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The relationship between noradrenaline-induced contraction and ^{45}Ca efflux stimulation in rabbit mesenteric artery

Paul A.A. Leijten[1] & Cornelis van Breemen

Department of Pharmacology School of Medicine, University of Miami, Miami, FL 33101, U.S.A.

1 Cellular Ca^{2+} recycling in a branch of the rabbit mesenteric artery was investigated by measuring the time- and concentration-dependent effects of noradrenaline (NA) on contraction and ^{45}Ca efflux in Ca^{2+}-free solution.

2 When NA was present continuously (15 min), both force development and ^{45}Ca efflux stimulation consisted of a fast and a slow (often oscillatory) component. These components were sensitive to caffeine and are probably both related to Ca^{2+} release from the intracellular Ca^{2+} store, presumably sarcoplasmic reticulum (s.r.).

3 When NA was applied for shorter time periods, both tension and stimulated ^{45}Ca efflux decreased similarly.

4 Repetitive short (30 s) NA applications resulted in repeated contractions and stimulations of ^{45}Ca efflux.

5 The NA-stimulated ^{45}Ca efflux was not inhibited when external Ca^{2+} was present or in Na^+-free medium.

6 Loading the cell with Ca^{2+} (with physiological salt solution for 3 h or with a high K^+ depolarizing solution) increases the number of subsequent NA-induced repeated contractions in Ca^{2+}-free solution.

7 The Ca^{2+} content of the sarcoplasmic reticulum (s.r.) in the smooth muscle cells of this small artery was estimated to be at least 50 μmol kg^{-1} wet weight, corresponding to an s.r. Ca^{2+} concentration of about 3.1 mM.

8 These results indicate that the NA-induced increase in cytosolic free Ca^{2+} (as measured by force development) is accompanied by an increase in Ca^{2+} extrusion (as measured by stimulation of ^{45}Ca efflux). This suggests that at least part of the activator Ca^{2+} cycles through the extracellular space during hormone-induced activation of vascular smooth muscle.

Introduction

Results of numerous experiments on vascular smooth muscle have suggested that intracellular Ca^{2+} release is an important step in excitation- contraction coupling. Direct evidence for this is found in recent data (Bond *et al.*, 1984; Kowarski *et al.*, 1985) which show that the sarcoplasmic reticulum (s.r.) is a Ca^{2+} store sensitive to neurotransmitters.

In addition, estimations of the Ca^{2+} concentration of the s.r. in smooth muscle cells suggest that it is sufficiently high to contribute to the initial force development (^{45}Ca flux studies: van Breemen & Deth 1976; Deth & van Breemen, 1977; Droogmans *et al.*, 1977; Casteels & Droogmans, 1981; Leijten & van Breemen, 1984; electron probe analysis: Bond *et al.*, 1984; Kowarski *et al.*, 1985).

It was inferred from experiments using ^{45}Ca flux measurements that hormone-released Ca^{2+} is ultimately extruded into the extracellular medium (Deth & van Breemen, 1977). This would imply that for maintained tension development Ca^{2+} influx is necessary. However, recent studies suggest that under certain experimental conditions hormone-induced responses can be maintained in the absence of external Ca^{2+}. For example, in vena cava strips, it was shown that shortlasting noradrenaline (NA)-induced stimuli produced repeated phasic contractions in Ca^{2+}-free

[1]Author for correspondence at present address: E.R. Squibb & Sons, Dept. of Clinical Pharmacology, P.O. Box 4000, Princeton N.J., U.S.A.

solution (Vonderlage, 1976). Also, in rabbit pulmonary artery (Ito *et al.*, 1977), rabbit mesentery artery (Itoh *et al.*, 1983) and rabbit aorta (Leijten *et al.*, 1985) repetitive drug-induced phasic contractions could be obtained in the absence of external Ca^{2+}. Similar findings, observed in guinea-pig portal vein (Bond *et al.*, 1984), led these authors to conclude that this phenomenon is consistent with the recycling of Ca^{2+} by the s.r. These results were extended to the hypothesis (Somlyo, 1985), that transmembrane Ca^{2+} fluxes make only a minor contribution to contractile activation during physiological smooth muscle contraction.

In the present study an attempt was made to clarify whether and to what extent released Ca^{2+} recycles in the cytoplasm. This was done by measuring force development and ^{45}Ca efflux under conditions of various degrees of Ca^{2+} release.

Methods

Adult New Zealand white rabbits were killed by CO_2 asphyxiation and exsanguinated from the carotid arteries. The first branch of the superior mesenteric artery was identified, carefully excised and placed in prewarmed, preoxygenated physiological salt solution (PSS). The artery was cleaned of fat and connective tissue under a binocular microscope and cut into rings of 1–3 mm length, weighing 0.2–0.8 mg. For each individual experiment comparing tension and ^{45}Ca efflux, rings of similar length, diameter and weight were used. The tension experiments were done either just before or just after the ^{45}Ca efflux experiment. Preliminary experiments in which force and ^{45}Ca efflux were measured simultaneously on the same preparation, gave similar results as those presented in this paper. Experiments were performed at 37°C.

