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THE EFFECT OF SODIUM DEFICIENCY ON THE RESPONSE OF THE ISOLATED MUSCLE SPINDLE

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There is now abundant evidence available showing that excitation in nerve and muscle is associated with movements of ions across the fibre membrane. Studies of the giant axon of the squid (Hodgkin, Huxley & Katz, 1952) have established that the depolarization is produced by a sudden increase in sodium permeability, and a similar change has also been demonstrated during activity of the muscle fibre (Fenn & Cobb, 1936; Hodgkin & Horowicz, 1959) and of the myelinated nerve fibre (Huxley & Stämpfli, 1951; Dodge & Frankenhaeuser, 1959). At the motor end-plate the situation is somewhat different as the depolarization of the end-plate membrane is due to a non-selective conductance change involving an increased permeability to sodium and potassium and possibly also to other ions (Fatt & Katz, 1951; del Castillo & Katz, 1955; Takeuchi & Takeuchi, 1959, 1960; Nastuk, 1959; Katz, 1962). As far as sensory organs are concerned but little is known about the mechanisms underlying the development of the electrical changes during activity. Katz (1950) noticed that the response of the muscle spindle persisted in sodium-free solution. Similar observations were also made on the Pacinian corpuscle (Gray & Sato, 1953), but later perfusion experiments showed that withdrawal of sodium was followed by a decline of the receptor response (Diamond, Gray & Inman, 1958).

The experiments to be described here were undertaken to see whether the response of the isolated muscle spindle is influenced by lowering the sodium concentration of the external fluid. A preliminary report of this study has been published (Ottoson, 1963).

METHODS

Muscle spindles of the frog's toe muscle (m.ext.dig.long. IV) were isolated and mounted in a small chamber, as described in an earlier paper (Ottoson, 1961). The spindle was clamped at each end to a small plastic rod connected to a micromanipulator, so that the resting length of the muscle could easily be changed. Stretches were applied by an electromagnet connected to one of the rods. The electrical response was recorded with silver-chloride-agar electrodes, one of which was placed in the fluid in the chamber while the other

was applied to the sensory axon which was lifted up in oil (Katz, 1950). The electrodes were connected to a DC-coupled amplifier. The normal Ringer's solution had the same composition as that used by Adrian (1956). Na^+-free solutions were obtained by replacing NaCl with choline chloride on a mole-for-mole basis.

RESULTS

The effect of low Na^+ concentration on the conducted activity

Effect on maintained activity. When the spindle was soaked in a solution containing less Na^+ than the Ringer's fluid the effect first observed was a slow decrease of the frequency of the spontaneous firing. This effect was

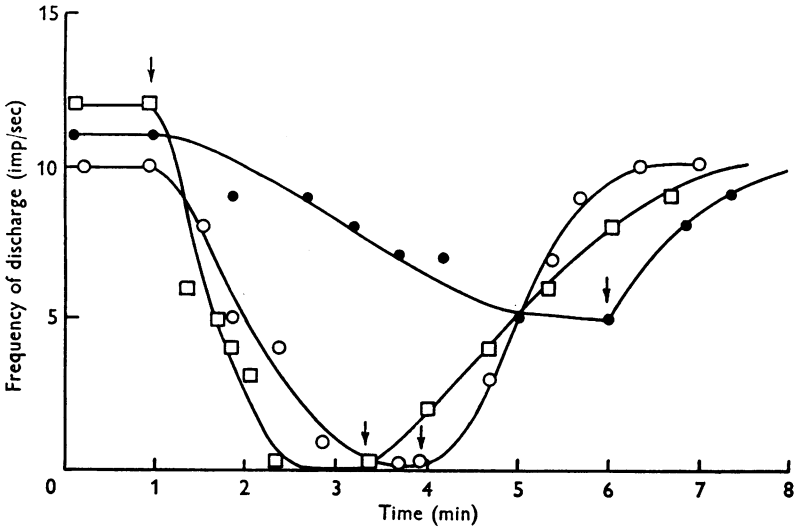

Fig. 1. Effect of change in Na^+ concentration on impulse discharge. Ordinate: average frequency of maintained discharge. Na^+ concentrations as per cent of value in normal Ringer's solution: ●—● = 50 %; ○—○ = 25 %; □—□ = 0 %. Change from normal to low Na^+ concentration marked by first arrow; the following arrows indicate return to Ringer's solution.

