-adrenergic receptors is responsible for both effects.

INTRODUCTION

It is generally accepted that the contractile machinery in muscle is activated by a rise of the intracellular free Ca concentration (Heilbrunn & Wiercinski, 1947; Sandow, 1952; Niedergerke, 1955; Edman & Schild, 1962; Bohr, 1964; Portzehl, Caldwell & Rüegg, 1964; Ridgway & Ashley, 1967; Ebashi & Endo, 1968; Somlyo & Somlyo, 1968). Studies of the contraction of arterial smooth muscle evoked by noradrenaline have shown that large tonic responses depend on the presence of extracellular Ca ions while small phasic contractions can still be produced in a Ca -poor solution. In accord with such observations, it has been proposed that the rise of intracellular calcium may have a double origin: an influx of Ca from the outside and a release from intracellular storage sites (Bohr, 1973; Godfraind & Kaba, 1969, 1972; Keatinge, 1972*a*, *b*; Peiper, Griebel & Wende, 1971).

Several attempts have been made to estimate cytoplasmic Ca changes occurring during excitation of smooth muscle. It was reported that La could replace Ca at superficial binding sites; and that it blocks transmembrane fluxes of calcium (Mayer, van Breemen & Casteels, 1972), it has therefore been proposed that the Ca content of muscle washed in La solutions was an estimate of cellular Ca (van Breemen, Farinas, Casteels, Gerba, Wuytack, Deth, 1973). Recent observations on intestinal smooth muscle and on rabbit aorta have shown that the La-resistant Ca is only one of several intracellular fractions (Burton & Godfraind, 1974; Freeman & Daniel, 1973). The present experiments were designed in order to study the action of La on Ca content and Ca exchange in rat aortae after treatment with noradrenaline. By using ^{45}Ca, it was possible to measure the Ca turnover after the extracellular Ca had been displaced by 50 mM-$LaCl_3$. Changes in this La-resistant Ca were more rapid when the tissue was stimulated by an α-adrenergic drug. The rate of change of the ^{45}Ca content of the tissue might provide an estimate of the Ca fluxes across the smooth muscle cell membrane.

Preliminary communications on some of this work have already been published (Godfraind, 1974*a*, *b*).

METHODS

Material

Wistar rats weighing about 220 g were stunned and bled. The thoracic aorta was rapidly dissected out and immersed in physiological solution at 37° C. The media was separated from connective tissue and from adventitia and the vessel was longitudinally opened. The aortae weighed about 20 mg.

Physiological solution

The physiological solution was prepared without phosphate ions in order to minimize Ca precipitation during incubation period (Goodford, 1967). The Ca concentration was half the normal amount (Godfraind & Kaba, 1969), it was close to the free plasma Ca. It contained (mM): NaCl 122, $NaHCO_3$ 15, KCl 5·9, $CaCl_2$ 1·25, $MgCl_2$ 1·25 and glucose 11. It was equilibrated at 37° C with a mixture of 95 % O_2 + 5 % CO_2 (pH 7·2). Radioactive ^{45}Ca was supplied as $CaCl_2$ solution by the Radiochemical Centre, Amersham. Radioactive solution had a specific activity between 0·2 and 0·5 μc/ml.

La solution

The following solution was used for experiments with La^{3+} (mM): NaCl 122, KCl 5·9, Mg Cl_2 1·25, glucose 11, $LaCl_3$ 1, 2 or 50, Tris-maleate (pH 6·8) 15. Due to the acidity of $LaCl_3$, the final pH of the solution was slightly more acid, but not lower than 6·6. The La solution was not gassed in order to avoid La precipitation. Generally, batches of three aortae were soaked in 200 ml. La solution.

Na, K, Mg and Ca determinations

At the end of the incubations, or after washing with La solution, the aortae were removed from the organ bath and each preparation was blotted on filter paper. It was placed between two sheets of filter paper and was pressed three times with a roller weighing 350 g. After weighing, the aorta was placed in a Pt crucible and kept overnight at 100° C; it was then weighed. To remove organic material, it was kept at 500° C for 18 hr. The residue was dissolved in 1 ml. HCl (1 N), and assayed by atomic absorption spectroscopy after dilution in water for Na, K and Mg and in the following solution for Ca (NaCl 1 g, KCl 0·2 g, $LaCl_3$ 5 g, water 1 l.) (Rousselet, 1966).