Solutions

The PSS contained (mM): NaCl 140, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.0, D-glucose 10, HEPES 5.0. The pH was adjusted to 7.3 with 0.1 N NaOH. In the Ca^{2+}-free solution Ca^{2+} was omitted and 2 mM EGTA added. In the high-K^+ solution (with or without Ca^{2+}), all NaCl was replaced with KCl isotonically.

Contraction

Two 40 μm tungsten wires were passed through the lumen of the isolated mesenteric artery. One wire was fastened to a fixed tissue mount, the other one was placed onto two parallel hooks, attached to a strain gauge force transducer (U-gauge, Shinko Co, Ltd). The position of the transducer could be set with a micromanipulator (Narishige). The tissue was stretched slightly above resting tension. The volume of the chamber was 1.5 ml. The solutions were added to one end of the chamber by rapid injection with a 10 ml syringe, while the solution already present was siphoned off by vacuum-suction on the other end. Tension was recorded isometrically on a Perkin-Elmer, model 56.

^{45}Ca efflux

Rings were equilibrated with ^{45}Ca for 3–4 h in the PSS, at the beginning of the washout placed on specially designed aerators and transferred into plastic scintillation vials. The tissues were passed through the vials at the appropriate intervals. At the end of the efflux experiment, the rings were blotted, weighed (on a Mettler microbalance) and placed overnight in 5 mM EDTA. The radioactivity in the tissues and effluent samples was counted by liquid scintillation and the rate of efflux was calculated. The amount of stimulated ^{45}Ca efflux was estimated by measuring the area under the stimulated portion of the ^{45}Ca efflux curve. This was done by weighing a graphical representation of the stimulated area and using the weight of a defined area as reference value. For the sake of clarity, in some figures the control rate of ^{45}Ca efflux is not shown.

For measuring the releasable ^{45}Ca content of the store, we used the same procedure as for rabbit aorta (Leijten & Van Breemen, 1984). Briefly, the amount of released ^{45}Ca was calculated after various exposure times to Ca^{2+}-free solution and the theoretical ^{45}Ca content at time zero was estimated by extrapolating backwards.

Drugs

NA ((−)-arterenol HCl) (Sigma) and caffeine (Sigma) were used.

Results

Tension and ^{45}Ca efflux in the continued presence of noradrenaline

Figure 1a shows typical examples of the NA- and caffeine-induced contractions in the absence of external Ca^{2+}. The time to peak force was shorter for caffeine than for NA. The caffeine-induced contraction relaxed rapidly, while the relaxation for NA consisted of a rapid and a slow phase. The slow component often showed oscillations with a frequency of about 2 per min. The ^{45}Ca efflux, stimulated by both drugs under identical conditions as the tension studies, is shown in Figure 1b. The pattern of ^{45}Ca efflux is similar to that of the force development. For NA, a rapid peak of ^{45}Ca efflux is followed by a slow return to

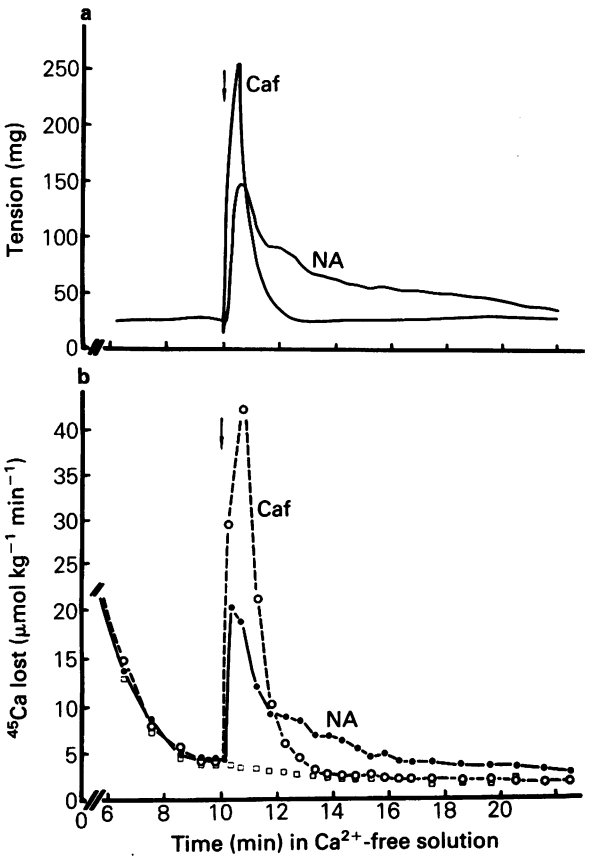

Figure 1 The effects of prolonged exposure to noradrenaline (NA, 3×10^{-5} M) and caffeine (Caf, 25 mM) on force development and ^{45}Ca efflux in Ca^{2+}-free solution. Drugs were added at 10 min (indicated by downward arrow) and left for the remainder of the experiment. (a) Superimposed NA- and caffeine-induced contractions. (b) Stimulated ^{45}Ca efflux by NA (●——●) and caffeine (O---O). (□) Control ^{45}Ca efflux rate.