usually not obvious until the Na^+ concentration was reduced to about 50 %. With further reduction of the Na^+ content of the bathing fluid the decay of activity became faster and the spontaneous firing usually ceased within a few minutes when the spindle was soaked in a Na^+-free solution. The time course of the effect of solutions of different Na^+ concentrations was difficult to determine because of the low and irregular rate at which the spindle fires when kept at resting length. A series of experiments were therefore done with the spindle slightly stretched so that it fired at a steady frequency of about 10/sec. The curves in Fig. 1 show the results obtained in one of these experiments. When the spindle was soaked in a

solution containing 50 % of the normal Na^+ concentration the activity declined slowly to about 5/sec and then remained practically constant at this frequency. Reduction of the Na^+ concentration to 25 % of the normal value led to a more rapid fall and the spindle ceased to fire in about 4 min. If NaCl was removed completely the effect developed still faster and the spindle became silent within less than 3 min. As is also shown in Fig. 1, return to Ringer's fluid was followed by a rapid recovery of the original firing frequency. With prolonged soaking time the rate of recovery became slower. If the spindle was exposed to repeated immersions in sodium-

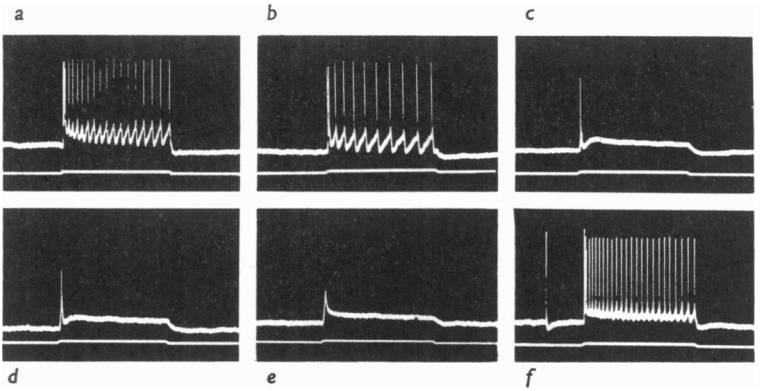

Fig. 2. Changes of response to brief (250 msec) stretching with changes in Na^+ concentration: *a*, response in Ringer's fluid, *b–e*, after 3 min in solution containing 50, 25, 10 and 0 % respectively of normal Na^+ concentration, NaCl being replaced by choline chloride; *f*, 5 min after return to Ringer's fluid.

deficient solutions the decline of the activity became successively more rapid.

Effects on the response to stretch. Immersion of the spindle in a solution in which the NaCl had been partially or completely replaced by choline chloride or sucrose was followed by a reduction of the number of spikes elicited by a given stretch. The effect of different concentrations of Na^+ is illustrated in Fig. 2, which shows the response obtained after soaking the spindle in the appropriate solution for 3 min. After each exposure to reduced Na^+ concentration the spindle was left to recover in Ringer's fluid until the original response was restored. It can be seen that lowering the Na^+ concentration of the bathing fluid to 50 % caused only a slight reduction of the impulse discharge. As the Na^+ content of the fluid was further reduced the effect become more conspicuous and in a solution of 25 % of the normal Na^+ concentration only one spike was left. The initial spike was still present when the spindle was soaked in a solution containing

10 % of the normal concentration of Na^+ and not until NaCl was completely replaced by choline chloride was this spike also abolished and only the pure receptor potential left.