Determination of ^{45}Ca

The strips were blotted as for atomic absorption. 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). This solution was heated for 15 min at 75° C, and after cooling was added to 10 ml. of a scintillation solution (bis MSB (*p*-bis-(*o*-methyl-styryl)-benzene) 0·2 g, PPO (2,5-diphenyl-oxazole) 4 g, naphthalene 50 g, toluene 600 ml., Triton X100 400 ml). When the radioactivity of ^{45}Ca physiological solution was estimated, 0·1 ml. was added to another scintillation solution (BBOT (2,5-bis-2-(5-tert-butyl-benzoxazolyl)-thiophene) 0·2 g, PPO 4 g, naphthalene 50 g, toluene 540 ml., Triton X100 360 ml., water 100 ml.).

The radioactivity of the samples was counted as usual with appropriate controls, and the efficiency was determined with internal standards. For the aortae, the results of each determination have been converted to the apparent tissue content of ^{45}Ca according to the formula

$$^{45}Ca \text{ (m-mole/kg wet wt.)} = \frac{\text{D.p.m. in muscle}}{\text{wet wt. (kg)}} \times \frac{\text{m-mole Ca/l. medium}}{\text{D.p.m./l. medium}}.$$

Determination of [^{14}C]*inulin or* [^{14}C]*sorbitol space*

[^{14}C]inulin or [^{14}C]sorbitol supplied by the Radiochemical Centre (Amersham), was added to the perfusion fluid so that its concentration was 0·1 %, w/v; the radioactivity of this solution was about 50,000 d.p.m./ml.

The procedure for [^{14}C]inulin or [^{14}C]sorbitol determination was similar to that followed for ^{45}Ca.

Drugs

Drugs used were (−) noradrenaline bitartrate, phentolamine methanesulphonate (Regitine^{R}, Ciba).

Statistical methods

Whenever possible, values are presented as means ± S.E. of mean. Significance of differences between means was checked by Student's t test.

RESULTS

Ionic content and ^{45}Ca uptake and loss in physiological solution and in La solution

The Na, K, Mg and Ca contents determined in steady-state conditions, after 2 hr incubation in physiological solutions, are reported in Table 1. The Na and K contents measured in these experiments were similar to

TABLE 1. Ionic content of rat aorta in various solutions. The estimations were obtained on preparations incubated for 2 hr at 37° C in the physiological solution and on preparations incubated under the same conditions and then transferred for either 5 or 15 min to La solution. The number of determinations is given between brackets. Two separate batches of La-treated aortae were used, one for Ca determinations and the other for Na, K and Mg

Ionic composition of the aortae m-mole/kg wet wt.		Na	K	Mg	Ca	Water ml./kg
Physiological solution 2 hr		86·8 ± 1·5 (40)	35·6 ± 0·4 (40)	3·7 ± 0·06 (20)	3·1 ± 0·08 (20)	672 ± 8 (40)
Then in 1 mM-La solution	5 min	79·7 ± 3·9 (16)	36·7 ± 1·8 (16)	3·7 ± 0·05 (16)	1·5 ± 0·08 (10)	672 ± 9 (26)
	15 min	82·4 ± 2·1 (13)	34·4 ± 1·2 (13)	3·6 ± 0·07 (13)	1·1 ± 0·06 (6)	640 ± 8 (19)
or 50 mM-La solution	5 min	43·8 ± 1·0 (16)	33·4 ± 0·3 (16)	3·3 ± 0·08 (16)	0·8 ± 0·04 (22)	683 ± 4 (38)
	15 min	53·6 ± 2·9 (16)	38·0 ± 1·5 (16)	3·3 ± 0·13 (16)	0·8 ± 0·05 (12)	555 ± 19 (28)

those found by others (Rorive, 1969; Kaba, 1973). ^{45}Ca uptake was measured in several batches of aortae incubated for various periods of time in radioactive physiological solution. The rate of the ^{45}Ca total uptake was fastest during the first 20 min (Table 2); when the incubation in radioactive solution was continued for 240 min, there was a further small gain ($P < 0·001$).