the basal ^{45}Ca efflux rate. The initial ^{45}Ca efflux peak with caffeine was larger than that with NA, and returned quickly to control rate (peak efflux rate for caffeine was 3.8 ± 0.5 times larger than that for NA, $n = 6$). The total amount of released ^{45}Ca was similar for NA and caffeine. The concentration-response curves for NA-induced contractions and ^{45}Ca release were parallel (not shown). After the application of 25 mM caffeine for 2 min, neither caffeine nor NA could induce any force or ^{45}Ca efflux. This suggest that the s.r. is depleted by this procedure. Subsequently, extended applications of 25 mM caffeine were used to estimate the releasable s.r. ^{45}Ca content in this small muscular artery. This was calculated according to the protocol we have used previously for rabbit aorta (Leijten & van Breemen, 1984). The results of these experiments are shown in Figure 2. The initial ^{45}Ca content of the store was about 50 μmol kg^{-1} wet weight. The ^{45}Ca was lost with a rate displaying a half-life of about 20 min. The caffeine-induced contractile responses in the Ca^{2+}-free solution decreased with an identical rate (not shown).

The possibility that the NA-stimulated ^{45}Ca efflux was an artifact due to any unphysiological external medium (EGTA), was excluded because the initial phasic stimulated ^{45}Ca efflux was also present in normal Ca^{2+} containing physiological salt solution (not shown). The slow phase of ^{45}Ca efflux was more pronounced in the washout solution containing Ca^{2+}. NA also stimulated ^{45}Ca efflux in zero Na^+ (140 mM K^+) Ca^{2+}-free solution (not shown).

The slow component of the NA transient may represent a second phase of Ca^{2+} release from the sarcoplasmic reticulum. This idea was supported by the finding (Figure 3) that this phase was abolished by

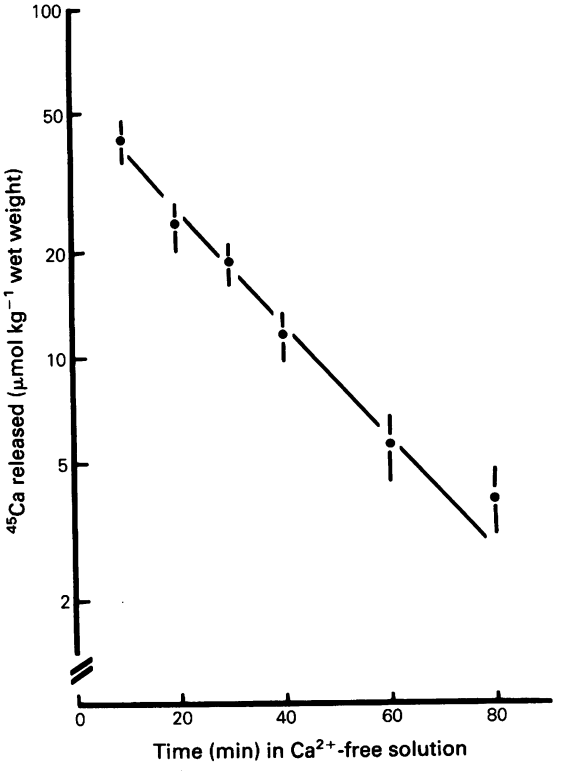

Figure 2 The rate of decline of the caffeine releasable ^{45}Ca in Ca^{2+}-free solution. At various times after exposure to Ca^{2+}-free solution 25 mM caffeine was added and the amount of ^{45}Ca efflux calculated. Vertical lines represent s.e.mean of 6 experiments.

