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The effect of drugs on the constriction of isolated depolarized blood vessels in response to calcium or barium

B. J. NORTHOVER

Leicester Regional College of Technology, Leicester

1. The rat isolated anterior mesenteric artery was perfused at a constant rate with a calcium-free depolarizing solution. Injection close to the cannula of 0.05–0.1 ml. of solutions of $CaCl_2$ (117 mM) or $BaCl_2$ (100 mM) caused a rise in perfusion pressure.

2. The responses to injected $CaCl_2$ solution could be obtained repeatedly but those to successive injections of $BaCl_2$ solution slowly declined. When the responsiveness to barium had almost disappeared, it could be restored by the addition to the perfusing fluid of a small amount of calcium (0.05 mM).

3. The contractile effects of calcium or barium were antagonized by the addition to the perfusing fluid of several anti-inflammatory substances, certain local anaesthetics and certain spasmolytic drugs.

4. Perfusion of the mesenteric artery with a depolarizing solution containing 0.2 mM-$CaCl_2$ caused a persistent rise of the perfusion pressure. This was rapidly and completely reversed by the addition of indomethacin (4 mg/100 ml.) or cinchocaine hydrochloride (2 mg/100 ml.) to the perfusing fluid.

5. The uptake of ^{45}Ca by rat aorta depleted of calcium was reduced by amethocaine hydrochloride (10 mg/100 ml.) or cinchocaine hydrochloride (2 mg/100 ml.) but not by indomethacin (10 mg/100 ml.) or desipramine hydrochloride (1 mg/100 ml.).

Ringer (1883) first showed that cardiac muscle requires calcium ions for contraction. Since then it has been shown that almost all types of muscle require calcium for contraction. It is now generally agreed that calcium ions are the link between excitation of the muscle cell surface and shortening of the contractile proteins within the cell. The very extensive literature on this subject has been reviewed by Shanes (1958), Eichna (1962), Honig (1963), Bohr (1964), Sandow (1965) and Caldwell (1968).

Vascular smooth muscle ceases to respond to constrictor agents in the absence of calcium (Evans, Schild & Thesleff, 1958; Briggs & Melvin, 1961; Bohr & Goulet, 1961; Briggs, 1962; Waugh, 1962; Hinke, Wilson & Burnham, 1964; Cuthbert & Sutter, 1965; Axelsson, Johansson, Jonsson & Wahlström, 1966; Briggs & Shibata, 1966; Shibata & Briggs, 1966; Alexander, 1967; Burks, Whitacre & Long, 1967; Hrdina & Garattini, 1967; Jhamandas & Nash, 1967; Mohme-Lundholm & Vamos,

1967 ; Northover, 1967a ; Shibata & Carrier, 1967 ; Hudgins & Weiss, 1968). Several of these reports also mention that calcium ions cause constriction of depolarized blood vessels. A contractile protein can be extracted from vascular smooth muscle which resembles actomyosin from skeletal muscle in that it develops ATP-ase activity and contracts under the influence of calcium ions (Bohr, Filo & Guthe, 1962). There is evidence that extracellular calcium ions enter the vascular smooth muscle cell in response to substances which produce contraction (Briggs, 1962 ; Waugh, 1962 ; Briggs & Shibata, 1966 ; Shibata & Carrier, 1967). In addition to the entry of extracellular calcium, the activity of some vasoactive drugs is best explained by the release of membrane-bound intracellular calcium in the vicinity of the contractile proteins (Hinke *et al.*, 1964 ; Cuthbert & Sutter, 1965 ; Jhamandas & Nash, 1967 ; Hudgins & Weiss, 1968).

Several non-steroid anti-inflammatory drugs inhibit constriction of isolated blood vessels in response to a variety of vasoactive drugs (Northover, 1967a). Constriction of depolarized blood vessels in response to calcium is also antagonized by these anti-inflammatory drugs. The present experiments were designed to investigate whether these antagonists prevent the entry of calcium into the muscle cell or whether they prevent the calcium which has entered the cell from causing the contractile proteins to shorten.