Preparations incubated for 20 min in radioactive physiological solution

at 37° C and then washed in non-radioactive solution, lost ^{45}Ca rapidly during the first 15 min of washing and thereafter at a progressively slower rate (Table 3). In 50 mM-La solution, ^{45}Ca efflux was very fast during the first 5 min of washout, thereafter, it was greatly reduced but not completely absent.

TABLE 2. ^{45}Ca content of rat aorta (m-mole/kg wet wt.) incubated for various times in radioactive physiological solution at 37° C containing noradrenaline 10^{-5} M. Preparations had previously been immersed for 2 hr in inactive solution at 37° C in order to equilibrate. Aortae treated with noradrenaline were preincubated for 5 min in the radioactive solution. The number of determinations is given between brackets

Time in radioactive solution (min)	Physiological solution	Time in the presence of noradrenaline (min)	Physiological solution + noradrenaline 10^{-5} M
2	1·62 ± 0·055 (6)	—	—
5	2·08 ± 0·046 (6)	0	—
7	2·17 ± 0·022 (6)	2	2·19 ± 0·047 (4)
9	2·07 ± 0·030 (6)	4	2·22 ± 0·047 (6)
13	2·21 ± 0·052 (6)	8	2·31 ± 0·040 (6)
20	2·22 ± 0·025 (20)	15	2·24 ± 0·051 (14)
21	2·15 ± 0·033 (6)	—	—
120	2·31 ± 0·022 (28)	—	—
240	2·43 ± 0·023 (8)	—	—

The initial ^{45}Ca efflux was faster in 50 mM-La solution than in physiological solution. This was substantiated by the observations that the ^{45}Ca content was lower after 2 min (Table 3: $P < 0·001$) and 5 min (Table 3: $0·001 < P < 0·01$; Table 4: $P < 0·001$) in La-solution than after the same period in physiological solution. The subsequent reduction in rate was more marked in La solution than in physiological solution and the ^{45}Ca content remaining after 60 min was higher in La solution than in physiological solution (Table 3: $0·001 < P < 0·01$). This indicates that whereas La displaced ^{45}Ca from one site, it tended to inhibit ^{45}Ca efflux from another tissue Ca fraction. It is likely that this initial fast ^{45}Ca loss originated from a fraction displaced by 50 mM-La and that most of this

fraction was lost after 5 min. Experiments with La concentration ranging between 1 and 70 mM have shown that maximum ^{45}Ca displacement occurred with 50 mM-La (Table 4). It was therefore possible to estimate the La-resistant Ca fraction by washing aortae in 50 mM-La solution for 5 min.

TABLE 3. ^{45}Ca content (m-mole/kg wet wt.) of rat aorta incubated for 2 hr in physiological solution then for 20 min in ^{45}Ca-physiological solution and finally washed during various periods either in physiological solution or in 50 mM-La solution. The data were obtained from one randomized group of rats. After loading in ^{45}Ca-physiological solution, batches of four aortae were incubated at 37° C in 300 ml. of non-radioactive solution. The washing solution was continuously changed at a rate of 60 ml./min. The number of determinations is given between brackets

^{45}Ca content of controls before washing: $2{\cdot}27 \pm 0{\cdot}045$ (6)

^{45}Ca content of aortae washed in non-radioactive solutions:

Time in non-radioactive solution (min)	Physiological solution (4)	50 mM-La solution (4)
2	$0{\cdot}49 \pm 0{\cdot}01$	$0{\cdot}25 \pm 0{\cdot}03$
5	$0{\cdot}17 \pm 0{\cdot}01$	$0{\cdot}10 \pm 0{\cdot}01$
10	$0{\cdot}08 \pm 0{\cdot}005$	$0{\cdot}08 \pm 0{\cdot}009$
15	$0{\cdot}064 \pm 0{\cdot}004$	$0{\cdot}069 \pm 0{\cdot}006$
30	$0{\cdot}033 \pm 0{\cdot}001$	$0{\cdot}043 \pm 0{\cdot}004$
60	$0{\cdot}017 \pm 0{\cdot}001$	$0{\cdot}028 \pm 0{\cdot}002$

