EFFECT OF MEMBRANE HYPERPOLARIZATION ON NOVOCAINIZED SKELETAL MUSCLE FIBERS

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This investigation, conducted on skeletal muscle fibers by means of intracellular microelectrodes, showed that hyperpolarization of the membrane with a constant current restores generation of the action potentials when disturbed by Novocain. This effect is the more marked, the stronger the hyperpolarizing current and the longer the duration of its action. Analysis of the results obtained, and comparison between them and the effects of action of the hyperpolarizing current on the novocainized nodes of Ranvier of nerve fibers [1, 2, 4] show that Novocain inactivates the sodium permeability of the membrane. Some differences in the mechanism of action of Novocain on muscle and nerve fibers are examined.

EXPERIMENTAL METHOD

The test objects were the sartorius muscles of the grass frog. Under a binocular loop, two glass microelectrodes, filled with 3 M KCl solution, were introduced separately into a muscle fiber so that the distance between their points was about 50 μ. One of the electrodes was used for recording the membrane potential and the other for transmitting the polarizing and stimulating currents.

The resistance of the microelectrodes varied from 10 to 40 mΩ. A two-channel electrometric dc amplifier with input capacitance compensation was used. One channel was used for recording the changes in the membrane potential, the other for recording the rate of these changes (dV/dt). For this purpose, the signal from the output of the first amplifier was fed into a differential circuit with a time constant of 20 μ/sec (P = 100 kΩ, C = 200 pF). Novocain hydrochloride, in concentrations of $3 \cdot 10^{-4}$–$5 \cdot 10^{-4}$ g/ml was dissolved in Ringer's solution. The experiments began with measurement of the resting potential (RP), the critical potential (CP), and the action potential (AP) of the muscle fibers in Ringer's solution, after which this solution was replaced by Novocain solution. The changes in the CP, RP, and AP produced by Novocain usually began to be investigated 30 min after exposure to the drug. The strength of the direct current used for producing hyperpolarization of the membrane varied from 10^{-8} to 10^{-7} A. The duration of action of the current varied from 5 msec to 1 sec. The experiments were carried out between January and May, 1966.

EXPERIMENTAL RESULTS AND DISCUSSION

In Ringer's solution of normal composition, the RP of the muscle fibers was 83.2 ± 4.5 mV (50).* The overshoot of the AP was 36.7 ± 16.7 mV, and of the CP it was 40.6 ± 15.5 mV. The maximal rate of increase of the AP was 245 ± 12 V/sec (10) and the maximal rate of its decrease 110 ± 4.41 V/sec.

Novocain in a concentration of $3 \cdot 10^{-4}$ depressed the RP to 73.3 ± 14.5 mV (25), and in a concentration of $5 \cdot 10^{-4}$ g/ml to 68.3 ± 8.6 mV (25). In these circumstances the AP fell considerably or disappeared completely, changing into a local response. Hyperpolarization of the membrane by a constant current restored AP generation. Its amplitude and the steepness of both the ascending and descending limbs increased more, the greater the increase produced in the membrane potential by the current applied. The CP was displaced toward the RP by the action of hyperpolarization (Fig. 1).

During hyperpolarization of the membrane to 100–110 mV, the overshoot and the steepness of its ascending and descending phases, although they came close to the mean values of these parameters in normal fibers, did not quite reach them. For instance, during the action of Novocain in a concentration of

*The number of fibers investigated is given in parentheses.

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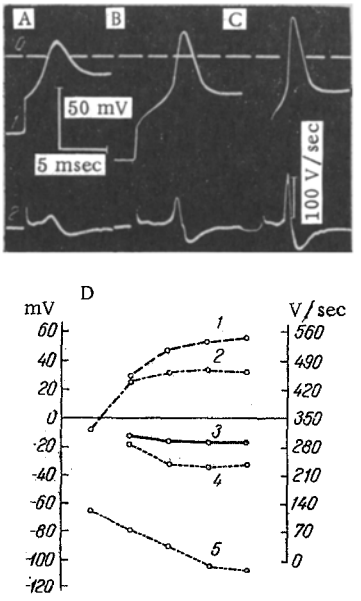

Fig. 1. Restoration of AP generation in a novocainized skeletal muscle fiber in relation to degree of hyperpolarization of the membrane by a constant current. A) Muscles in Novocain solution in a concentration of $3 \cdot 10^{-4}$ g/ml; B, C) the same fiber during the action of a hyperpolarizing current of varied strength; test stimuli were applied 1 sec after the beginning of hyperpolarization. 0) Zero line; 1) RP; 2) rate of change of membrane potential dV/dt; D) graph showing relationship between maximal steepness of ascending limb of the AP (1), the overshoot (2), the maximal steepness of descending limb (3), and the CP (4) and the magnitude of the RP (5) of the novocainized muscle fiber.