Methods

Anterior mesenteric artery

The dissection and cannulation of the artery have previously been described in detail (Northover, 1967a, b). The cannulated blood vessel was perfused with an aerated electrolyte solution at 38° C. The rate of perfusion was controlled with a roller pump and was adjusted at the beginning of an experiment to give a perfusion pressure of 30 cm H_2O. The flow rate was usually in the range 4–10 ml./min and was held constant for the duration of any one experiment. The perfusion pressure was recorded with a water manometer, the float of which marked a smoked paper kymograph.

When adrenaline was used to produce constriction the perfusing fluid had the following composition (mM): NaCl 138, KCl 2.74, $NaHCO_3$ 10.1, $MgCl_2$ 1.06, $CaCl_2$ 0.582, NaH_2PO_4 0.416, glucose 5.68. For all other experiments the following depolarizing solution was used (mM): K_2SO_4 92, $KHCO_3$ 10.0, $MgCl_2$ 1.06, NaH_2PO_4 0.416, glucose 5.68, with or without $CaCl_2$. For experiments in which barium was used K_2SO_4 was replaced by KCl (138 mM). Solutions from which $CaCl_2$ was intentionally omitted are referred to as calcium-free. Although " Analar " grade reagents (British Drug Houses Ltd.) were used, a small amount of calcium may have been present as a contaminant.

Aorta

The thoracic aorta was removed from freshly killed rats which had been injected intravenously with 1,000 units of heparin 5 min before death. Each aorta was opened lengthwise and the strips so formed were cut transversely into three pieces. With fine scissors and forceps the tributaries of the aorta and the loosely adherent connective tissue were carefully removed. Failure to take this precaution produced erratic results. Each piece of aorta was blotted gently with paper tissue and

weighed in a tared stoppered bottle containing 5 ml. of calcium-free depolarizing solution. The pieces usually weighed 8–16 mg. A length of cotton thread was attached to each piece of aorta so that it could be moved rapidly from one solution to another. Subsequent steps were conducted at 38° C. Each piece of aorta was incubated for 2 hr in 30 ml. of calcium-free depolarizing solution, the bathing fluid being replaced by fresh solution at 20 min intervals.

To load the tissue with ^{45}Ca, each piece of aorta was incubated with 2.5 ml. of depolarizing solution containing calcium (0.36 mM) labelled with 20 μc ^{45}Ca/ml. (obtained from the Radiochemical Centre, Amersham, Buckinghamshire). To measure the amount of ^{45}Ca in the piece of aorta, it was blotted lightly with paper tissue, ashed in a crucible in a muffle furnace at 600° C for 1 hr, and the ash dissolved in 3.2 ml. of a mixture consisting of 1 part by volume of concentrated nitric acid and 3 parts by volume of 0.1% $CaCl_2$ solution. The radioactivity of this solution was measured by transferring 3 ml. to a transparent plastic scintillator cup (Nuclear Enterprises NE 102) mounted directly on top of a photomultiplier tube in the manner described by O'Kelley (1962).

Results

Effect of calcium on the mesenteric artery perfused with calcium-free depolarizing solution

The mesenteric artery was first perfused with calcium-free depolarizing solution. Without interruption of the perfusion, the solution was changed to one containing calcium, causing the artery to constrict. When perfusion with calcium-free solution was resumed the artery slowly dilated again. The threshold concentration of $CaCl_2$ necessary to cause a detectable constriction was 0.04–0.08 mM, higher concentrations producing a greater effect. Constrictor responses to 3.49 mM-$CaCl_2$ were obtained repeatedly at 40 min intervals for several hours, provided the artery was perfused with a calcium-free solution between responses. The inclusion of indomethacin in the perfusing fluid inhibited the responses to calcium (Fig. 1). Table 1 gives the approximate minimum concentrations of indomethacin and other drugs required to abolish the response to calcium.

