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SODIUM CHLORIDE AND SMOOTH MUSCLE

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It was shown by McDowall & Zayat (1952) that the contraction of isolated strips of cardiac muscle in a bath of Krebs's solution is profoundly affected by the concentration of the sodium chloride of the solution, especially after anoxia and rapid stimulation. This was subsequently analysed by McDowall, Munro & Zayat (1955) and by Hercus, McDowall & Mendel (1955) who found that this could be explained partly by increases and decreases in the sodium content of the muscle, but not wholly so; and they suggested that the magnitude of the contraction of the muscle depended rather on the ease with which it could extrude sodium. The present investigation was carried out to see how far these results were applicable to smooth muscle.

METHODS

In most of the experiments the uterus of the guinea-pig was used because of the extensive literature regarding its reactions, but a few experiments were carried out on the uterus of the rat. The uterus was suspended in the usual way in a bath of Krebs's solution having the following composition: NaCl 0·69, KCl 0·0354, $CaCl_2$ 0·0282, $NaHCO_3$ 0·21, KH_2PO_4 0·0162, $MgSO_4$ 0·029 g/100 ml., kept at 37° C and aerated with 5% CO_2 and 95% O_2. When the sodium chloride of the solution was reduced a corresponding amount of sucrose was added to maintain the osmotic pressure. The method used for the determination of sodium has recently been described in this *Journal* by Hercus *et al.* (1955).

For sodium and potassium estimations the following procedure was used. Muscles after removal from the bath were dried with blotting-paper, weighed in glass tubes, dried for 24 hr at 120° C, reweighed after cooling in a desiccator (to obtain the total water content), and then dissolved in 1 ml. 50% (v/v) nitric acid in a boiling water-bath. This solution was diluted appropriately with distilled water for estimation of sodium and potassium by flame photometry.

RESULTS

The responses of the normal uterus

In acute experiments the concentration of the sodium chloride in the bath can be varied between 1 and 0·3% without there being any material changes in the response to small doses of histamine or acetylcholine. Over 1% sodium chloride reduces the uterine contractions, as found by Dale (1913), but since this result

is also produced by increasing the osmotic pressure with sucrose it was concluded that it is probably an osmotic effect. A similar osmotic action in the rat diaphragm has been described by Lüllmann & Muscholl (1954).

If the uterus is immersed in normal Krebs's solution for 3 hr the response to small doses of histamine is much reduced or may disappear altogether, provided the preparation has not been stimulated during the period (Fig. 1).

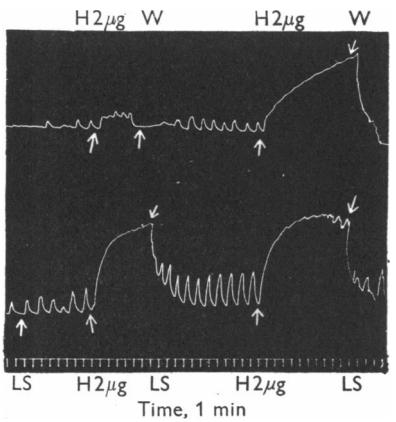

Fig. 1

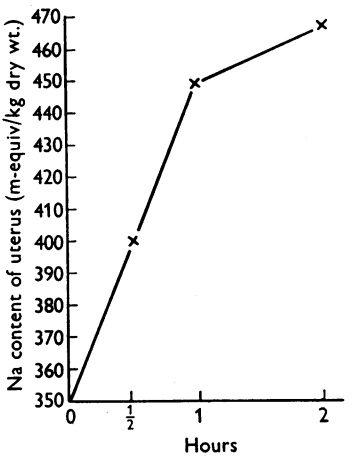

Fig. 2

Fig. 1. Upper record: one horn of the uterus was soaked in Krebs's solution for 2 hr and subsequently at each H 2μg histamine was applied. The fluid in the bath was changed at *W*. Lower record: the other horn of the same uterus was similarly soaked; but at L.S., before the histamine was applied, the solution in the bath was reduced to a modified Krebs's solution containing 0·3% NaCl and sucrose. The same doses of histamine were given at each H.