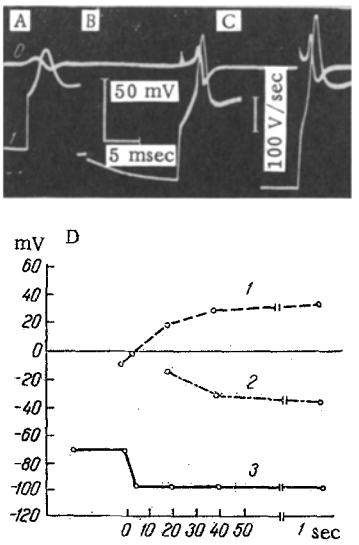

Fig. 2. Effect of duration of action of hyperpolarizing current on effect of restoration of AP of a muscle fiber in Novocain solution in a concentration of $3 \cdot 10^{-4}$ g/ml. A) Muscle in Novocain solution before switching on hyperpolarizing current; B) the same, 13 msec after beginning of hyperpolarization; C) the same, 1 sec after hyperpolarization. 0) Zero line and result of differentiation of potential changes in time; 1) level of RP; D) dynamics of changes in the peak of the AP (1), CP (2), and RP (3) during the action of the hyperpolarizing current. The moment of switching on the current corresponds to the point 0 on the abscissa.

$3 \cdot 10^{-4}$ g/ml, the maximal steepness of the ascending phase of the response was 25.5 ± 7.3 V/sec, while the maximal steepness of the descending phase of the response was 19.5 ± 6.9 V/sec (10). Under the influence of hyperpolarization of the membrane, the maximal steepness of the depolarization phase rose to 208 ± 6.8 V/sec, and that of the repolarization phase to 81.2 ± 2 V/sec. The overshoot of the AP of these fibers was 35.4 ± 5.5 mV (30), and the CP also remained low by comparison with the original value, namely 32.7 ± 10.3 mV.

A series of experiments was carried out to investigate the relationship between the effects of restoration of the AP and the duration of action of the hyperpolarizing current. The results of these experiments showed that when hyperpolarization of the membrane lasted 5 and 10 msec, a small increase in the amplitude and steepness of the responses took place, but with a further increase in the duration of action of the constant current the AP increased and reached a more or less constant value toward the end of the first second of hyperpolarization.

The relationship between the effects of restoration of AP generation in the novocainized muscle fibers and the duration of hyperpolarization is illustrated in Fig. 2.

In the previous investigation [3] it was shown that the depressant action of Novocain on skeletal muscle fibers may be considerably diminished by increasing the calcium ion concentration in the medium. The results of the present investigation showed that hyperpolarization of the membrane by a constant current had an even stronger restorative action on the novocainized muscle fibers. In this respect, the effects of hyperpolarization and of an excess of calcium ions in the medium were very similar to those ob-

tained earlier [2, 4, 5] in experiments in which these agents were applied to novocainized nodes of Ranvier of the frog's nerve fibers. The suggestion may naturally be made that the mechanism of action of Novocain on nerve and muscle tissue is the same in principle.

A number of facts have been described in the recent literature indicating that Novocain, after penetrating into the membrane of nerve and muscle fibers, prevents the increase in its sodium permeability in response to depolarization [2, 7, 8, 9]. The results of the present investigation support this hypothesis. We have seen that under the influence of Novocain the steepness of increase of the AP (which is known to be directly dependent on the strength of the incoming sodium flow) was considerably reduced and the amplitude of the AP and CP was lowered.

According to the Hodgkin–Huxley theory, changes of this type may be due either to a decrease in the sodium permeability constant ($\bar{P}_{Na}$) or to an increase in the inactivation of the sodium carriers. The action of the hyperpolarizing current on the membrane assists with the differentiation of these phenomena. The sodium permeability constant is independent of the membrane potential. For this reason, a decrease in the constant cannot be abolished by hyperpolarization of the membrane. Conversely, inactivation of the sodium "carriers" is completely abolished by strong hyperpolarization. In the present experiments, hyperpolarization of the membrane to −100 (−115) mV led to restoration of AP generation suppressed by Novocain. The fact that the steepness of the ascending phase of the AP and its overshoot increased to values close to the initial levels suggests that inactivation of the sodium "carriers" plays the leading role in the mechanism of action of Novocain on skeletal muscle fibers.

Although stressing the similarity between the changes in the properties of the excitable membrane of muscle and nerve fibers during exposure to Novocain, certain differences between them must also be mentioned, mainly concerned with the effect of Novocain on the membrane RP.

In nerve fibers, Novocain shifts the RP toward hyperpolarization (by 3-5 mV), while in muscle fibers it produces some degree of depolarization [6]. The question naturally arises, to what extent this depolarization is responsible for inactivation of the sodium permeability of the membrane of muscle fibers under the influence of Novocain. As pointed out above, Novocain in concentrations of $3 \cdot 10^{-4}$-$5 \cdot 10^{-4}$ g/ml lowered the absolute value of the membrane RP by 10-15 mV. To restore the AP in novocainized muscle fibers by a direct current, however, it was not sufficient to raise the RP to its initial level but, as a rule, it was necessary to exceed this level by approximately 20 mV. It may evidently be concluded from this that the inactivation produced by Novocain is complex in nature, for it is due both to the direct action of the drug on this process and also to its indirect effect through the change in the membrane potential. The significance of each of these factors is evidently not the same in different muscle fibers. In some fibers, depolarization of the membrane under the influence of Novocain is very weak or absent altogether, but nevertheless the depression of the AP is very considerable, while in other fibers depression of the AP takes place against the background of appreciable depolarization of the membrane.

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