As described by Katz (1950) the response of the spindle consists of an initial dynamic component and a later static component. The results obtained in the experiments with brief stretching showed that the static component was more susceptible to lowering of the Na^+ content of the external fluid than the dynamic component. This is also illustrated in Fig. 3, which shows the effect of sodium deficiency on the responses to stretches of 5 sec duration. The spindle was in this case subjected to a relatively strong stretch, so as to make the difference between the response

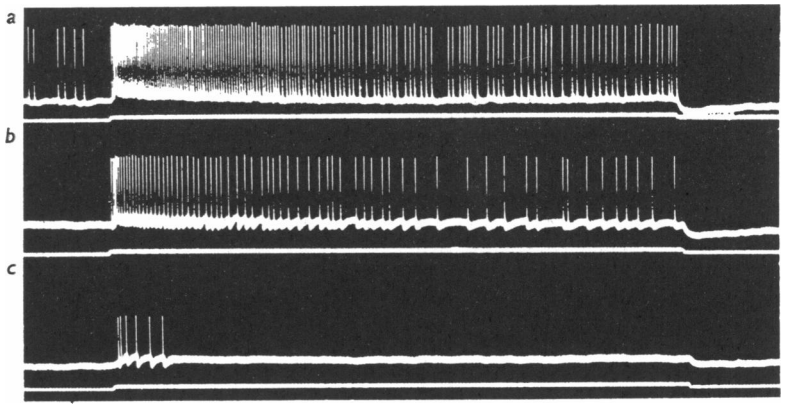

Fig. 3. Response to sustained stretching (5 sec): *a*, response in Ringer's fluid, *b*, after 3 min in solution containing 50 % of the normal Na^+ concentration, *c*, in Na-free solution.

in Ringer's solution and those in sodium-deficient solutions more marked. As can be seen, immersion of the spindle in a solution containing 50 % (Fig. 3*b*) of the normal Na^+ concentration is followed by a marked fall of the sustained discharge. Record 3*c* shows for comparison the effect of complete removal of Na^+. It may be noted that in this record the receptor potential remains relatively unaltered, though the impulse discharge is almost completely extinguished. Similar results were obtained when the spindle was subjected to repeated short stretching at intervals of 1 sec. In a solution containing 50 % of the normal Na^+ concentration the first stretching in a series gave almost the same response as in Ringer's fluid, while the later ones gave smaller responses than those obtained in a similar run in normal solution.

Effects of prolonged immersion. In the experiments described above the soaking time usually did not exceed 5 min. The effect of prolonged

exposure was studied in another series of experiments with a standard soaking time of 30 min during which the fluid of the bath was frequently changed. Figure 4 gives the result of one of these experiments. The curves show the changes of the impulse discharges elicited by a brief test stretching; the number of spikes obtained in Ringer's fluid before immersion in the appropriate solution has been taken as 100 % response. As is seen, a lowering of the Na^+ concentration of the bathing fluid to 50 % produces a gradual fall of the response to about 40 % of the original value

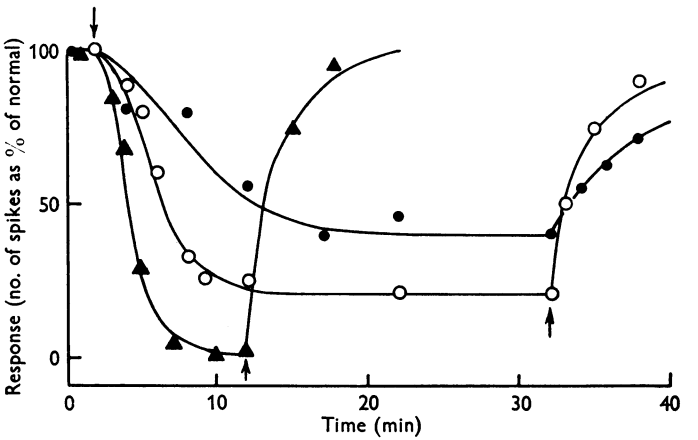

Fig. 4. Effect of change of Na^+ concentration on response to brief (250 msec) stretching. Ordinate: response expressed as per cent of response in Ringer's fluid. Na^+ concentrations: ●—● = 50 %; ○—○ = 25 %; ▲—▲ = 0 % of value in normal solution.