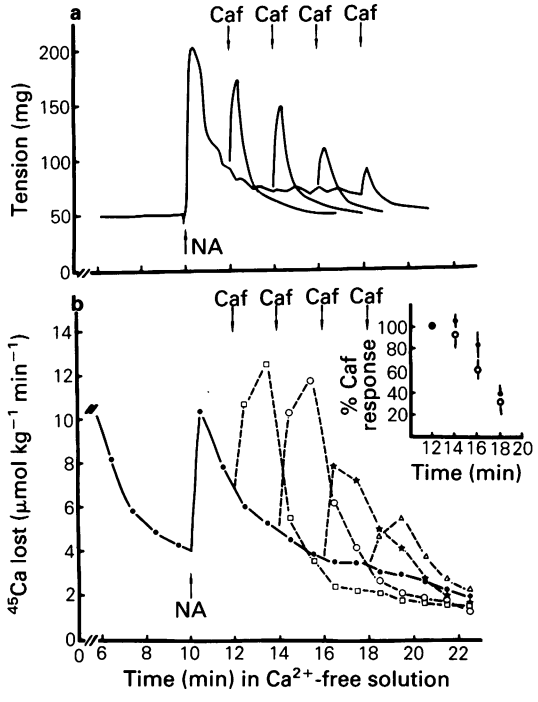

Figure 3 The effects of caffeine (Caf) on the slow phase of the noradrenaline (NA)-induced contraction and ^{45}Ca efflux. NA (3×10^{-5} M) was applied at 10 min (upward arrow) and left for the remainder of the experiment. Caffeine (25 mM) was added at 12, 14, 16 and 18 min. (a) Superimposed tracings of 4 individual tension experiments. (b) Caffeine-stimulated ^{45}Ca efflux during the slow NA-stimulated ^{45}Ca efflux. Inset: decline of the caffeine-induced contraction (○) and caffeine-stimulated ^{45}Ca efflux (●). $n = 4$, vertical lines indicate s.e.mean.

depletion of the s.r. by caffeine. Caffeine, applied at various times during the NA-induced slow phase, transiently increased force (Figure 3a) and ^{45}Ca efflux (Figure 3b), but the responses declined as the slow phase approached control tension and ^{45}Ca efflux rate.

This series of experiments indicate: (1) in this small muscular artery the sparse s.r. (2% of total cell volume: Devine *et al.*, 1972) contains a considerable amount of ^{45}Ca (50 μmol kg^{-1} wet wt.). Assuming that the smooth muscle cells occupy 80% of total weight, the s.r. Ca^{2+} concentration would be $0.05/(0.8 \times 0.02) = 3.1$ mM, (2) the s.r. is more rapidly depleted by caffeine than by NA, as had been suggested from mechanical studies (Itoh *et al.*, 1983), (3) there is a tight relationship between force and stimulated ^{45}Ca efflux, (4) the s.r. still contains Ca^{2+} as long as NA-induced tension and ^{45}Ca efflux remain elevated above the controls.

Tension and ^{45}Ca efflux with short applications of noradrenaline

In the following experiments the effects of short lasting NA exposures on force and ^{45}Ca efflux were tested. Figure 4a is an example of such an experiment. NA was applied for only 30 s, at which time peak force was reached. It is apparent that, parallel to the rapid relaxation upon NA removal, the ^{45}Ca efflux rate quickly returned to control levels. Figure 4b graphically shows how the relationship between force and ^{45}Ca efflux is affected by decreasing the time of NA application (from 900 to 5 s). With shorter NA exposures both force and ^{45}Ca efflux are decreased similarly. From these results it follows that a short NA 'pulse' (e.g. 30 s) partially depletes the Ca^{2+} available in the store. The partial depletion by a short NA pulse is specifically tested in the experiments illustrated in Figures 5 and 6. In Figure 5 it is seen that with repeated short NA exposures, partial depletion of the labelled Ca^{2+} store occurs parallel with the partial decline in force development. Thus the relationship between successive contractions and ^{45}Ca released is similar to that shown in Figure 4b. From this we would predict that omission of one shortlasting NA exposure in a trian of stimuli would increase the size of the subsequent contraction. This was tested in the experiment shown in Figure 6. It is seen that the test contraction (i.e. the stimulus at 16 min) was about 30% larger than the control one. The inset of Figure 6 shows that the amplitude of the test response increased when the number of previous pulses was reduced. In Ca^{2+}-containing PSS, the amplitudes of repeated 30 s NA-induced contractions remained constant (not shown).

It follows from the above experiments that during the initial peak of force development only 30% of the total Ca^{2+} store was released and that a relatively large amount of Ca^{2+} was released during the slow phase. We studied whether the slow phase of the NA-induced release response could be due to the loading of a slowly-exchanging cellular compartment during the prolonged exposure to the radiolabelled PSS. Figure 7a shows the NA-induced contraction after a Ca^{2+} loading period of 10 min or 3 h duration. It is clear that the slow phase is more pronounced after the latter condition. A similar result was obtained when the tissue was pre-exposed to a high K^+ solution for 5 min (not shown). Although the amplitude of the NA-induced phasic contraction in the Ca^{2+}-free solution recovers within approximately 10 min of Ca^{2+} repletion (not shown), the number of repeated contractions (30 s pulses) was larger than 3 h loading in PSS (Figure 7b). This could suggest that upon NA removal, Ca^{2+} from a slow compartment refills the sites responsible for the fast response.

