J. Physiol. (1961), **157**, *pp.* 90–96
With 4 text-figures
Printed in Great Britain

THE CALCIUM DEPENDENCE OF TENSION DEVELOPMENT IN DEPOLARIZED SMOOTH MUSCLE

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(*Received* 28 *November* 1960)

It is known that several types of smooth muscle contract on exposure to acetylcholine (ACh), even when depolarized by immersion in isotonic solutions of potassium chloride or sulphate (Evans, Schild & Thesleff, 1958). The present study originated from the finding that the magnitude of this response was dependent on the calcium content of the bathing fluid. It seemed possible that this might provide a clue to the mechanism of the contracture, for there is evidence to suggest that tension development in the heart (Niedergerke, 1956; Lüttgau & Niedergerke, 1958), and possibly in skeletal muscle (Bianchi & Shanes, 1959; Frank, 1960) is initiated by additional entry of calcium into the cells. If smooth muscle is similar in this respect, it is conceivable that the ACh contracture is a direct consequence of the action of the drug in increasing the permeability of the membrane to calcium (Durbin & Jenkinson, 1961). It thus seemed of interest to examine the effects of a reduction in the calcium concentration on the magnitude of (*a*) the contracture and (*b*) the simultaneous increase in membrane permeability which follow the application of a stable analogue of ACh, carbachol, to a smooth muscle bathed in potassium-rich solution.

METHODS

The experiments were made at room temperature (19–23° C) with isolated portions of the taenia coli of the guinea-pig. For measurements of the rate of potassium exchange a strip of muscle was attached to a stainless-steel frame and exposed for several hours to a solution containing ^{42}K. It was then transferred to a continuously flowing stream of inactive fluid and the loss of tracer followed; the tension developed by the strip was recorded simultaneously by means of a transducer valve coupled to a pen recorder. Throughout such experiments the preparations were bathed in solutions containing for the most part potassium sulphate (76 mM) and potassium bicarbonate (16 mM). It should be noted that a considerable part of the total concentration (7·5 mM) of calcium normally included may have been in a combined form, because of binding by carbonate and by sulphate (Hill & Howarth, 1957; Hodgkin & Horowicz, 1959). Further details of the composition of this and other solutions used, and of the procedure in tracer experiments, have been given in the preceding paper (Durbin & Jenkinson, 1961).

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RESULTS

The first experiments were made to determine the calcium dependence of the response of the taenia coli to prolonged depolarization by application of the potassium-rich solution used in much of the present work. A typical 'potassium contracture' is illustrated in Fig. 1. It may be seen that the tension first rose rapidly, and then fell more slowly to an inter-

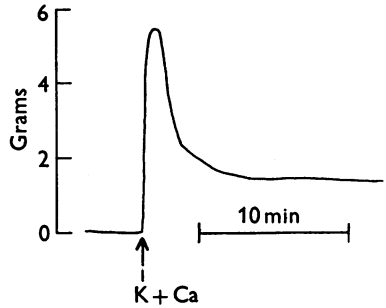

Fig. 1. Mechanical response of the taenia coli to replacement of the Krebs's bathing fluid by a solution containing 168 mM potassium and 7·5 mM calcium. Isometric recording at room temperature.

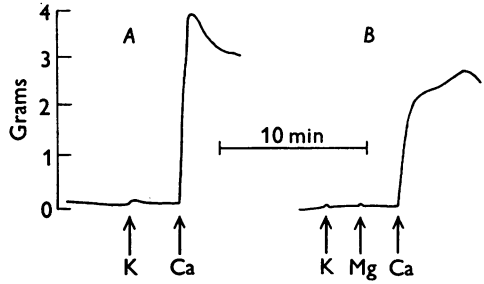

Fig. 2. Calcium dependence of 'potassium contracture'. In *A*, the muscle strip had been bathed for 15 min in calcium-free Krebs's fluid. At first arrow, this was replaced by calcium-free potassium-rich solution. At second arrow, calcium chloride (7·5 mM) was added. In *B*, obtained using another strip, the reintroduction of calcium was preceded by the addition of 30 mM magnesium chloride.

mediate level, hereafter referred to as 'resting' tension. This rarely declined further, and with some preparations tended to increase over a period of several hours. Its value was not affected by omission of the 5·7 mM sodium normally included in the potassium-rich fluid.

On repeating the experiment of Fig. 1, but with Ca-free solutions, it was found that the 'potassium contracture' did not then occur. A similar observation has been reported for frog skeletal muscle (Denton, 1948; Frank, 1960), for the heart (Niedergerke, 1956) and, most recently, for rat uterine muscle (Edman & Schild, 1961). Restoration of calcium, but not of

magnesium, was followed by an immediate contracture, as illustrated in Fig. 2. As might be expected, subsequent alterations in the calcium concentration produced corresponding changes in 'resting' tension (Fig. 3*a* and *b*).