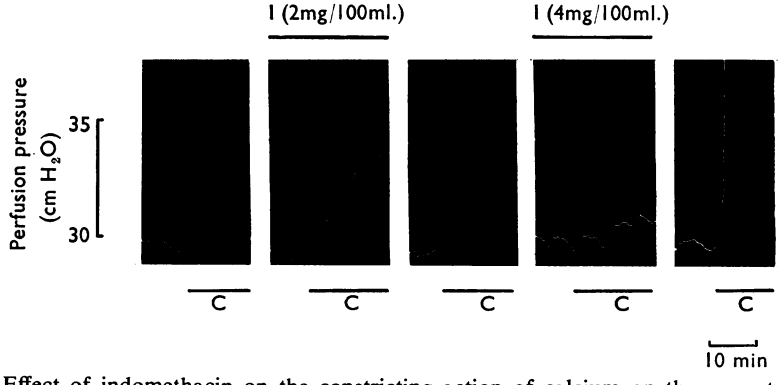

FIG. 1. Effect of indomethacin on the constricting action of calcium on the mesenteric artery perfused with calcium-free depolarizing solution. $CaCl_2$ (3.49 mM) was present during the periods marked C. Indomethacin was present in the perfusing fluid during the periods marked I.

Continued perfusion with calcium-containing (0.2 mM) depolarizing solution caused a persistent elevation of the perfusion pressure. The addition of indomethacin (4 mg/100 ml.) to the calcium-containing perfusing fluid caused a rapid and complete relaxation of the vessel (Fig. 2). Cinchocaine hydrochloride (5 mg/100 ml.) also completely relaxed the calcium-induced spasm of the artery.

When calcium-free depolarizing solution was used continuously for perfusion, the injection of 0.05–0.1 ml. of a $CaCl_2$ solution (117 mM, approximately isotonic with the perfusing fluid) close to the cannula caused a rise in perfusion pressure. The rise was immediate, quite brief, and could be measured accurately from the kymograph record. The rise in perfusion pressure was reduced by the inclusion

TABLE 1. *Antagonist effects of drugs on the constricting actions of adrenaline, $CaCl_2$ and $BaCl_2$ on the mesenteric artery*

Antagonist	Concentration required to reduce by 50% the response to injection into the cannula of 0·05–0·10 ml. of solutions of			Approximate concentration required to abolish response to $CaCl_2$ (3·49 mM) (mg/100 ml.)
	Adrenaline (10 μg/ml.)	$CaCl_2$ (117 mM) (mg/100 ml.)	$BaCl_2$ (100 mM)	
Indomethacin	3·5	3·1	3·0	4
Flufenamic acid	1·8	0·64	0·76	1
Aminopyrine	29	15	Not tested	Not tested
Imipramine hydrochloride	0·092	0·11	0·071	0·4
Desipramine hydrochloride	0·061	0·017	0·043	0·1
Papaverine	0·074	0·58	0·20	0·7
Benzindamine hydrochloride	0·14	Not tested	0·44	1
Oxolamine citrate	5·1	Not tested	12	40
Chloroquine diphosphate	0·61	0·25	1·2	6
Lignocaine hydrochloride	15	4·6	16	30
Amethocaine hydrochloride	3·9	0·58	3·5	9
Cichocaine hydrochloride	1·7	Not tested	1·0	2
Caffeine	8·9	23	11	20
Aminophylline	10	12	>100	>100

Responses to calcium and barium were obtained with a depolarizing perfusion fluid and those to adrenaline with a non-depolarizing perfusion fluid.