This set of experiments shows (1) during brief NA stimuli (even before peak force is reached, i.e. at 5 s)

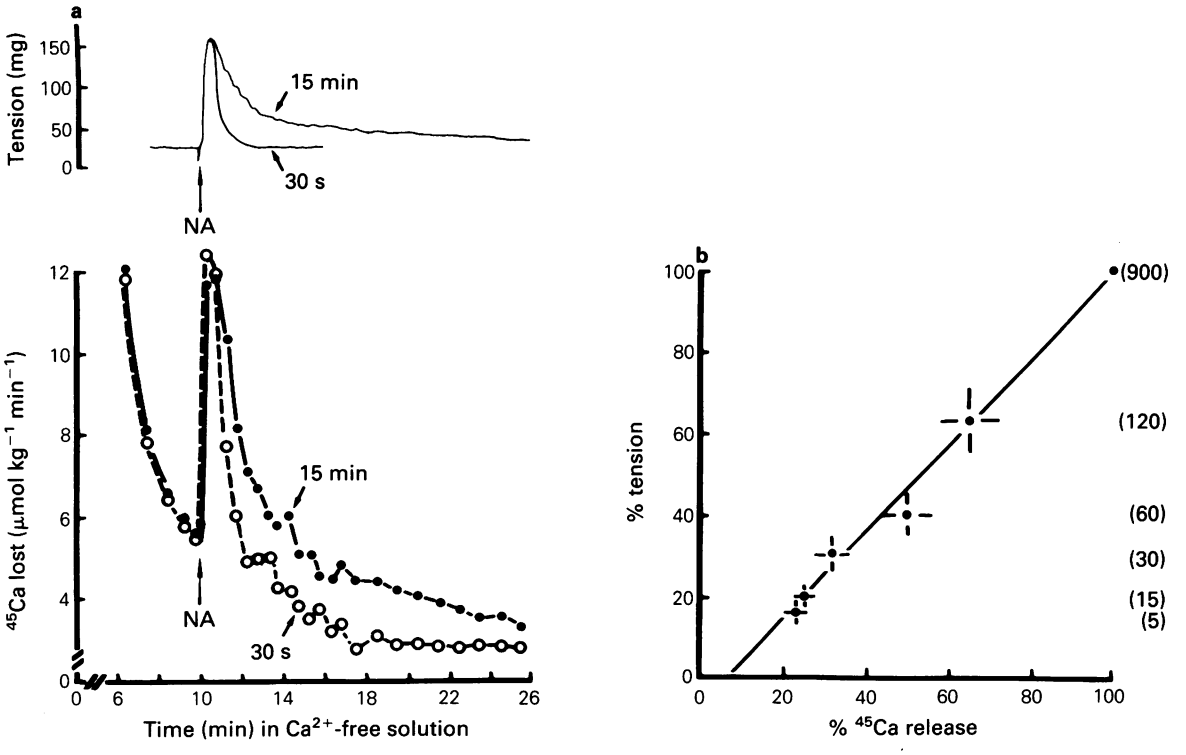

Figure 4 The effects of decreasing the time of noradrenaline (NA) application on contraction and stimulated ^{45}Ca efflux. (a) Comparison of the time course of the NA-induced contraction (top trace) and stimulated ^{45}Ca efflux (bottom trace) when the drug was applied for 30 s or 15 min. NA (3×10^{-5} M) was given at 10 min. (b) Relation between force (area under the tension curve) and stimulated ^{45}Ca efflux (area under the stimulated ^{45}Ca efflux curve) for various exposure times to NA. The exposure time (s) is indicated by number in parentheses. A 15 min application time was taken as 100%. Clearly, with shorter exposure times both force and ^{45}Ca efflux decreased. $n = 5-8$ for each point ± s.e.mean. Linear regression of the data using the least squares method yielded a straight line with a correlation coefficient (r) of 0.995 and a slope of 1.068. The line intersected the abscissa scale at + 7.2%.

activator Ca^{2+} is rapidly extruded into the extracellular space; (2) after cellular Ca^{2+} loading the repeated contractions are better maintained.

Discussion

The main finding of these experiments is that, in Ca^{2+}-free solution, NA-induced force development was always accompanied by a stimulation of ^{45}Ca efflux. This was the case for (1) all durations of NA exposure, (2) all concentrations of NA, (3) both the initial fast NA response and the slow phase.

