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The stimulant action of acetylcholine and catecholamines on the uterus

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The α action of catecholamines on oestrogen dominated guinea-pig uterus is stimulant. The cell membrane is depolarized, membrane conductance is increased, spike discharge is accelerated and tension develops. This action resembles that of acetylcholine though catecholamines are less potent, and, in equiactive concentrations, catecholamines have a longer latency and a longer duration of action.

Evidence, obtained by modifications of the ionic environment, indicates that the depolarization by acetylcholine is due to an increase in sodium and calcium permeability and that acetylcholine can release calcium from intracellular stores. The depolarization by catecholamines is due to an increase in chloride permeability and, in addition, sodium is required for the ensuing increase of spike discharge.

Catecholamines produce an increase in the force of contraction, long outlasting their immediate stimulation. Moreover, their effect on membrane potential and membrane conductance persists in the presence of lanthanum. These results suggest that Ca release from intracellular stores may be the primary effect produced by the α action of catecholamines and that the increase in the cytoplasmic Ca^{2+} concentration may cause the changes at the cell membrane.

The muscarinic action of acetylcholine and and α action of catecholamines on oestrogen-dominated guinea-pig uterus are superficially similar. Both cause contraction. The underlying electrical changes consist of depolarization, increased membrane conductance, and initiation or acceleration of spontaneous spike discharge (Bülbring, Casteels & Kuriyama 1968; Bülbring & Szurszewski 1971).

The differences between the cholinergic and adrenergic action include the difference in potency, the different latency and duration of the effects they produce, and differences in the long-term changes which follow the drug responses.

The purpose of this paper is to give a short description of a detailed investigation into the ionic basis for the responses of the guinea-pig myometrium to acetylcholine and noradrenaline (Bülbring & Szurszewski 1971, 1973*a*, *b*; Szurszewski 1973).

The results to be described were obtained from the myometrium of immature guinea-pigs which had been treated by daily injections of 5 μg oestradiol for 8 days. On the 9th day a strip of myometrium was set up in a double sucrose gap apparatus (Bülbring & Tomita 1969*a*), to record changes in membrane potential, membrane conductance and isometric tension. Electrotonic potentials were evoked by applying constant current pulses of alternating polarities for 3 s every 10 s. Drugs were applied for a period of 1 min by injection through a narrow side tube into the stream of the bathing solution. All solutions contained propranolol (1×10^{-6} g/ml) to exclude the β action of catecholamines.

Typical examples of the response to acetylcholine (1×10^{-6} g/ml) in the normal Krebs solution are shown in figures 1*a*, 2*a* and 4*a*. This concentration of acetylcholine caused a rapid discharge of spike potentials which led to a sustained depolarization during which the electrotonic potential was reduced to near zero. When the injection was stopped, the effect quickly wore off. Lower concentrations (10^{-9} to 4×10^{-7} g/ml) depolarized the membrane by 10 to

15 mV, reduced the electrotonic potential by 10 to 15 % and accelerated spontaneous spike discharge. These weaker effects were similar to those produced by a high concentration (1×10^{-6} g/ml) of catecholamines. Typical examples of responses to noradrenaline are shown in figures 4*c* and 6, and to adrenaline in figures 7*a* and 8*a*. In some tissues the increase in spontaneous spike discharge was small or absent, whereas in others there was a definite acceleration. Higher concentrations of catecholamines could not be used because the tissues did not recover and, therefore, the effects were not consistently reproducible.