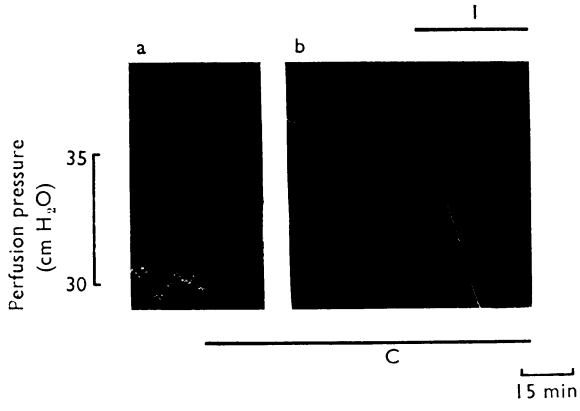

FIG. 2. Relaxation by indomethacin of calcium-induced spasm of the mesenteric artery perfused with calcium-free depolarizing solution. $CaCl_2$ (0.2 mM) was present during the period marked C. Indomethacin (4 mg/100 ml.) was present during the period marked I. The drum was stopped for 30 min between (a) and (b). The pressure calibration is the same for (a) and (b).

of indomethacin in the perfusing fluid (Fig. 3). The percentage inhibition of the response to calcium was calculated by the method described earlier (Northover, 1967a). Table 1 gives for various drugs the concentrations required to reduce the response to calcium by 50%.

For comparison, Table 1 also includes the concentrations of the various antagonists which were required to reduce by 50% the constricting effect of adrenaline (0.5 μg) on the artery when it was perfused with a calcium-containing non-depolarizing solution (Northover, 1967a). The concentrations of a particular drug required to inhibit the responses to adrenaline and to calcium were usually similar. Aminophylline was an exception, being ineffective against the response to calcium even in a concentration ten times greater than that required to abolish the response to adrenaline. On the other hand, caffeine, a related xanthine derivative, was equally effective against both adrenaline and calcium.

If an inhibitor of constriction prevented the entry of calcium ions into the muscle cell, the concentration of calcium ions in the perfusing fluid might be expected to influence its action. Indomethacin was tested against the response to adrenaline

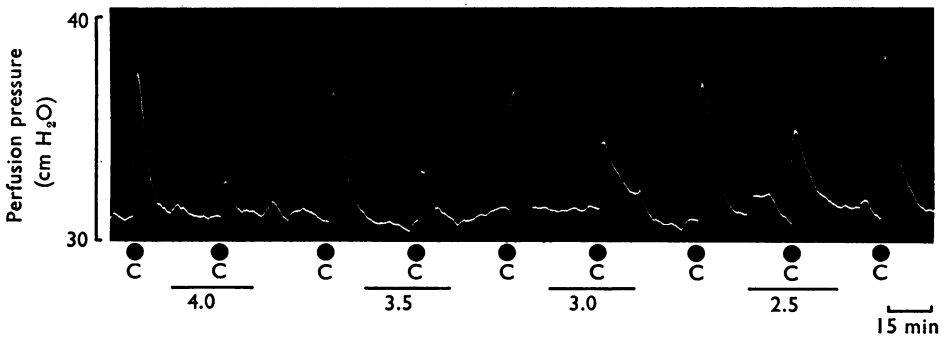

FIG. 3. Effect of indomethacin on the constricting action of calcium on the mesenteric artery perfused with calcium-free depolarizing solution. At C, 0.1 ml. of $CaCl_2$ solution (117 mM) was injected into the cannula. Indomethacin (mg/100 ml.) was present during the periods indicated by the horizontal bars.