Several findings, presented here and in earlier publications (van Breemen & Deth, 1976; Deth & van Breemen, 1977; Droogmans *et al.*, 1977; Leijten & van Breemen, 1984) clearly show that tension development and stimulation of ^{45}Ca efflux are closely correlated (e.g. the similar decay rate of the contractile and ^{45}Ca efflux responses to caffeine in Ca^{2+}-free solution and the similar concentration-dependence of force development and stimulated ^{45}Ca efflux in response to NA). The experiment in which the NA exposure time was varied (Figure 4b) demonstrates that a linear relation exists between NA-induced contraction and stimulated ^{45}Ca efflux. The explanation for this linearity may lie in the fact that once Ca^{2+} is released from the s.r. into the cytoplasm it activates both contraction and its own extrusion through calmodulin-stimulated enzymes: the myosin light chain kinase (Kamm & Stull, 1985) and the ATP-dependent Ca^{2+}-pump in the plasmalemma (Wuytack *et al.*, 1984; Daniel, 1985), respectively. This conclusion is supported by recent work with intracellular Ca^{2+}-indicators in vascular smooth muscle cells, which showed that the threshold and half-maximal response

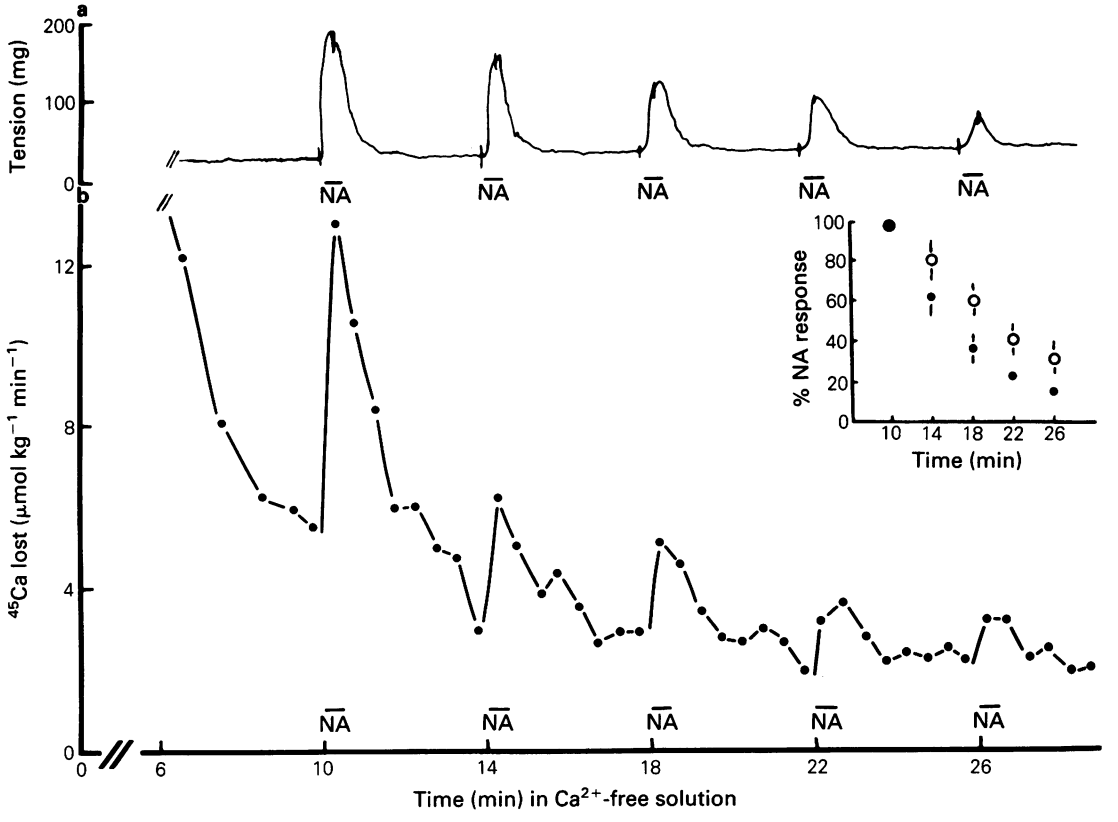

Figure 5 The effect of repeated applications of noradrenaline (NA) (3×10^{-5} M) on contraction (a) and stimulated ^{45}Ca efflux (b) in Ca^{2+}-free solution. Duration of exposures: 30 s. Inset: decline of successive NA-induced contractions (○) and stimulated ^{45}Ca efflux (●). $n = 2-4$ for each point with vertical lines indicating s.e.mean.

for angiotensin II-induced increases in cytosolic free Ca^{2+} and ^{45}Ca efflux were similar (Alexander *et al.*, 1985) and that stimulation with caffeine both raised intracellular free Ca^{2+} and depleted the Ca^{2+} store (Kobayashi *et al.*, 1985). It thus appears that at least some of the Ca^{2+} released from the s.r. by agonists is extruded into the extracellular space. In order to complete the Ca^{2+}-cycle, Ca^{2+} would need to be replenished by a process of Ca^{2+}-influx.