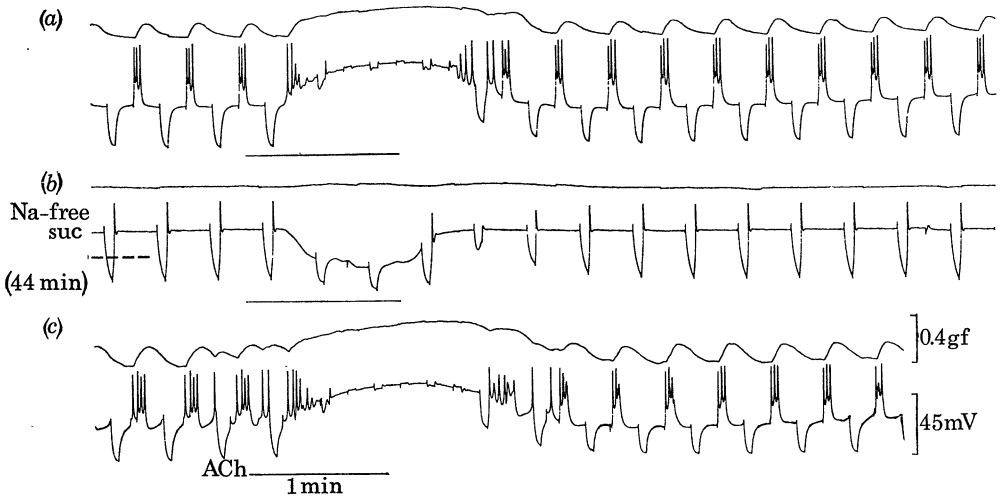

FIGURE 1. Effect of sodium-free solution (sucrose substitution) on the action of acetylcholine (1×10^{-6} g/ml). In each panel, the top tracing shows the tension, and the bottom tracing the electrical potential; the black bar indicates the period of drug application. In this and figures 2, 4, 6, 7 and 8, hyperpolarizing and depolarizing current pulses were applied alternately for 3 s duration every 10 s. (*a*) Control; (*b*) 44 min after changing to sodium-free solution. Dotted line in (*b*) represents original potential level; (*c*) control, 45 min after returning to Krebs solution.

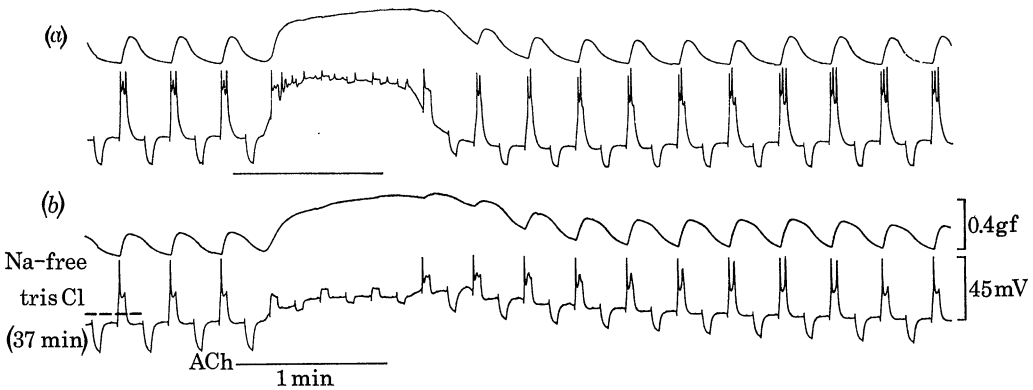

FIGURE 2. Effect of sodium free solution (tris Cl substitution) on action of acetylcholine (1×10^{-6} g/ml). (*a*) Control; (*b*) 37 min after changing to sodium-free solution.

The stimulant action of acetylcholine depended on the external sodium concentration. In sodium-free solution, using sucrose as the substitute for sodium chloride, the depolarization produced by acetylcholine was reversed to a hyperpolarization the extent of which depended on the external potassium concentration. An example of the reversal is shown in figure 1*b*.

When the impermeant cation, tris, was used to replace sodium, the depolarization by acetylcholine was reduced but was not abolished or reversed (figure 2*b*). Moreover, the increases in conductance and in tension were similar to those in the normal Krebs solution (figure 2*a*).

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The effect of varying the external sodium concentration on the depolarization produced by acetylcholine is shown in figure 3, left panel. When sucrose was used to replace NaCl, the maximum depolarization was related to the external concentration of sodium chloride. When tris was used as the substitute for sodium, the reduction of the peak level of depolarization in sodium-free solution was never as great as with sucrose. These observations suggested that the depolarization observed in the normal Krebs solution was due to an increase in sodium conductance but possibly, as will be shown below, also to an increase in calcium conductance, while there was no evidence for an increased chloride conductance.