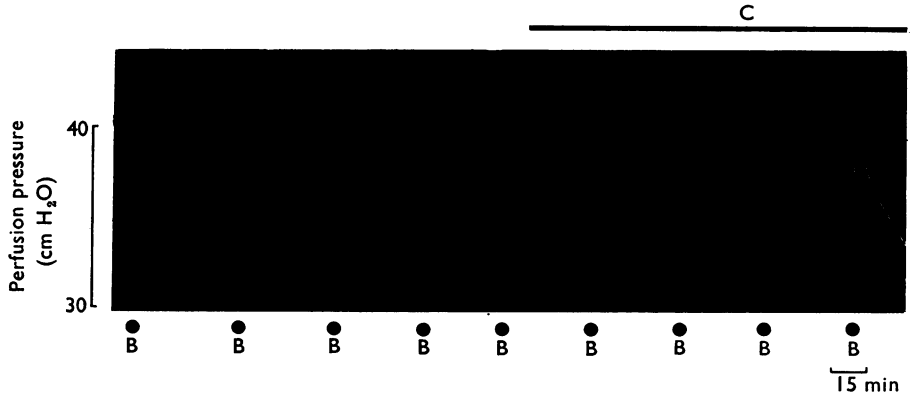

FIG. 4. Effect of $BaCl_2$ on the mesenteric artery perfused first with a depolarizing solution which was calcium-free and later contained calcium (0.05 mM) during the period marked C. At B, 0.05 ml. of $BaCl_2$ solution (100 mM) was injected into the cannula.

in a non-depolarizing solution ; variation of the concentration of calcium between 0.5 and 1.8 mM did not alter the depressant effect of indomethacin.

Effect of barium on the mesenteric artery perfused with calcium-free depolarizing solution

The injection of 0.05–0.1 ml. of a $BaCl_2$ solution (100 mM, approximately isotonic with the perfusing fluid) into the stream of calcium-free depolarizing solution perfusing the mesenteric artery caused constriction of the vessel. During several hours' perfusion with calcium-free solution, however, the response to barium gradually waned (Fig. 4). The addition of a little $CaCl_2$ (0.05 mM) to the perfusing fluid, when the response to barium had almost disappeared, caused a small but maintained constriction of the vessel and the restoration of the response to barium. The response to barium would therefore seem to depend on tissue stores of calcium which are lost only after prolonged perfusion with calcium-free solution.

Several drugs were tested for their ability to inhibit the response to barium. A depolarizing solution containing a small amount of $CaCl_2$ (0.05 mM) was used throughout to prevent tachyphylaxis to barium. It can be seen from Table 1 that the effects of barium or calcium were usually antagonized by the same drugs and in approximately the same concentrations. In some earlier experiments indomethacin and flufenamate were tested against the response to barium when the perfusing fluid was calcium-free. Although the responses to barium were less consistent than those in arteries perfused with a calcium-containing solution, the inhibitory effects of indomethacin and flufenamate were still clearly seen.

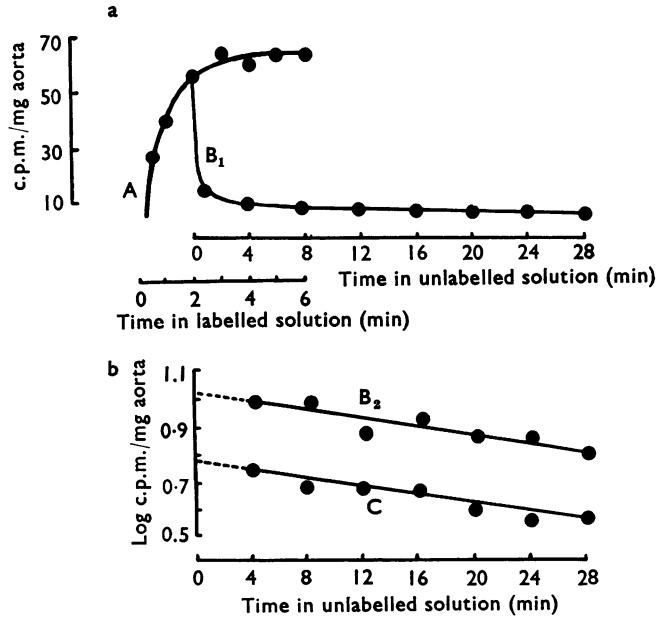

FIG. 5. Uptake of ^{45}Ca and its subsequent loss from aorta soaked for 2 hr in calcium-free depolarizing solution. Radioactivity is expressed as c.p.m./mg of aorta. (a) Line A represents uptake of ^{45}Ca, and line B_1 its subsequent loss. (b) Radioactivity on a logarithmic scale. Line B_2 is the slow phase of line B_1. Line C is the slow phase of the loss of ^{45}Ca from pieces of aorta treated with amethocaine (10 mg/100 ml.) during both uptake and loss of ^{45}Ca.