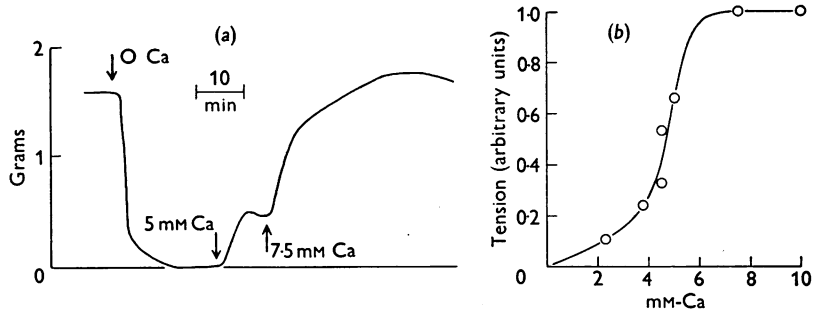

Fig. 3. (*a*) Calcium dependence of maintained phase of 'potassium contracture'. Bathing fluid contained 168 mM potassium throughout; initial and final calcium concentration, 7·5 mM. (*b*) 'Resting' tension developed by another strip of the taenia coli as a function of calcium concentration. Values expressed as fractions of that in a potassium-rich fluid containing 7·5 mM calcium.

Calcium dependence of the response to carbachol

It has been shown in the preceding paper (Durbin & Jenkinson, 1961) that the taenia coli of the guinea-pig contracts in response to carbachol even when bathed in potassium-rich fluid; at the same time, an increase in the permeability to certain inorganic ions occurs. The experiments described in this section were made to determine the effects of changes in calcium concentration on these actions of carbachol. For this purpose the rate of efflux of ^{42}K in exchange for inactive potassium was taken as a measure of membrane permeability.

A first experiment showed that 15 min after the introduction of a calcium-free solution both the increase in tension, and less expectedly, the change in permeability produced by a standard application of carbachol, had fallen reversibly to less than 5 % of the control values. The effects of intermediate reductions in the calcium content of the bathing fluid were then tested, as illustrated in Fig. 4, where it may be seen that there was little mechanical response to carbachol applied in a solution containing one fifth of the previous calcium concentration, although an appreciable increase in the rate of efflux of ^{42}K still occurred. The results of this and four other such experiments are listed in Table 1. Although the sensitivity to calcium varied considerably from preparation to preparation, the reduction in the contracture tension exceeded that in the change in permeability produced by carbachol in every case.

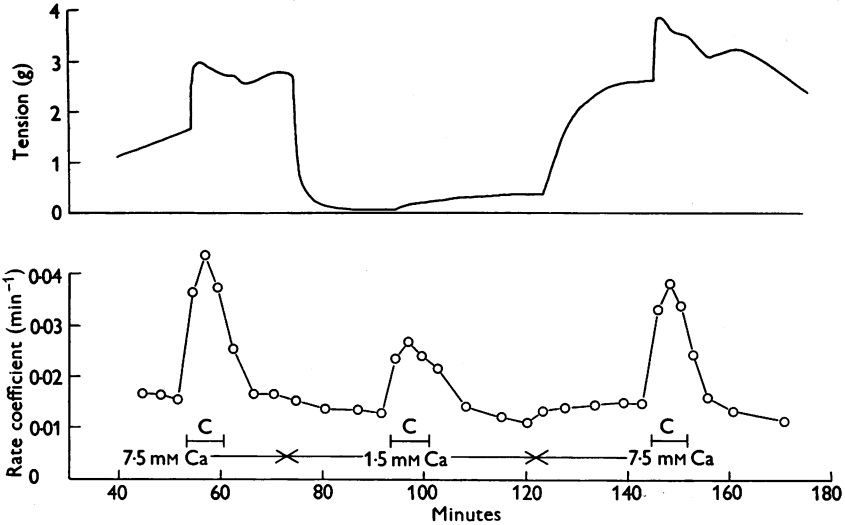

Fig. 4. Effect of a reduction (from 7·5 to 1·5 mM) in calcium concentration on the increases in tension and in rate of exchange of labelled potassium produced by a standard application of carbachol (3×10^{-7} g/ml. for 7 min). Potassium concentration 168 mM throughout; tonicity of low calcium solutions maintained with sucrose. Upper and lower ordinates, respectively, isometric tension (g), and rate of loss of ^{42}K, measured as described in preceding paper (Durbin & Jenkinson, 1961).