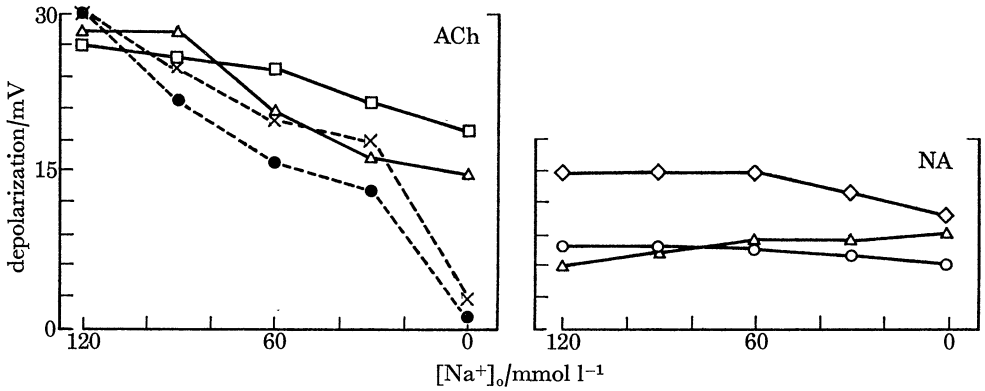

FIGURE 3. Effect of varying the external sodium concentration on the maximum depolarization produced by acetylcholine (ACh; 1×10^{-6} g/ml) and (*b*) noradrenaline (NA; 1×10^{-5} g/ml). Measured responses to acetylcholine and noradrenaline obtained 50 min after changing to the sodium-deficient solution. Symbols connected by the same line represent data obtained from same experiment. —, tris; – – –, sucrose.

The depolarization caused by noradrenaline or adrenaline was not sodium dependent; it was still observed in sodium-free solution (tris substitution). The effect of partial reduction and complete replacement of the external sodium by tris on the depolarization produced by noradrenaline is shown in the right hand panel of figure 3. There was no significant change and the greatest reduction of the noradrenaline depolarization was only 4 mV. However, the acceleration of spontaneous spike discharge was greatly reduced in Na-free solution, indicating that sodium was required for the stimulation of membrane activity.

Figure 4 shows the response of the myometrium to acetylcholine and noradrenaline in chloride-deficient solution, when glutamate was used as the substitute for chloride. It can be seen that even though the sodium concentration was 137 mmol/l, the depolarization and increase in conductance produced by noradrenaline was abolished after 42 min exposure (figure 4*d*), whereas the depolarization, increase in conductance and increase in tension produced by acetylcholine was little affected after 53 min exposure (figure 4*b*). Both the depolarization produced by noradrenaline and that caused by adrenaline was highly dependent on the external chloride concentration, whereas the stimulant action of acetylcholine was not.

Figure 5 summarizes the effects of varying the external chloride concentration, using different impermeant anions as substitutes, on the depolarization produced by acetylcholine and noradrenaline. In all instances when the concentration was lowered, and a steady state was reached after about 45 min, the depolarization by noradrenaline was reduced, abolished or reversed to hyperpolarization. From these results it was concluded that the depolarization produced by noradrenaline was mainly due to an increase in chloride conductance. In contrast, chloride ions do not seem to be necessary for the depolarization and reduction in membrane resistance

produced by acetylcholine since they were similar in the normal Krebs solution and in chloride-deficient solution.

Bülbring & Tomita (1969*b*, *c*) and more recently Bülbring (1972) proposed that both α and β responses in smooth muscle may not only depend on the presence of calcium in the external solution, but also affect the cellular distribution of calcium. For example, high external calcium