Fig. 2. Uptake of sodium (m-equiv/kg dry wt.) after soaking in normal Krebs's solution containing 0·69% NaCl.

Sometimes a very short-lived contraction is produced. If, however, successive doses of histamine are added to the bath, there is usually a rapid recovery of the sensitivity of the preparation to histamine even although it is washed between doses. Washing alone with normal Krebs's solution does not have this effect. It can readily be shown, using the method of Hercus *et al.* (1955), that sodium has been taken up during the soaking (Fig. 2); but it cannot be shown that the magnitude of the response to histamine is proportional to the sodium content of the muscle, although preparations soaked in Krebs's solution containing histamine contain less sodium than those soaked in Krebs's solution only.

If, on the other hand, the uterus is soaked in a modified Krebs's solution in which the sodium chloride is reduced to 0·3%, the response to histamine is not affected; nor is it if, after soaking in normal Krebs's solution, the sodium is reduced before the histamine is applied.

The effect of anoxia. Unlike cardiac muscle, uterine muscle is remarkably resistant to oxygen lack and it has not been possible to differentiate between the effect of anoxia and that of prolonged immersion in Krebs's solution.

The response of the tachyphylactic uterus

If a uterus is rendered insensitive to a small dose of acetylcholine (4μg) or histamine (2·5μg) by a large dose of either drug, the subsequent response to small doses of these drugs becomes very markedly affected by the concentration of the sodium chloride in the bath. Thus, in a bath of normal Krebs's solution containing 0·69% NaCl a tachyphylactic uterus may be quite insensitive to a standard small dose of histamine, but it will contract normally in a bath containing 0·3% NaCl, the osmotic pressure being maintained by sucrose. On returning to 0·69% NaCl it is again insensitive to the same small dose (Fig. 3).

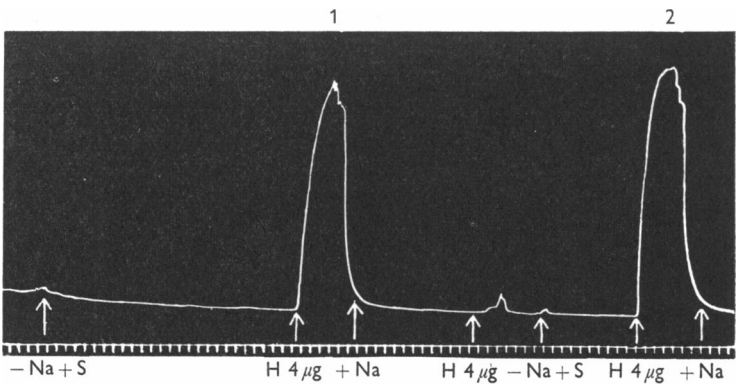

Fig. 3. Response of the tachyphylactic uterus to histamine (H 4μg); −Na+S, introduction of 0·3% NaCl+sucrose; +Na, introduction of normal Krebs's solution.

It is of interest that Cantoni & Eastman (1946) found an increase of potassium in the bath to be as effective as low sodium in abolishing tachyphylaxis. We have confirmed this and find that the two effects supplement each other; that is to say that a less reduction of sodium is effective in the presence of a small additional amount of potassium. Chemical estimations fail to demonstrate that any additional sodium has been taken up during the exposure to histamine; indeed it is found that a muscle so exposed does not take up so much sodium as a muscle similarly soaked in normal Krebs's solution.

These responses of the tachyphylactic uterus may be repeated over and over again on the same preparation until it ceases to be tachyphylactic, usually after 1 or 2 hr. The change over from the insensitive to the sensitive state occurs in 1 or 2 min.

The effect of temperature on the responses

It was found by McDowall *et al.* (1955) that the depressant effects of rapid stimulation on the heart were increased by increase in temperature. On the uterus it was found that although it might be tachyphylactic to histamine at 37° C, it was no longer so at 32° C (Fig. 4). Similarly, raising the temperature to 42° C so increased the tachyphylaxis that low sodium would no longer render the preparation sensitive.