TABLE 1. Summary of five experiments of the type illustrated in Fig. 4. In each, a strip of taenia coli previously loaded with ^{42}K has been bathed successively in inactive potassium-rich solutions containing the concentrations of calcium listed in columns 1, 2 and 3. Carbachol (3×10^{-7}g/ml.) was applied in each solution, and the corresponding tension responses are listed in columns 4, 5 and 6 respectively. Columns 7, 8 and 9 give the simultaneously measured increases in the rate of loss of ^{42}K, expressed in terms of R_e, as defined in the preceding paper (Durbin & Jenkinson, 1961). For comparison, the effects on tension and ^{42}K flux produced by the standard concentration of carbachol when applied in low calcium solutions have been expressed as percentages of the mean values of the controls in columns 10 and 11 respectively

	1	2	3	4	5	6	7	8	9	10	11
	[Ca] (mM)			ΔT (g)			R_e			%	%
Expt.										tension	flux
1	3·5	0·70	7·0	0·7	0·1	0·7	1·7	1·3	1·9	14	72
2	7·5	0·20	7·5	2·9	0·25	2·7	2·0	0·5	2·6	9	22
3	7·5	0·85	7·5	3·5	0·05	3·2	5·1	1·7	5·0	1·5	34
4*	7·5	1·50	7·5	1·25	0·20	1·2	2·2	1·6	2·3	16	71
5	7·5	0·23	7·5	3·15	0·15	2·8	2·2	0·95	2·7	5	39

* Fig. 4.

DISCUSSION

The necessity of calcium for 'potassium contractures' of the taenia coli of the guinea-pig, and of the uterus of the rat (Edman & Schild, 1961), suggests that calcium plays an important part in the mechanical response

of these varieties of smooth muscle. Further evidence on this point has recently been obtained by Axelsson & Bülbring (1959), who have shown that as with heart muscle (Mines, 1913), the response of the taenia to propagated action potentials may be abolished by a suitable reduction in the external calcium concentration. In general, the actions of calcium on the taenia and on the heart are so similar that it seems reasonable to suppose that the process whereby contraction is activated is the same, and is initiated by a net movement of calcium from a superficial to a deeper part of the cell, as proposed by Lüttgau & Niedergerke (1958) on the basis of experiments with ventricular muscle (cf. Niedergerke, 1959). Such an influx of calcium may arise in different ways; Lüttgau & Niedergerke suggested that depolarization may cause heart muscle to contract by altering the distribution in the membrane of a negatively charged complex which acts as a 'carrier' for calcium. In the present experiments, however, the muscle fibres were already depolarized, so that it is improbable that this process can have contributed greatly to the contractures evoked by carbachol. A more likely explanation is to be found in the action of the drug in increasing the permeability of the membrane to calcium. This would give rise to a net influx, and so presumably initiate contraction, provided that a concentration gradient existed across the membrane. Although direct evidence for a low internal concentration of unbound calcium has so far been obtained with only one excitable tissue, the giant axon of the squid (Hodgkin & Keynes, 1957), it seems likely that this may be a rather general phenomenon; Gilbert & Fenn (1957) have shown that skeletal muscle can maintain the intracellular calcium concentration against an electrochemical gradient, probably by 'active transport'.

On this view the size of the carbachol contracture might be expected to vary with (*a*) the concentration gradient for calcium and (*b*) the extent of the increase in permeability. The finding that the effect of carbachol on ion flux is itself calcium dependent suggests that reductions in both (*a*) and (*b*) contribute to the failure of the mechanical response which occurs in solutions containing little calcium. However, the fall in (*a*) appears to be the more important factor, for it was possible, on suitably lowering the calcium concentration, to observe a relatively large action of carbachol on permeability, in the almost complete absence of tension development. It had been hoped to obtain more exact information on this point by using ^{45}Ca to make direct measurements of the effect of carbachol on the movement of calcium. Although the expected increase in uptake was observed, as described in the preceding paper (Durbin & Jenkinson, 1961), the complexity of the kinetics of exchange in this tissue made it difficult to study the effect quantitatively. It is possible that other varieties of smooth muscle may prove more satisfactory for this purpose; Robertson (1960)

has reported in a preliminary communication that ACh markedly increases the uptake of ^{45}Ca by depolarized longitudinal muscle from the ileum of the rabbit.

The rather variable effect of a reduction in the concentration of calcium on the permeability change produced by carbachol was of interest as it is known that the actions of ACh in depolarizing the end-plate region of frog skeletal muscle (del Castillo & Stark, 1952), and in increasing the ionic permeability of denervated muscle from the diaphragm of the rat (D. H. Jenkinson & J. G. Nicholls, unpublished observations), are not calcium dependent. This may reflect some difference in the receptors of smooth and skeletal muscle, but this can only be decided by a more detailed study of the actions of ACh and carbachol on these tissues.

SUMMARY

1. The mechanical responses of the taenia coli of the guinea-pig both to replacement of the Krebs's bathing fluid by potassium-rich solutions, and to subsequent applications of carbachol, are abolished in the absence of calcium.

2. Carbachol becomes less effective in increasing the membrane permeability of the depolarized taenia when the calcium content of the bathing fluid is lowered. However, over a certain range of calcium concentrations, the reduction in the accompanying contracture is greater, so that the two actions of the drug can be almost dissociated.

3. These findings are discussed in relation to the hypothesis that the carbachol contracture of depolarized smooth muscle is a consequence of net movement of calcium into the cells, following the increase in permeability produced by the drug.

We are indebted to Professor B. Katz for constant help and encouragement and to Mr J. L. Parkinson, Miss A. Paintin and Mr L. Ward for frequent assistance.

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