and then the response remains almost constant at this level for the duration of the exposure. If the spindle is soaked in a solution containing 25 % of the normal concentration of Na^+ the effect appears earlier, the decline is more rapid, and the response drops to a maintained level of about 20 % of the original value within about 8 min. In a Na^+-free solution the fall is still faster and all signs of impulse activity are abolished within about 5 min.

The effect of blocking agents. It is probable that the observed changes of the response are due to a combined action of low Na^+ concentration on the sensory endings and on the afferent nerve fibre. Now it is difficult to measure the changes in amplitude of the receptor potential when the spikes are also recorded. However, as was shown by Katz (1950) it is possible to block the conducted activity with local anaesthetics without causing any significant reduction of the receptor potential. To do this, one must carefully grade the concentration of the blocking agent. To find

the critical dose at which impulse activity became blocked, the action of different concentrations of procaine and lignocaine were examined. With 0·05 % lignocaine all spikes except a single initial one were usually blocked; while procaine of the same concentration left two or three spikes of the response to a brief stretch unblocked. With 0·1 % lignocaine all signs of conducted activity were regularly extinguished, while with 0·1 % procaine a single initial impulse was constantly left. The fact that application of lignocaine of still higher concentrations did not produce any further immediate reduction of the amplitude of the initial component of the response suggested that no residue of the first spike was left. The blocking effect usually developed in less than 1 min, after which the response

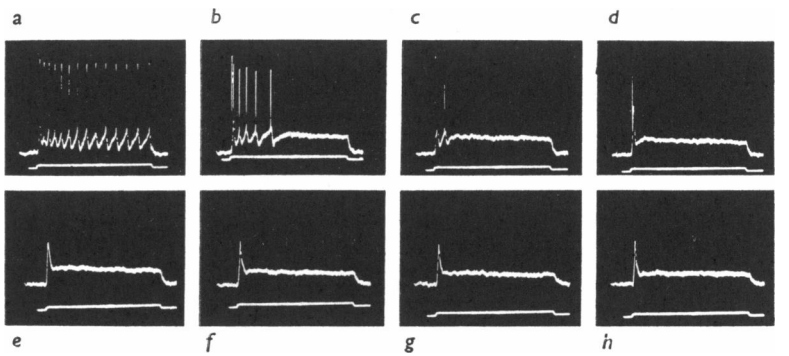

Fig. 5. Effect of prolonged exposure to lignocaine on response to brief (250 msec) stretching. *a*, response in Ringer's fluid, *b–d*, in 0·005, 0·01 and 0·05 % lignocaine-Ringer's solution. Records *e–h* obtained after 5, 10, 20 and 30 min in the 0·05 % solution.

remained unchanged. Sometimes a partial recovery of the response occurred. This effect was usually abolished if the solution in the bath was stirred. After the spikes had been removed the changes in amplitude of the receptor potential could readily be followed for the rest of the time of immersion. As a rule the response remained unchanged for 30–60 min in 0·05 % lignocaine (Fig. 5) or in 0·2–0·3 % procaine. With higher concentrations there was a slow gradual decline of the response. Since lignocaine was more effective than procaine, the former agent was used in most of the experiments described in the following section.