Uptake of ^{45}Ca by the aorta

Aortic segments which had been soaked for 2 hr in calcium-free depolarizing solution were incubated in calcium-containing (0.36 mM) depolarizing solution labelled with ^{45}Ca. After varying times in the labelled solution the pieces of aorta were removed, blotted lightly with paper tissue and ashed. Figure 5 shows that the content of ^{45}Ca increased with increasing time of incubation in the labelled solution (line A), saturation being reached in 3–5 min. If, however, after 2 min in the labelled solution the pieces of aorta were removed and placed for varying lengths of time in unlabelled calcium-containing (0.36 mM) depolarizing solution, the efflux of ^{45}Ca could be followed. Figure 5 shows that ^{45}Ca is lost rapidly at first (line B_1), but much more slowly later. The radioactivity in the aorta during the slow phase of efflux declined almost exponentially, so that when it was plotted on a logarithmic scale (line B_2), a straight line was obtained. This line was extrapolated to time zero in order to obtain the amount of radioactivity which was present in the pool of slowly lost ^{45}Ca at the end of incubation in the labelled solution (Liew, 1962).

Feinstein (1963) reported that amethocaine retards the influx of calcium into skeletal muscle in certain conditions. The effect of amethocaine was tested, therefore, on the uptake of ^{45}Ca into the depolarized aorta. When amethocaine (10 mg/100 ml.) was present throughout the experiment, it reduced the content of slowly lost ^{45}Ca present in the aorta at the end of 2 min incubation in the labelled solution (Fig. 5, line C); this concentration of amethocaine also inhibited constriction of the depolarized artery in response to calcium. In nine experiments the mean content of ^{45}Ca of the amethocaine-treated tissues was 69% of that of the controls; this difference was significant ($P<0.05$). The efflux rate of the slowly lost ^{45}Ca was, however, unchanged. In a concentration of 50 mg/100 ml. amethocaine did not have a significantly greater effect, and in a concentration of 5 mg/100 ml. the effect on the uptake of ^{45}Ca was erratic.

Because in these experiments the aorta had been soaked in calcium-free solution for 2 hr before incubation in the calcium-containing labelled solution, it may be assumed that its content of slowly lost ^{45}Ca at the end of the 2 min incubation was probably due partly to the exchange of ^{45}Ca with residual stores of calcium and partly to a net influx of calcium. When the content of slowly lost ^{45}Ca was measured in twelve pieces of aorta incubated for 2 min in labelled solution after washing for 2 hr in depolarizing solution containing the same concentration of $CaCl_2$ (0.36 mM)

TABLE 2. *Effect of drugs on content of slowly-lost ^{45}Ca in aorta*

Drug	Concentration (mg/100 ml.)	Extrapolated ^{45}Ca content (controls=100)
Amethocaine hydrochloride	10	69*
Cinchocaine hydrochloride	5	74*
Indomethacin	10	105
Desipramine hydrochloride	1	103

The aorta was depleted of calcium by soaking it in calcium-free depolarizing solution for 2 hr before 2 min incubation in calcium-containing (0·36 mM) depolarizing solution containing ^{45}Ca; the ^{45}Ca content at the end of the incubation period was extrapolated from the regression line for the efflux of ^{45}Ca.