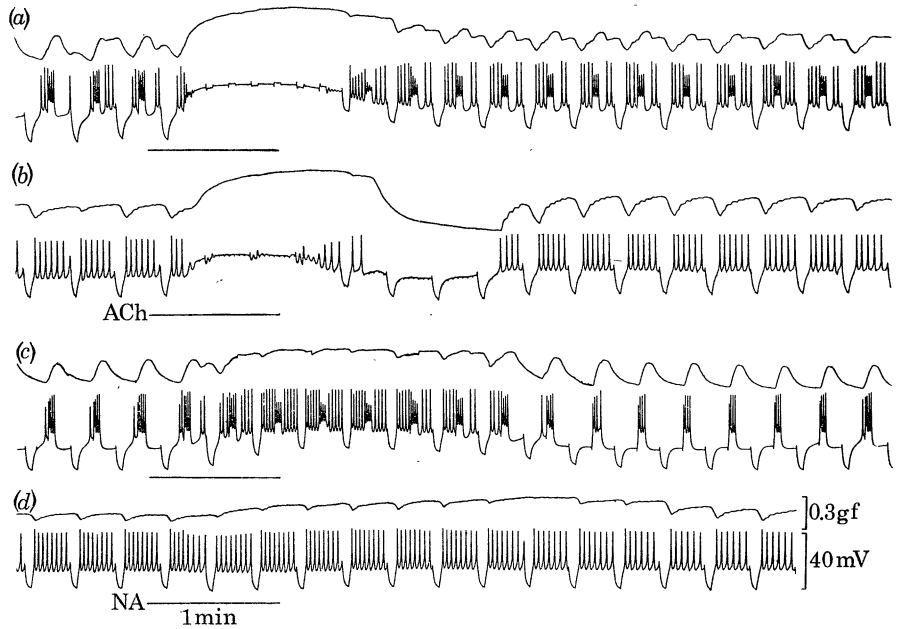

FIGURE 4. Effect of chloride-deficient (13 mmol/l) solution on the stimulant action of acetylcholine (1×10^{-6} g/ml) and noradrenaline (1×10^{-6} g/ml). (*a*) Effect of acetylcholine, and (*c*) effect of noradrenaline in Krebs solution; (*b*) 53 min and (*d*) 42 min after replacement of NaCl with sodium glutamate (Bülbring & Szurszewski 1971).

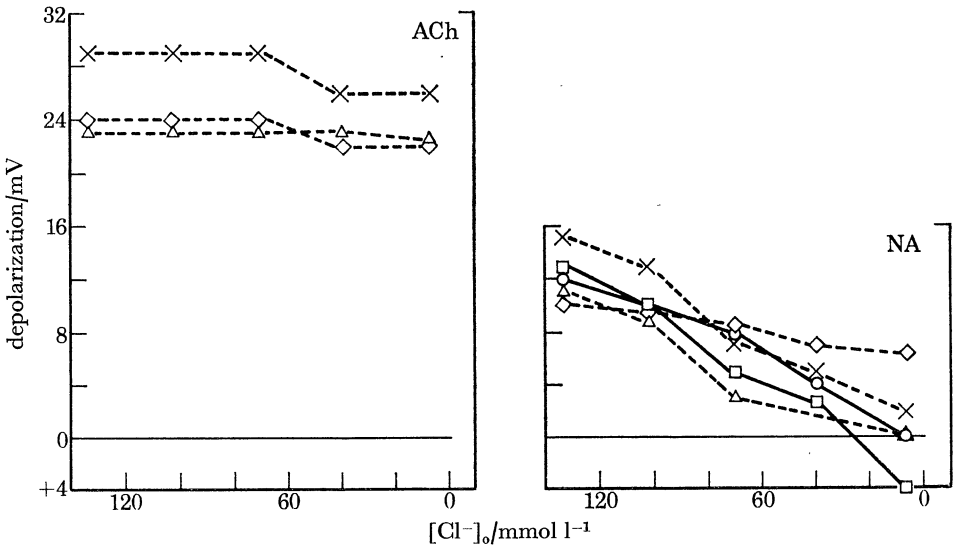

FIGURE 5. Effect of varying the external chloride concentration on the maximum depolarization produced by acetylcholine (ACh; 2×10^{-6} g/ml) and noradrenaline (NA; 1×10^{-5} glml). Measured responses to acetylcholine and noradrenaline obtained 50 min after changing to the chloride-deficient solution. Symbols attached by the same line represent data obtained from the same experiment. —, benzenesulphonate; - - -, isethionate.