Effects of reduced Na^+ concentration on the isolated receptor potential

The effect of a diminution of the Na^+ concentration on the amplitude of the dynamic component of the receptor potential, after blocking the impulse activity with 0·1 % lignocaine, is illustrated in Fig. 6. A com-

parison with the curves in Fig. 4 clearly shows that a reduction of the Na^+ content of the external fluid has much less effect on the receptor response than on the conducted activity of the spindle. Thus the dynamic component of the receptor potential is only reduced to about 30 % of its original value after immersion in a Na^+-free solution for 30 min, whereas the impulse discharge elicited by stretch is extinguished within less than 10 min. The fall of the receptor potential varied a great deal from one preparation to another. However, with soaking time of 30 min the receptor potential never disappeared in Na^+-free solution. Even when the soaking time was prolonged to 60 min and the solution was frequently changed,

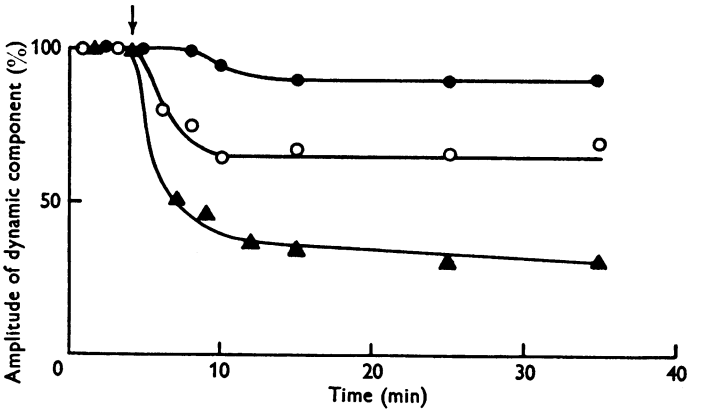

Fig. 6. Effect of lack of sodium on receptor potential. Ordinate: amplitude of dynamic component of receptor potential as per cent of amplitude of same component in Ringer's solution after blocking the conducted activity with 0·1 % lignocaine. Concentrations of Na^+ (% of normal value): ●—● = 50; ○—○ = 25; ▲—▲ = 0.

the response was not extinguished. To ensure that the recorded potential did not include any artifacts the spindle was killed after each experiment and the test stretching thereafter applied again.

As a rule the static component suffered the greatest reduction and was often barely detectable after 60 min in Na^+-free solution, while the dynamic component still was of appreciable amplitude. The changes of the static component were often difficult to determine because of the low amplitude of this portion of the potential. However, in some preparations with unusually large receptor potential the effects of different concentrations of Na^+ could be measured with a reasonable degree of accuracy. The curve in Fig. 7 gives the results of such an experiment. A comparison with the curve in Fig. 6 shows that the static component undergoes a gradual decay during prolonged soaking.

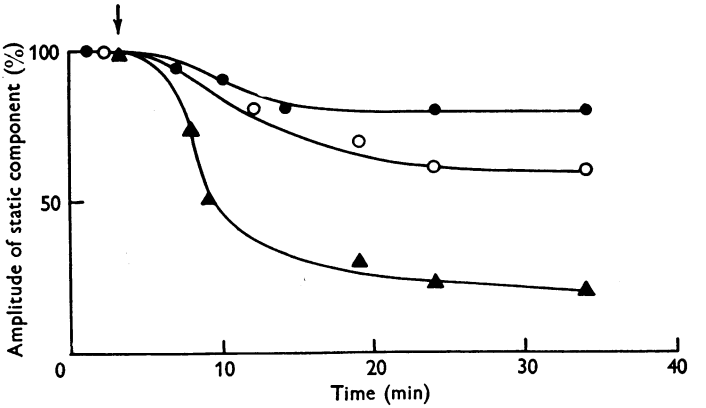

Fig. 7. Effect of lack of sodium on static component of receptor potential. Ordinate: amplitude of static component as per cent of amplitude of same component in Ringer's fluid. Concentration of Na^+ (% of normal value): ●—● = 50; ○—○ = 25; ▲—▲ = 0.