This hypothesis is in contrast to one proposed by Bond *et al.* (1984) who suggested that under physiological conditions all Ca^{2+} released from the s.r. by NA is directly returned from the cytoplasm to the s.r. Their hypothesis was based upon the finding that repeated NA-induced contractions could be evoked in Ca^{2+}-free solution, if the drug was removed at the peak of the tension response. The rationale was that during brief NA exposures the increased cytoplasmic Ca^{2+} would be resequestered into the store. The gradual decrease in the amplitude of successive brief NA-induced contractions, observed in this type of experiment, must be explained then by the rate at which Ca^{2+} leaks from the store in Ca^{2+}-free solution, receptor desensitization or an undefined form of excitation-contraction uncoupling. However, the experiments described in this paper show, as expected from the relation between tension and ^{45}Ca efflux, that repeated NA-induced contractions in Ca^{2+}-free solution are accompanied by repeated fractional release of ^{45}Ca from the store. The fact that the repeated contractions decrease in amplitude is thus not only due to the rate of loss of Ca^{2+} from the s.r. (leak), but also to repeated extrusion of activator Ca^{2+} with each stimulus (see also Figure 6). This makes it unnecessary to invoke the hypothesis that the diminished responsiveness to NA is caused by desensitization (Bolton, 1979), unless somehow the ability to release Ca^{2+} is related to the amount of (activator?) Ca^{2+} near the

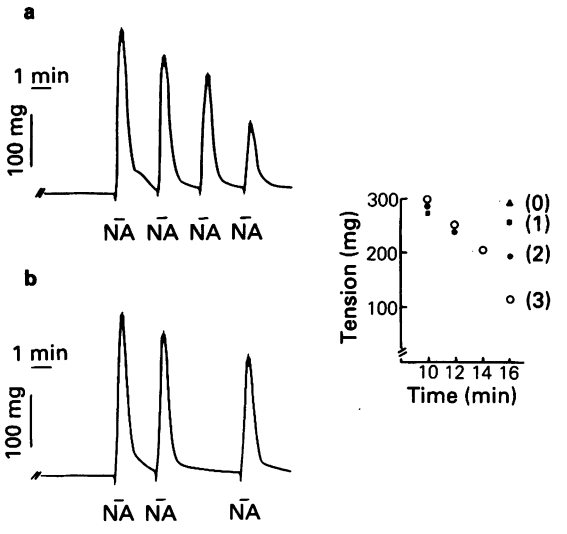

Figure 6 The effect of omitting one shortlasting noradrenaline (NA) exposure on the amplitude of the subsequent contraction. (a) Control experiment: NA was applied four times, at 10, 12, 14 and 16 min in the Ca^{2+}-free solution. Duration of each exposure: 30 s. (b) Test experiment: the tissue was left unstimulated at 14 min and the subsequent contraction at 16 min was larger than the fourth application in the control experiment. Inset: the effect of the number of previous stimulations on the NA test response at 16 min. The number of previous applications is shown in parentheses. Note that with less pre-applications, the amplitude of the test response increased.

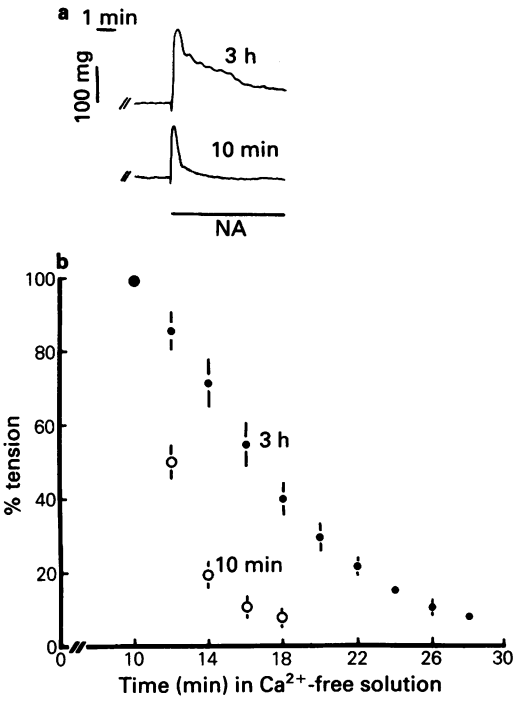

Figure 7 (a) The effect of prolonged exposure to physiological salt solution on the slow phase of the noradrenaline (NA)-induced contraction in Ca^{2+}-free solution. The slow phase was more pronounced and often oscillatory after the long pre-incubation period (top trace). The tissue relaxed more rapidly after the short loading period (lower trace). Before the different loading periods the store was depleted with 25 mM caffeine. NA (3×10^{-5} M) was added at 10 min and kept present throughout. (b) The effect of prolonged exposure to physiological salt solution on the number of repeated contractions induced by NA in Ca^{2+}-free solution. NA was applied every 2 min, starting at 10 min. The amplitudes of the successive contractions were plotted as % of the amplitude of the initial contraction at 10 min. Duration of each exposure: 30 s. Clearly, after long loading more contractions can be elicited. $n = 3$ for each point and vertical lines indicate s.e.mean.

adrenoceptor or in the store itself. The former hypothesis is unlikely since the rate of loss of responsiveness to caffeine was also related to the number of stimuli, i.e. with more pulses the responsiveness was lost faster (unpublished observation). This is in agreement with experiments from cultured rat aortic cells (Kobayashi *et al.*, 1985), in which it was found that the repeated application of caffeine in Ca^{2+}-free solution resulted in repeated transient increases in cytosolic Ca^{2+} (measured by quin-2). The level of these transients was reduced progressively by each application of caffeine, again suggesting that with every stimulus a net loss of activator Ca^{2+} has occurred.