TABLE 4. ^{45}Ca content (m-mole/kg wet wt.) of rat aorta incubated for 2 hr in physiological solution then for 20 min in ^{45}Ca-physiological solution and finally washed for 5 min in the quoted La solution

La^{3+}	^{45}Ca content (8)
0 (+EGTA 1 mM)	$0{\cdot}57 \pm 0{\cdot}011$
1 mM	$0{\cdot}49 \pm 0{\cdot}010$
2 mM	$0{\cdot}45 \pm 0{\cdot}006$
10 mM	$0{\cdot}18 \pm 0{\cdot}013$
30 mM	$0{\cdot}11 \pm 0{\cdot}004$
50 mM	$0{\cdot}10 \pm 0{\cdot}003$
70 mM	$0{\cdot}13 \pm 0{\cdot}006$

^{45}Ca content of aortae washed for 5 min in non-radioactive physiological solution instead of La-solution: $0{\cdot}17 \pm 0{\cdot}004$ (8).

Batches of aortae were preincubated for 2 hr in physiological solution and were transferred to La solution containing $LaCl_3$ 1 or 50 mM. As shown in Table 1, after washing in La solution, preparations have lost Na and Ca, the amount depending on the concentration of La ($P < 0{\cdot}01$). There was no change in K content. A small loss of Mg was observed in the 50 mM-La solution. Tissue water loss was observed after 15 min in La

TABLE 5. Diffusion space for ^{45}Ca, [^{14}C]inulin and [^{14}C]sorbitol of isolated rat aortae in various incubation solutions. The pieces of aorta were preincubated for 2 hr in the physiological solution at 37° C and were transferred to La solution 10 min before the addition of the markers. Diffusion spaces are given in ml./kg wet wt. The number of determinations is between brackets

Time (min) after adding the marker	Physiological solution		1 mM-La solution			50 mM-La solution		
	[^{14}C]inulin	[^{14}C]sorbitol	[^{14}C]inulin	[^{14}C]sorbitol	^{45}Ca	[^{14}C]inulin	[^{14}C]sorbitol	^{45}Ca
15	—	—	—	—	—	—	—	421 ± 10 (6)
30	—	—	353 ± 6 (6)	504 ± 17 (12)	1240 ± 17 (6)	258 ± 13 (6)	480 ± 11 (6)	416 ± 10 (12)
40	371 ± 6 (26)	554 ± 6 (10)	—	—	1222 ± 16 (6)	—	—	440 ± 12 (6)
60	—	—	364 ± 5 (6)	512 ± 5 (12)	1241 ± 24 (6)	274 ± 10 (6)	519 ± 6 (12)	445 ± 9 (18)

solutions ($P < 0.01$), it increased with La concentration ($P < 0.001$). The diffusion spaces of the non-penetrating saccharides, sorbitol and inulin were lower in La solutions than in physiological solution (Table 5). The reduction of diffusion space was more important for inulin than for sorbitol. This might be due to an exclusion of the larger marker molecule by La deposits in the extracellular space. La did not precipitate in solution. In the more alkaline extracellular space, insoluble La hydroxide and carbonate might be formed. This is consistent with the electron microscopic observation of dense particles between smooth muscle cells of aortae treated with 50 mM-La solution for 5 min. Therefore, changes in extracellular space might be better estimated by changes in sorbitol space. As the latter was only slightly reduced, tissue water loss was likely due to intracellular dehydration caused by the hypertonicity of the 50 mM-La solution.

In intestinal smooth muscle, it was found that La displaced Ca and Na (Burton & Godfraind, 1974; Widdicombe, 1974). In the present experiments, the loss of Ca and Na correspond to a counter-cation displacement of 144 m-mole/kg dry wt. of Na and of 7·6 m-mole/kg dry wt. of Ca after 15 min in 50 mM-La solution. The diffusion space of ^{45}Ca was reduced in 50 mM-La solution, it was intermediate between the values found for the [^{14}C]sorbitol space and for the [^{14}C]inulin space (Table 5). This indicates that in 50 mM-La solution, ^{45}Ca diffused into the extracellular space and that there was no Ca binding to superficial sites nor Ca entry into the cell.