DISCUSSION

Among the various suggestions put forward to explain the production of receptor potentials (Katz, 1950; Diamond *et al.* 1958; Gray, 1959) the most plausible is that the mechanical stimulus gives rise to a change in permeability of the sensory membrane. If this assumption is true it seems reasonable to assume that Na^+ ions are involved in the transfer of charge across the membrane. Earlier experiments, in which the effect of sodium withdrawal was studied, did not provide evidence supporting this view. Thus Katz (1950) found that the receptor potential of the muscle spindle, unlike the nerve spikes, persisted when sodium in the bathing fluid was replaced by choline chloride, and similar observations were also made by Gray & Sato (1953) on the Pacinian corpuscle. In later experiments Gray & Sato (1955) found that the diffusion through the lamellae of the Pacinian corpuscle takes place slowly. This finding suggested that the failure to produce any appreciable effect on the receptor potential by soaking the Pacinian corpuscle in Na^+-free solution might be explained by the lamellae acting as a diffusion barrier. By perfusing the Pacinian corpuscle, Diamond *et al.* (1958) were able to demonstrate that the amplitude and rate of rise of the receptor potential were related to the concentration of Na^+ in the perfusion fluid. However, the response was not abolished by complete removal of Na^+; usually there remained a residue of about 10% of the original response after perfusion for up to 30 min. These authors therefore concluded that Na^+, though essential for the production of a full-sized response, was not the only ion responsible for the transport of charge across the membrane.

The results obtained in the present study are in close agreement with the observations made on the Pacinian corpuscle (Gray & Sato, 1955; Diamond *et al.* 1958). As has been shown, removal of Na^+ from the bathing fluid results in the abolition of the conducted activity and a substantial reduction of the receptor potential. As a rule the spontaneous activity disappeared in less than 3 min after immersion in Na^+-free solution, while the impulses elicited by the test stretch persisted for a longer period, usually 4–8 min. It is interesting to note that this is also the time it takes to block the conducted activity of the frog's sciatic nerve by perfusion with Na^+-free solution (Krnjevic, 1954).

The activity of the sensory endings is less dependent on the external concentration of sodium than the nerve fibre, as is shown by the fact that the receptor potential could be obtained for a considerable time after the conducted activity was blocked. After soaking for 60 min in Na^+-free solution, about 20 % of the response obtained in Ringer's fluid usually remained. This may be due to Na^+ being retained in the intercellular spaces. An alternative explanation is that other ions besides Na^+ participate in the production of the receptor potential. An implication of the latter hypothesis would be that the conductance change produced in the sensory membrane by the mechanical stimulus is not Na^+-selective. If this is true, the behaviour of the spindle when exposed to sodium-deficient or sodium-free solutions would thus seem to be akin to that of the motor end-plate under similar conditions. As has been shown by Fatt (1950), ACh produces a local depolarization at the end-plate also when the muscle is kept in Na^+-free solution. Recent experiments by Takeuchi & Takeuchi (1960) provide evidence that ACh increases both the Na^+ and K^+ conductance of the end-plate membrane. It remains to be investigated whether similar permeability changes occur in the sensory membrane during production of the receptor potential. It appears from the experiments of the present study that the conversion of mechanical energy into the receptor potential of the muscle spindle is brought about by a change in permeability to Na^+ and most likely also to other, not yet identified, ions.

SUMMARY

1. A study has been made of the effects of sodium-deficient and sodium-free solutions on the isolated muscle spindle of the frog.

2. The impulse discharge evoked by a test stretch was gradually curtailed in sodium-free solution. After immersion for 4–8 min all signs of conducted activity were abolished, leaving the pure receptor potential behind.

3. The effect of lack of sodium on the receptor potential was studied after blocking of the impulse activity with local anaesthetics. Immersion

in sodium-free solution produced a fall of the receptor potential to about 30–20% in about 10 min. With prolonged soaking (30–60 min) there was only a slight further decay.

4. The conclusion is drawn that the production of the receptor potential depends on the presence not only of Na^+ but of other ions also.

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