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mimics the α action on the cell membrane both in the taenia coli (Bülbring & Tomita 1969*b*) and in the guinea-pig myometrium (Bülbring 1972). On the other hand, a high calcium concentration in the external solution antagonizes the β effect in both tissues, suggesting that part of the β action may be to reduce the concentration of free intracellular calcium (see Fleckenstein, Grün, Tritthardt & Byon 1971; Bülbring & Kuriyama, this volume, p. 115). There is evidence that the α action raises the intracellular level of ionized calcium by intracellular redistribution of calcium (see van Breemen *et al.*, this volume, p. 57). Such an action may be the cause for the frequent observation of a long-term positive inotropic change following the actual response of the myometrium to catecholamines. An example is shown in figure 6. After the initial stimulant effect of noradrenaline had subsided, there was a prolonged increase in the force of the phasic contractions and of the basic tone, although the membrane potential, the membrane resistance and the membrane excitation evoked by the depolarizing current pulses had returned to the same level as before the noradrenaline application. The prolonged potentiation of the tension response suggests that noradrenaline might cause an increase of the cytoplasmic calcium ion concentration, and that this effect can last for some time.

The relatively slow onset and the long duration of the response, outlasting the presence of the catecholamine, indicates the possibility that the increase of membrane permeability to chloride may be a secondary effect, produced primarily by the intracellular release of calcium.

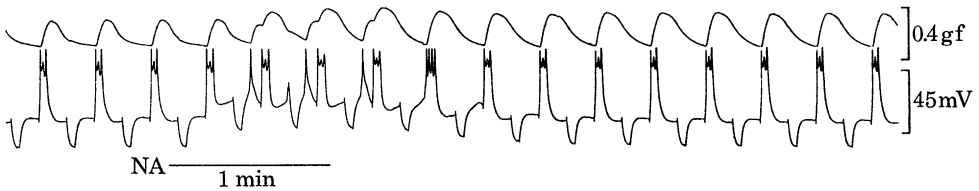

FIGURE 6. Effect of noradrenaline (1×10^{-6} g/ml) on the force of the phasic contractions in Krebs solution. Note that, after the initial depolarizing effect by noradrenaline, the size of phasic contraction is potentiated even though the membrane potential and the responses to hyperpolarizing and depolarizing current are similar to those before injection of noradrenaline.

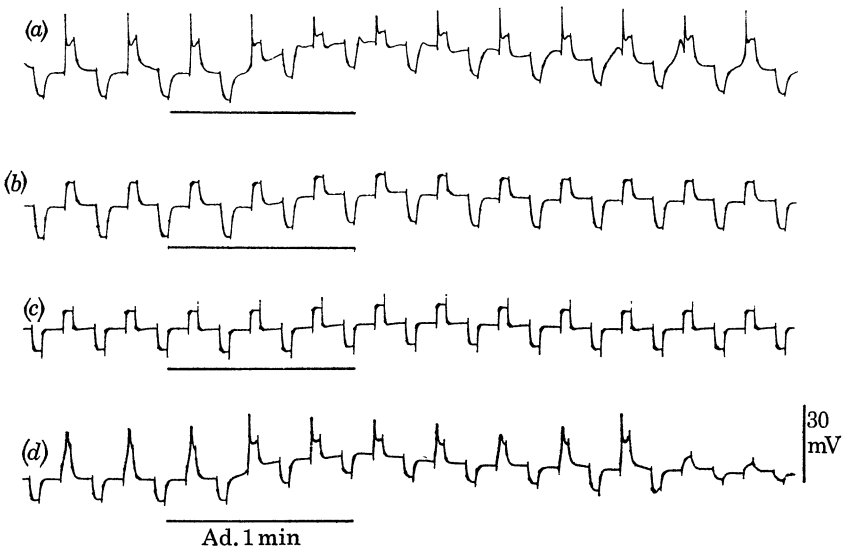

FIGURE 7. Effect of calcium-free solution on the action of adrenaline (Ad.; 1×10^{-6} g/ml) (*a*) control (2 mmol/l Ca); (*b*) 30 min after removal of calcium; (*c*) 6 min after addition of EGTA (0.5 mmol/l); (*d*) 8 min after restoring the normal calcium concentration.