The effect of noradrenaline on the ionic content of rat aortae and on ^{45}Ca uptake into a site resistant to release by La

Noradrenaline 10^{-5}M evokes a maximum sustained contraction of isolated rat aorta (Godfraind & Kaba, 1972) but does not cause significant changes in ionic content. Aortae were preincubated for 5 min in ^{45}Ca-physiological solution, the addition of noradrenaline 10^{-5}M to this solution did not significantly change the ^{45}Ca uptake (Table 2). Aortae preincubated for 5 min in ^{45}Ca-physiological solution were incubated in the same solution, in the presence or in the absence of noradrenaline for periods up to 2 hr. They were then washed for 5 min in the 50 mM-La solution. As Fig. 1 illustrates, the rate of ^{45}Ca uptake into the La-resistant Ca fraction was faster for noradrenaline treated aortae than for controls. This increase in the rate of ^{45}Ca uptake was dependent upon the noradrenaline concentration. The maximum ^{45}Ca uptake into the La-resistant Ca fraction was not modified by noradrenaline. When noradrenaline 10^{-5}M was added to the ^{45}Ca-physiological solution after a pre-incubation time of 60 min or more, no additional ^{45}Ca gain was seen in the La-resistant Ca fraction.

When aortae were equilibrated in physiological solution and then transferred to ^{45}Ca-solution already containing noradrenaline 10^{-5}M, the ^{45}Ca uptake was slower than in aortae preincubated for 5 min in the radioactive solution before adding noradrenaline (Fig. 2). The action of various doses of noradrenaline on ^{45}Ca uptake into the La-resistant Ca fraction

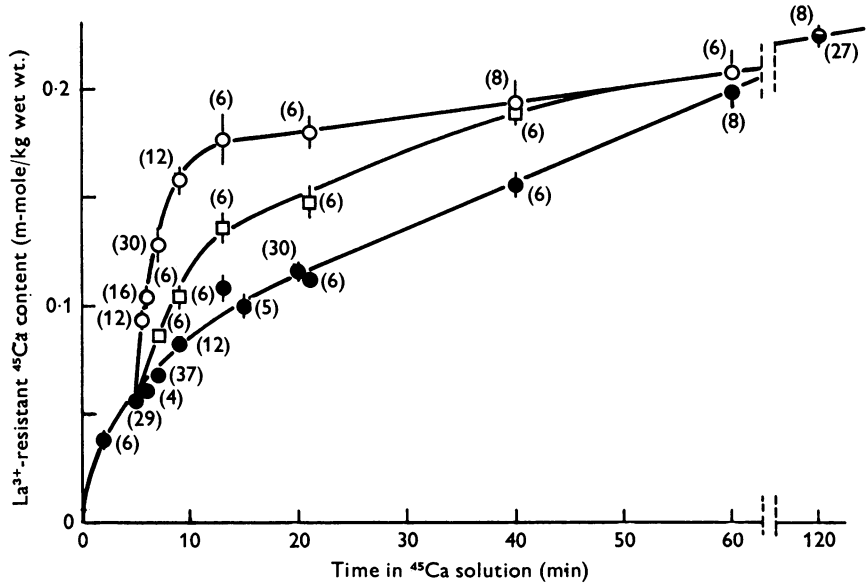

Fig. 1. The uptake of ^{45}Ca into the La-resistant Ca fraction of rat aorta. Aortae preincubated for 2 hr in physiological solution were transferred to ^{45}Ca solution for 5 min. They were then transferred to ^{45}Ca solution containing noradrenaline 10^{-5}, 10^{-8}M or none. After various periods of time, they were washed in 50 mM-La solution for 5 min. Abscissa: time (min) in the radioactive solution. Ordinate: ^{45}Ca content of the La-resistant Ca fraction in m-mole/kg wet wt., for controls in ^{45}Ca solution (●), for aortae treated by noradrenaline 10^{-8}M (□) or 10^{-5}M (○). The number of determinations is between brackets. The limits of S.E. are shown when they exceeded the diameter of the symbol.