The finding that the number of repeated contractions in response to activators is increased in Na^+-free, Ca^{2+}-free solution (Itoh *et al.*, 1983; 1985) or in the presence of La^{3+} (Bond *et al.*, 1984), has been interpreted as being caused by a block of neurotransmitter-stimulated Ca^{2+} efflux. Two findings argue against this hypothesis. Firstly the NA-stimulated ^{45}Ca efflux is not abolished in Na^+-free solution (this study; Droogmans & Casteels, 1979) in La^{3+} solution (Deth & Lynch, 1981) or at room temperature (Droogmans & Casteels, 1981). In the second place, in the former experiments (Itoh *et al.*, 1985) it was necessary to pre-incubate the tissue for 180 min in Na^+-free physiological salt solution and in the latter study (Bond *et al.*, 1984) the preparation was pre-exposed to a high-K^+ depolarizing solution before the repeated contractions were produced. These procedures cause increased Ca^{2+} loading of intracellular organelles (Aaronson & van Breemen, 1981; Leijten & van

Breemen, 1984) which in fact is consistent with our finding that an increase in cellular Ca^{2+} increases the number of repeated contractions. Lowering the temperature might also be expected to increase cellular Ca^{2+} by inhibition of the Ca^{2+}-extrusion pump. The degree of Ca^{2+} loading had its greatest effect on the slow phases of NA-induced contraction and ^{45}Ca efflux.

The response to a maintained presence of NA in the Ca^{2+}-free solution consisted of a fast component, followed by a slow one. The pool of NA-released Ca^{2+}, which is related to the phasic contraction is probably located in the superficial s.r. This is strongly suggested by the direct demonstration (electron probe analysis measurements) that the Ca^{2+} content in the superficially located s.r. was decreased at the peak of NA-induced contractions (Bond *et al.*, 1984). The close relation between the amplitude of the fast response and the external Ca^{2+} concentration during refilling experiments (Casteels & Droogmans, 1981), support this store being located in close proximity to the surface membrane. The slow NA component is probably also related to the s.r. because it is sensitive to caffeine, a drug with some selectivity for the s.r. (Weber & Herz, 1968). Furthermore, the display of oscillatory behaviour resembles the spontaneous s.r. Ca^{2+} release observed in heart muscle (Fabiato, 1985). It is noteworthy that these force oscillations in heart were accentuated by cellular Ca^{2+} overload.

It is now appropriate to discuss two models, not mutually exclusive, which could explain the data. Firstly, it is possible that initially, NA releases Ca^{2+} from a more superficially located s.r. and later from deeper located s.r. The rate of Ca^{2+} release (and consequently of ^{45}Ca efflux) from the superficial site would be faster than that of the release from the deep site. In this model no recycling of released Ca^{2+} occurs. A similar observation was made in heart muscle (Hess & Wier, 1984), where Ca^{2+}-indicator experiments have suggested that the fast and slow Ca^{2+}-transient are related to Ca^{2+} release from two separate parts of the s.r. Secondly, it could be that part of the Ca^{2+} released from the superficial s.r. is extruded and part is rebound in another part of the s.r. This Ca^{2+}, recycling over deeper s.r. membranes, causes the slow response. This process is made possible because in the continued presence of NA, Ca^{2+} cannot be resequestered in the superficial s.r. This is supported by the finding that the site responsible for the fast release-response cannot be refilled in the presence of NA (Karaki *et al.*, 1979, Leijten, unpublished results). Only upon removal of NA can the superficial s.r. be refilled with Ca^{2+}, which may be derived from external or internal sources. The latter may be relatively more important when slowly exchanging stores (deep s.r., nucleus, mitochondria) have a high Ca^{2+} content. The 'fast' Ca^{2+} store can be refilled both from the external medium and internally in smooth muscle cells from the taenia caeci (Brading *et al.*, 1980).

In conclusion, the results in this paper indicate that during the contraction-relaxation cycle in vascular smooth muscle at least part of the activator Ca^{2+} is extruded into the extracellular space. Thus, the Ca^{2+} cycle cannot be completed solely intracellularly, i.e. by cycling of all activator Ca^{2+} between the cytoplasm and the s.r. However, the present results cannot exclude the possibility that under certain conditions a slower process of activation is due to internal Ca^{2+}-recycling.

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