There are perhaps two kinds of experiments one can do with the double sucrose gap to examine this hypothesis. The first is to test the ability of noradrenaline to produce an increase in tension in chloride-deficient solution. In this ionic environment, the depolarization by noradrenaline should be abolished but not the increase in tension. Figure 4*d* shows, indeed, that in chloride-deficient solution, noradrenaline produced an increase in tension even though there was no depolarization, no acceleration in spontaneous spike discharge and no increase in conductance.

The second test of the above hypothesis is to remove calcium and then measure the ability of catecholamines to stimulate the tissue. The result from such an experiment is shown in figure 7. It can be seen that the depolarization and increase in conductance produced by adrenaline in normal Krebs solution (*a*) was much reduced in calcium-free solution (*b*) and abolished in calcium-free solution containing EGTA (*c*). This suggests that calcium is necessary for the increase in the membrane conductance to chloride.

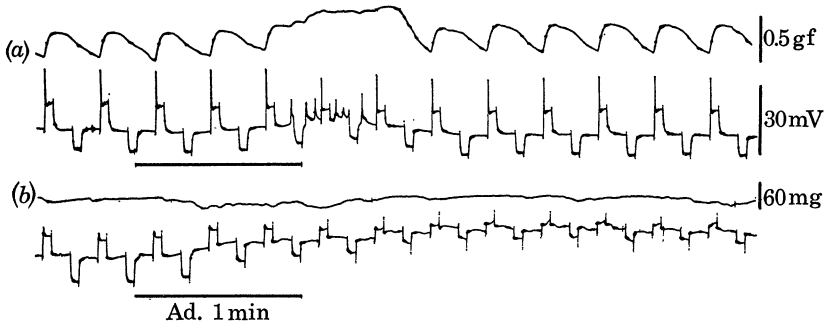

FIGURE 8. The effect of lanthanum on the response to adrenaline (1×10^{-6} g/ml). (*a*) Control; (*b*) 21 min after addition of 1 mmol/l La^{3+} to the bathing solution.

A number of workers have recently shown that lanthanum ions block both calcium efflux and influx in a variety of smooth muscle tissues (Weiss & Goodman 1969; Goodman & Weiss 1971; van Breemen, Farinas, Gerba & McNaughton 1972; van Breemen *et al.*, this volume, p. 57). If external calcium were necessary for the depolarizing action of noradrenaline, then the presence of lanthanum should reduce or abolish the stimulant action of catecholamines. It was found that lanthanum in a concentration of 1 mmol/l, abolished the evoked spike and mechanical responsiveness within 3 min after its addition. Figure 8 shows the effect of adrenaline before and 21 min after adding 1 mmol/l lanthanum. Although there was no tension response, adrenaline still caused depolarization and increased membrane conductance. Both were prolonged and, in some tissues, maintained for as long as lanthanum was present. The sustained increase in conductance and depolarization by noradrenaline or adrenaline in lanthanum containing solution suggests that the calcium which is released inside the cell cannot be pumped out.

Calcium also participates in the stimulant action of acetylcholine. It was shown in figures 2 and 3 that, after sodium replacement by the impermeant cation tris, the depolarization by acetylcholine was reduced, but not abolished. The ions likely to be responsible for this depolarization in the absence of sodium are either chloride or calcium. Chloride was shown not to be necessary for the depolarizing action in the Krebs solution. This leaves calcium. The effect of removing the external calcium from Na-free solution was investigated and the result of such an experiment is shown in figure 9. 57 min after removing calcium (and 112 min after removing sodium), the depolarization and increase in membrane conductance was largely

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abolished. However, acetylcholine still produced an increase in tension. These observations suggest that acetylcholine increases the membrane permeability to calcium ions, at least in sodium-free solution, and, in addition, acetylcholine releases calcium from intracellular organelles.