has been studied after a preincubation of 5 min in radioactive solution. The rate of ^{45}Ca uptake into the La-resistant Ca fraction has been estimated by measuring the difference in La-resistant ^{45}Ca content after 5 min (n = 29) and 7 min (n = 37) in ^{45}Ca-physiological solution, the gain in La-resistant Ca content was equal to 6 μmole ^{45}Ca kg^{-1} min^{-1}. In the presence of noradrenaline 10^{-5}M (n = 29) this rate increased to 36 μmole ^{45}Ca kg^{-1} min^{-1}. 50% of the maximum effect (noradrenaline 10^{-5}M) was produced by 2×10^{-8}M noradrenaline (Fig. 3) as for contraction (Kaba,

1973). In the presence of phentolamine, an α-blocking agent, the dose–effect curves for the action of noradrenaline on ^{45}Ca uptake were displaced to the right (Fig. 3). The antagonism of noradrenaline by phentolamine appeared to be competitive.

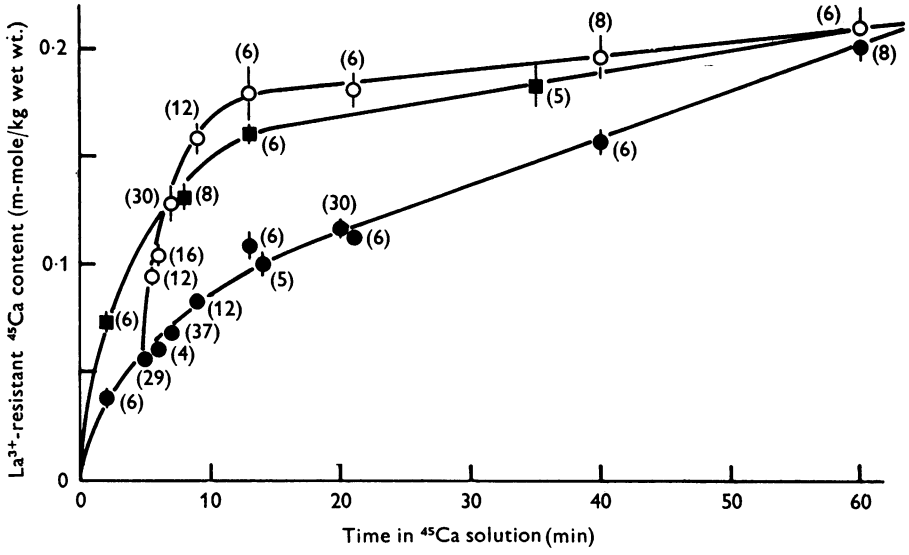

Fig. 2. The uptake of ^{45}Ca into the La-resistant Ca fraction of rat aorta. Aortae preincubated for 2 hr in physiological solution were transferred to ^{45}Ca physiological solution containing noradrenaline 10^{-5} M with or without preincubation in ^{45}Ca solution without noradrenaline. After various periods of time, they were washed for 5 min in 50 mM-La solution. Abscissa: time (min) in the radioactive solution. Ordinate: ^{45}Ca content of the La resistant Ca fraction, in m-mole/kg wet wt., of controls in ^{45}Ca physiological solution (●) of aortae transferred to ^{45}Ca physiological solution containing noradrenaline 10^{-5} M, without preincubation in the absence of noradrenaline (■) with a preincubation of 5 min (○). S.E. are shown when they exceeded the diameter of the symbol.

Phentolamine did not change the ^{45}Ca uptake in the absence of noradrenaline. The pA_2 for phentolamine estimated according to Arunlakshana & Schild (1959), was 7·8 for ^{45}Ca uptake. The difference pA_2–pA_{10} was equal to 0·95. By measuring the contraction of the aorta, the pA_2 for phentolamine was 7·9 (Kaba, 1973).