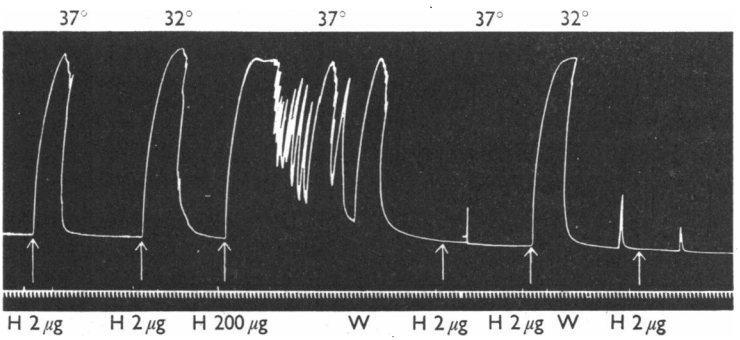

Fig. 4. The response of the uterus to 2 μg histamine at 37 and 32° C before and after exposure to 200 μg histamine. The fluid in the bath was changed at W. Note the return of the response at 32° but not at 37° C.

The action of strophanthin

It was pointed out by Clark (1913) and by Daly & Clark (1921) that a heart weakened by long perfusion benefited by perfusion with a solution containing low sodium, and they remarked that it appeared to act like strophanthin, the well-known cardiac tonic. McDowall & Zayat (1952) also observed that strophanthin, like a low sodium solution, enhanced the contractions of the heart strip. It is now found that strophanthin has a similar action on the tachyphylactic uterus and renders it sensitive again to small doses of histamine or acetylcholine (Fig. 5). This observation can readily be repeated. This anti-tachyphylactic action, like that of low sodium, is also absent at 42° C. When the strophanthin is washed out the tachyphylactic state returns. If, however, the histamine is placed in the bath before the strophanthin, the addition of the latter does not always cause a contraction.

Sodium and the anaphylactic reaction

Twenty-five guinea-pigs were sensitized to egg albumin by peritoneal injection and were tested as with histamine. When a first dose of antigen (5 mg crystalline egg albumin) was added to the bath the usual anaphylactic contraction was produced. Normally, if at this stage the fluid in the bath is changed and a second dose of antigen is added, no further response is obtained. In our

experiments the normal Krebs's solution was now changed to one of low sodium, and a third dose of antigen added to the bath. In twenty out of twenty-five experiments no further response was obtained but in five there was a further contraction (Fig. 6). This positive response is, however, to be considered important because of the difficulty in obtaining any fixed degree of

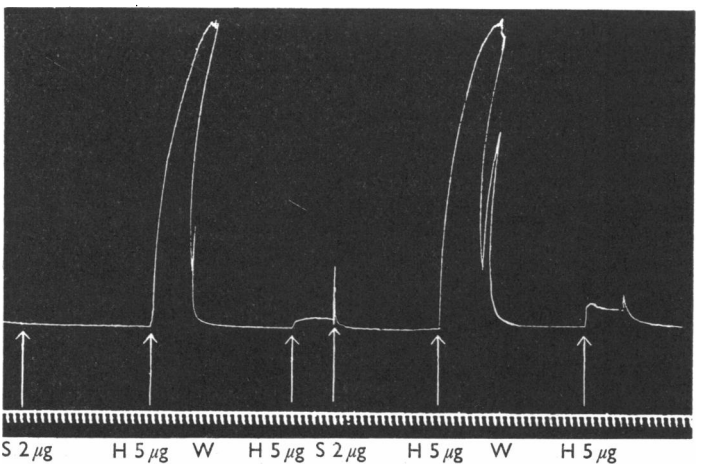

Fig. 5. The response of the uterus to 5μg histamine after and before 2μg strophanthin (S). At each W the normal Krebs's solution in the bath was changed.