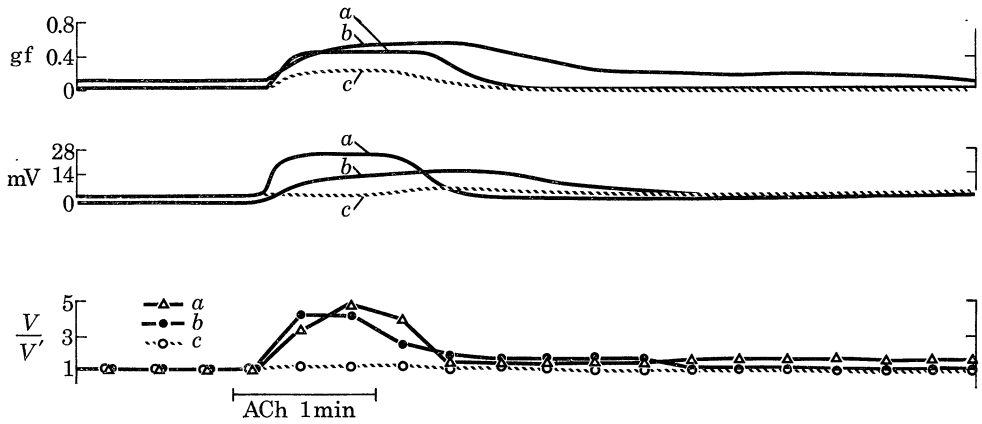

FIGURE 9. Response of myometrium to acetylcholine in Krebs solution (*a*), sodium-free (*b*), and sodium-free, calcium-free (*c*) solution. Response in (*b*) obtained 55 min after changing to sodium-free solution; in (*c*) 57 min after changing to sodium-free, calcium-free solution. Upper panel, tension; middle panel, change in membrane potential; lower panel, change in size of electrotonic potential: V, Size of electrotonic potential before injecting acetylcholine; V', size during and after injection. Value 1 before injection obtained by dividing V into itself. $T = 37$ °C. Black line under bottom panel indicates period of application of drug (2×10^{-6} g/ml).

CONCLUSIONS

The preliminary evidence presented in this symposium suggests that acetylcholine increases the membrane permeability to sodium and calcium but not to chloride ions. In addition, acetylcholine can release calcium from an intracellular store. The depolarization by catecholamines, however, is due to an increase in chloride permeability, whereas the acceleration of the spontaneous spike discharge is sodium dependent. The data also suggest that noradrenaline, or adrenaline, mobilizes an activator substance for contraction, presumably calcium, and that the rise in the intracellular calcium ion concentration may be the primary cause for the increased chloride conductance of the membrane produced by catecholamines.

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Discussion

W. R. KEATINGE (*London Hospital Medical College*): How long after removal of chloride was the depolarizing response to noradrenaline lost? If this response was brought about by increased chloride permeability one would expect the immediate effect of removing extracellular chloride to be an increase in the depolarizing action of noradrenaline, followed by loss of this response as intracellular chloride was depleted.

J. H. SZURSZEWSKI: An increase in the depolarizing action of noradrenaline could be observed if it was administered within 5 to 10 min following the change to Cl-deficient solution. This increase was greater in the presence of high $[K]_o$. The noradrenaline response was diminished or lost after 10 to 15 min exposure and all the results represented on the graph were obtained after 45 min exposure to Cl-deficient solution.

W. R. KEATINGE: Your finding that lanthanum abolishes mechanical responsiveness after 15 to 20 min implies that it is not acting simply by blocking all transmembrane flux of calcium. Does it block influx more effectively than efflux? This could cause difficulties in the use of lanthanum to measure intracellular Ca. Perhaps you and Dr van Breemen would comment.

J. H. SZURSZEWSKI: La in a concentration of 1 mmol/l abolishes mechanical responsiveness within 3 min. Nevertheless, after 15 to 20 min exposure the conductance change caused by adrenaline is still observed. However, it is prolonged and membrane resistance remains low. This may indicate, that also the Ca-efflux is suppressed. Whether La blocks influx more effectively than efflux cannot be decided on the basis of our evidence.

C. VAN BREEMEN: La^{3+} and closely related lanthanide ions have been shown to block calcium efflux from squid axon, red blood cells, aortic smooth muscle cells, previously loaded with ^{45}Ca during metabolic inhibition or high K^+ depolarization, taenia coli smooth muscle cells, loaded with ^{45}Ca during metabolic inhibition, and the anterior byssal retractor muscle of *Mytilus edulis*. The 10 mmol/l La^{3+} which we use to measure intracellular Ca^{2+} is well above the maximal inhibitory concentration.

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