Effect of noradrenaline on ^{45}Ca loss

Aortae preincubated for 120 min in ^{45}Ca-physiological solution were transferred to non-radioactive solution and 5 min later to the same

solution with or without noradrenaline. The ^{45}Ca content of the 50 mM-La-resistant Ca fraction declined with the time of incubation in non-radioactive solution (Fig. 4). When noradrenaline was added to the non-radioactive bathing fluid, ^{45}Ca loss from the La-resistant Ca fraction

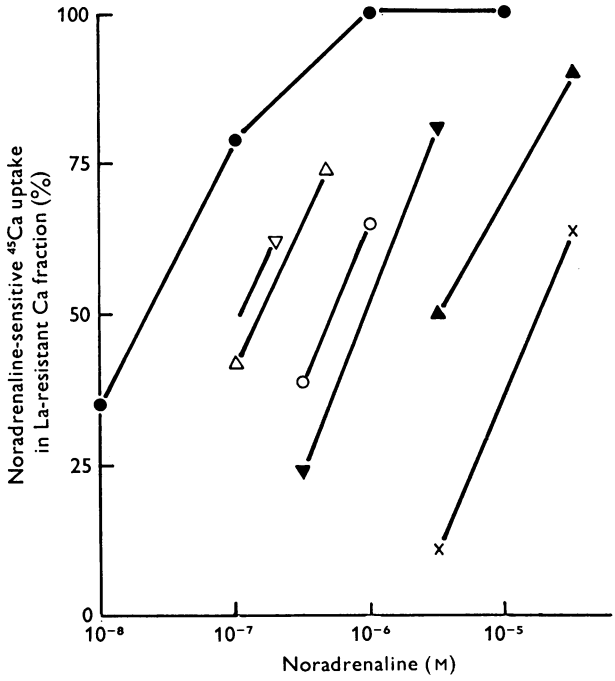

Fig. 3. Dose–response relation of the effect of noradrenaline on ^{45}Ca uptake into the La-resistant Ca fraction in the absence and the presence of various concentrations of phentolamine. Abscissa: noradrenaline concentration. Ordinate: noradrenaline sensitive ^{45}Ca uptake into the La-resistant Ca fraction. Noradrenaline was added from the 5th to the 7th minutes in ^{45}Ca-physiological solution; the noradrenaline sensitive Ca uptake was obtained by subtracting the La-resistant ^{45}Ca content of controls incubated during the same period without noradrenaline. Aortae were preincubated for 2 hr in physiological solution. Phentolamine was added 60 min before transferring the preparation to ^{45}Ca solution and it was also present in this solution. Each point is the mean of at least six determinations in the absence of phentolamine (●) or in the presence of phentolamine 3×10^{-8} (▽), 10^{-7} M (△), 3×10^{-7} M (○), 10^{-6} M (▼), 3×10^{-6} M (▲) and 10^{-5} M (X).

was increased (Fig. 4). The initial rate of ^{45}Ca loss has been estimated by measuring the difference in La-resistant Ca content after 5 and 7 min, in physiological solution, it was equal to 7 μmole ^{45}Ca kg^{-1} min^{-1}. In the presence of noradrenaline 10^{-5} M, it increased to 34 μmole ^{45}Ca kg^{-1} min^{-1}. This increase in the rate of ^{45}Ca efflux was dose-dependent, 50 %

of the maximum effect was produced by noradrenaline 2×10^{-8} M, a value already found for influx and contraction. The action of noradrenaline on Ca efflux was sensitive to phentolamine which displaced the dose-effect curves to the right, without altering the slope of the log dose–response curve. The dose ratios measured for phentolamine 10^{-7} M and 10^{-6} M