* The value was significantly different from that of the control group ($P<0·05$). Each value is the mean of nine experiments.

as the labelled solution, it was found to be only 57% of that of pieces of aorta soaked in calcium-free solution for 2 hr before the experiment. In these conditions the content of slowly lost ^{45}Ca probably represents the ^{45}Ca which exchanges with the slowly lost tissue stores of calcium during the 2 min incubation in the labelled solution. Because amethocaine (10 mg/100 ml.) did not significantly reduce the content of slowly lost ^{45}Ca in experiments in which the calcium concentration was maintained constant throughout, amethocaine did not hinder the uptake of ^{45}Ca due to exchange with unlabelled calcium but reduced the net uptake of calcium into a calcium-depleted aorta.

The uptake of calcium into a calcium-depleted aorta probably involves not only the smooth muscle but also the connective tissue of the wall (Keatinge, 1968). It was therefore necessary to determine the effect of amethocaine on the uptake of ^{45}Ca into connective tissue. Pieces of hairless skin were taken from the plantar surface of the hind feet of freshly killed rats. After incubation for 2 hr in calcium-free depolarizing solution they were transferred to labelled calcium-containing solution (0.36 mM). The pieces of skin were then transferred to unlabelled calcium-containing solution (0.36 mM) and the efflux of ^{45}Ca measured. The changes in the content of ^{45}Ca with time were strikingly similar to those obtained with pieces of aorta. Amethocaine, however, even when present throughout the experiment in a concentration of 10 mg/100 ml., failed to have any effect on the content of slowly lost ^{45}Ca in the skin. Because amethocaine appeared to have no effect on the uptake of calcium into connective tissue, albeit from the skin, it was concluded that the ability of amethocaine to inhibit uptake of calcium in the aorta was due to the inhibition of uptake of calcium into the calcium-depleted smooth muscle cells.

Cinchocaine, in a final concentration of 5 mg/100 ml. was found to reduce the uptake of ^{45}Ca into the slowly lost pool of the calcium-depleted aorta (Table 2). Indomethacin (10 mg/100 ml.) and desipramine (1 mg/100 ml.) were also tested, but even in these high concentrations neither drug had a significant effect on the uptake of ^{45}Ca (Table 2).

Discussion

In the present experiments, contraction of vascular smooth muscle was produced in one of three ways—namely, by the administration of adrenaline or by the administration of calcium or barium ions to muscle depolarized by potassium. The results obtained by Briggs (1962) and Waugh (1962) indicate that vascular smooth muscle which is depolarized by potassium, in contrast to normally polarized muscle, permits the rapid entry of extracellular calcium ions into the interior of the muscle cell. The contraction which results from the application of calcium ions to muscle previously bathed in calcium-free depolarizing solution can be accounted for by the penetration of the muscle cell by the calcium ions supplied in the extracellular fluid. Contraction of vascular smooth muscle in response to adrenaline seems to involve a different mechanism. The experiments of Hinke (1965), Jhamandas & Nash (1967) and Hudgins & Weiss (1968) indicate that during contraction in response to catecholamines the majority of the calcium ions which reach the contractile proteins come from a previously membrane-bound intracellular store. Barium ions may contract smooth muscle both by causing the contractile proteins to shorten directly and by releasing intracellular membrane-bound calcium (Daniel, 1964). In the conditions of the present experiments, however, the second

mechanism seems to operate almost exclusively. The store of membrane-bound calcium which is involved is lost at a very slow rate during treatment with a calcium-free depolarizing solution. Similar findings have been reported recently by Karaki, Ikeda & Urakawa (1967) and Hotta & Tsukui (1968).

Thus it may be said that agents which cause contraction of vascular smooth muscle make calcium ions available to the contractile proteins, but the origin of the calcium ions varies with the nature of the excitor agent. The effectiveness of the antagonists studied did not, however, appear to be related to the nature of the excitor drug. In fact, most of the antagonists studied in the present experiments were equally effective against the constriction caused by adrenaline in the artery perfused with normal salt solution and against that produced by calcium or barium in the artery perfused with a depolarizing solution. This suggests that the antagonists were not preventing calcium ions from being made available to the contractile proteins, but rather preventing the response of the contractile proteins to calcium ions. Furthermore, indomethacin rapidly relaxes the spasm observed in an artery previously perfused for a long period with calcium-containing depolarizing solution, that is, in a situation in which calcium ions must have already gained entrance to the vicinity of the contractile proteins.