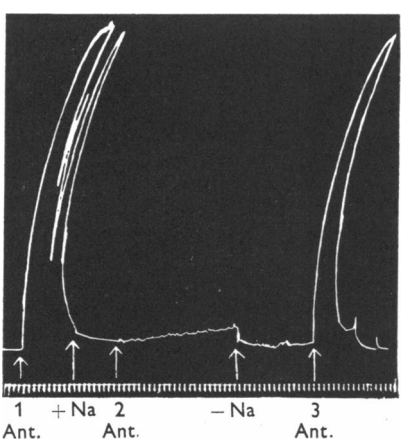

Fig. 6. Showing the responses to a first, second and third dose of antigen (ant.); at −Na sodium was reduced to 0·54%.

sensitivity and of adjusting the amount of the first dose. Thus, if the first dose used up all the available antibody, no result could be expected. On the other hand, if by the first dose the uterus is rendered tachyphylactic to substances released in the anaphylactic reaction, the low sodium might act as in the case of histamine.

DISCUSSION

It has been shown that if the isolated guinea-pig uterus is immersed in Krebs's solution it takes up sodium and its responses to submaximal doses of histamine are reduced. Since the responses cannot be shown to be directly related to the sodium uptake and rapidly return to normal if the sodium in the bath is reduced, it may be assumed that—as has been shown in relation to cardiac muscle (McDowall *et al.* 1955)—the depression is due primarily to a difficulty in extruding sodium.

It has also been shown that tachyphylaxis is relieved by low sodium and returns in high sodium, but here also it has not been possible to demonstrate chemically that the insensitivity is associated with any sodium uptake. It is possible that the amount of sodium taken up at the specific receptors is so small that it cannot be detected chemically, but it may be that the large dose so damages the membranes of the specific receptors that they have simply become more permeable to sodium than normally, and as a result the receptors have difficulty in extruding sodium and have not sufficient energy essential for some part of the excitation process. This would account for the very rapid effects of lowering the external sodium. Such an explanation has been put forward to explain a similar phenomenon in cardiac muscle (McDowall *et al.* 1955). These results go to support the idea of there being special receptor areas which are specially concerned in the transmission of excitation somewhat like the end-plates of voluntary muscle; and in this connexion it may be recalled that Awad & McDowall (1952) have described a neuromuscular block in the rat diaphragm, which is produced by anoxia and relieved by low sodium.

The effect of temperature indicates that the tachyphylaxis is more intense at 42° than at 32° C. This might be expected since higher temperature could cause increased movement of ions and so make it more difficult to extrude sodium against a gradient. It was observed by Hercus & Mendel (unpublished) that heat increased the uptake of sodium by cardiac muscle soaked in Krebs's solution.

The action of strophanthin is perhaps unexpected in view of the known effects of the cardiac glucosides in reducing the potassium uptake and sodium extrusion by red blood cells (Witt & Schatzmann, 1953; Joyce & Weatherall, 1955; Glynn, 1955). On the other hand, the action of strophanthin and low sodium on the uterus is very like their action on the heart, and it may be suggested that somehow the strophanthin facilitates the extrusion of sodium; but it has not been possible to show, either on the heart or the uterus, that strophanthin affects the sodium content of normal tissue. Its action may therefore be a purely membrane effect.

SUMMARY

1. It is shown that the isolated uterus soaked in Krebs's solution or rendered tachyphylactic by exposure to large doses of histamine or acetylcholine is very sensitive to changes in the sodium content of the bath at 37° C.

2. Tachyphylaxis is abolished by low sodium but increased by high sodium. It is also abolished by reduced temperature, by potassium and by strophanthin, but these effects are not present at 42° C.

3. Since the reduction of the responses after soaking or in tachyphylaxis cannot be shown to be directly related to a sodium uptake yet are rapidly relieved by lowering the sodium in the bath, it is suggested that they are due to a difficulty in extruding sodium.

A demonstration of the effects of low sodium on the tachyphylactic uterus was given to the Physiological Society in October 1953. We are grateful to Dr A. Fadl for confirming the observation of the effects of soaking the uterus in Krebs's solution.

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