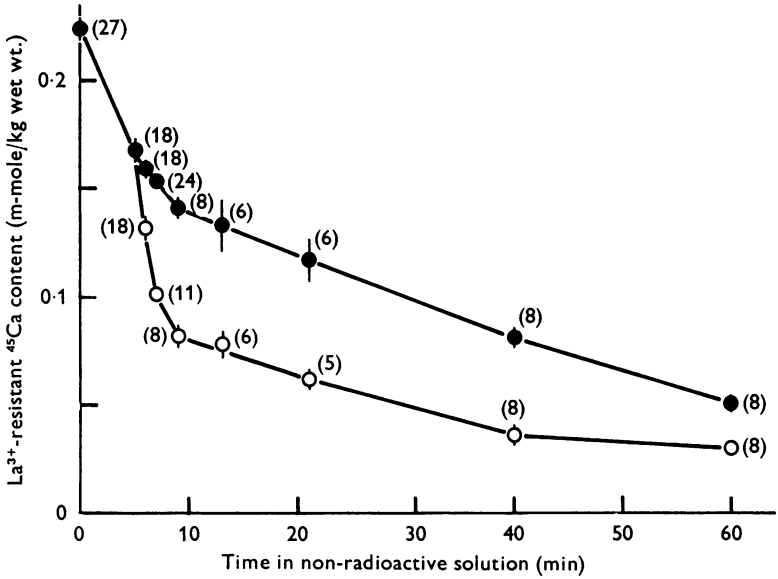

Fig. 4. The release of ^{45}Ca from the La-resistant Ca fraction of rat aorta. Aortae were preincubated for 2 hr in ^{45}Ca-physiological solution. They were washed for 5 min in 100 ml. non-radioactive solution, and then, for the period shown, in the same solution with or without the addition of noradrenaline 10^{-5} M. They were finally washed in 50 mM-La solution for 5 min before evaluation of ^{45}Ca. Abscissa: time (min) in non-radioactive physiological solution. Ordinate: ^{45}Ca content of the La-resistant Ca fraction in m-mole/kg wet wt., for controls in physiological solution (●), for aortae treated with noradrenaline 10^{-5} M (○). The number of determinations is between brackets. S.E. of mean are shown when they exceeded the diameter of the symbol.

were not significantly different from those estimated for ^{45}Ca influx. These observations suggest that noradrenaline increased, in a similar manner, Ca influx and efflux in the La-resistant Ca fraction.

DISCUSSION

In physiological solution, in the absence or in the presence of noradrenaline, there was a similar amount of total tissue calcium which was exchangeable for ^{45}Ca.

Treatment with La stopped Ca uptake but not Ca loss. Ca loss in La-solution might be accounted for by Ca displacement from extracellular sites or by Ca efflux from intracellular stores. In 50 mM-La solution most of Ca displacement occurred during the first 5 min and Ca efflux from intracellular stores appeared to be delayed. This allowed an estimation to be made of a La-resistant Ca fraction.

Noradrenaline evoked an increase in ^{45}Ca uptake into the La-resistant Ca fraction. ^{45}Ca uptake appeared to be slower when simultaneous application was employed than when the ^{45}Ca application preceded noradrenaline. The difference found might be accounted for by a difference in specific activity of ^{45}Ca in the extracellular space, as its complete equilibration with perfusion fluid might require a longer time than that needed for the diffusion of noradrenaline to receptors. The increased uptake of ^{45}Ca was not due to a net gain of tissue Ca but rather to an increase in the rate of exchange for extracellular ^{45}Ca. In view of the similarity of pA_2 when phentolamine was used as an antagonist, it appears that the activation of α-adrenergic receptors was responsible both for the contraction and for the increased rate of Ca exchange in the La resistant Ca fraction. It is likely that the rate of exchange from this fraction is the biologically important component of Ca exchange across smooth muscle cell membrane.

Keatinge (1972*b*) has observed that in sheep carotid arteries, contractions elicited by noradrenaline in Ca-free saline were not associated with any increase in the rate of loss of Ca greater than 1 μmole kg^{-1} min^{-1}; he attributed these contractions to the release of Ca from cellular stores. The contraction of rat aorta in response to noradrenaline 10^{-5} M is biphasic. The initial component is insensitive to changes in external calcium, whereas the amplitude of the subsequent tonic component is dependent on the calcium concentration of the bathing fluid (Godfraind & Kaba, 1972). The increased influx of Ca thus appears to control the tonic component of the contraction. The existence in smooth muscle of a dual source of Ca responsible for the activation of contractile proteins as shown by studies on Ca flux is in agreement with Mironneau's recent (1973) observation in voltage clamped rat uterus that a component of the contraction was dependent on a slow inward current and that a second component was observed without inward current.

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