The experiments with ^{45}Ca suggest that amethocaine and cinchocaine reduce the rate at which calcium ions enter the calcium-depleted smooth muscle cell. In the same conditions, however, indomethacin and flufenamate were ineffective. By exclusion, it is inferred that the ability of indomethacin and related drugs to antagonize vascular constriction is dependent on their ability to prevent calcium ions from causing the contractile proteins to shorten.

The mode of action of amethocaine and cinchocaine is complex. In addition to preventing the entry of ^{45}Ca into the muscle cell, they probably also antagonize the action of calcium ions on the contractile proteins, since they are capable of rapidly relaxing a calcium-induced spasm of the artery perfused with depolarizing solution. Local anaesthetics generally antagonize the contractile effects of a variety of agents (Bloom & Schoepfle, 1963). There are few reports, however, of their action on vascular smooth muscle. Fleckenstein (1952) found that several local anaesthetics antagonized the constrictive action of adrenaline in the blood vessels of the perfused rabbit ear. Åström (1964) found that local anaesthetics had an inhibitory effect on adrenaline-induced contractions of aortic strips. Contractions due to histamine and acetylcholine were also inhibited. Hudgins & Weiss (1968) found that procaine depressed the response of aortic strips to adrenaline more effectively than the response to depolarization by potassium. Nava-Rivera, Gutiérrez-López, Ferez & Eisenberg (1967) found that procaine reduced the response of aortic strips to adrenaline, whereas lignocaine and prilocaine potentiated it. In the present experiments no such potentiation was seen and the responses to adrenaline, calcium or barium were all inhibited equally.

The action of local anaesthetics on the efflux of calcium has been studied in various types of smooth muscle. Kuperman, Altura & Chezar (1968) found that procaine and amethocaine increased the rate of the slow component of calcium efflux from resting muscle. Hudgins & Weiss (1968) found that procaine overcame the retarding effect of adrenaline on the slow phase of efflux from aortic strips. No acceleration by local anaesthetic drugs of efflux of ^{45}Ca was seen, however, in

the present experiments. The fact that depolarized muscle was used in the present experiments may possibly account for the discrepancy.

In the present experiments the xanthine derivatives, caffeine and aminophylline, were found to antagonize the response to adrenaline. Caffeine, but not aminophylline, also antagonized the action of calcium on the artery perfused with a depolarizing solution. The experiments of Somlyo & Somlyo (1968) indicate that caffeine has both stimulant and inhibitory actions on several types of vascular smooth muscle. The resultant effect will depend on which of these opposing actions predominates in a particular set of circumstances. The present experiments suggest that the inhibitory action is predominant in vascular smooth muscle. This conclusion is supported by the findings of Axelsson & Högberg (1967).

Schild (1967) has provided evidence that the inhibitory action of isoprenaline on the depolarized rat uterus is due to the promotion of uptake of calcium ions into storage sites within the muscle cell, fewer calcium ions then being available to the contractile proteins. It seems unlikely, however, that a similar mechanism could account for the inhibitory actions of the drugs used here. In the first place, isoprenaline in concentrations of up to 0.1 mg/ml. failed to reduce the vascular constriction produced by adrenaline, calcium or barium (unpublished observations). Second, in the experiments of Schild isoprenaline was ineffective against contractile responses to barium, whereas the antagonists studied in the present experiments showed as much effect against the response to barium as they did against the responses to calcium or adrenaline. Finally, it is hard to see how calcium storage sites within the smooth muscle cell could have sufficient capacity to account for the fact that the mesenteric artery perfused with a depolarizing solution containing calcium (1 mM) and indomethacin (5 mg/100 ml.) failed to constrict, even when the perfusion was continued without interruption for 3 hr.

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