INTERACTION BETWEEN CALCIUM IONS AND NOVOCAIN ON THE SKELETAL MUSCLE FIBER MEMBRANE

E. G. Vornovitskii

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Investigations conducted on the neurons of the spinal ganglia [3] and on single nodes of Ranvier of the isolated frog's nerve fibers [1, 2] have shown that an increase in the concentration of calcium ions ($[Ca]_n$) in the solution sharply reduces the depressant action of Novocain on generation of the action potential (AP).

When the concentration of $[Ca]_n$ is lowered, on the other hand, the effect of the blocking action of Novocain on the node of Ranvier is strengthened.

The object of the present investigation was to determine to what extent this rule governing the interaction between Novocain and calcium ions is applicable to skeletal muscle fibers.

EXPERIMENTAL METHOD

The test object was the sartorius muscle of the frog (Rana ridibunda). The electrical activity of single muscle fibers was recorded intracellularly by means of glass microelectrodes filled with a 3 M solution of KCl; the impedance of the electrodes varied from 10 to 40 MΩ. Two microelectrodes were introduced separately into one muscle fiber. One of these microelectrodes was used for detecting the potentials and the other for stimulating the fiber. The potential difference on the membrane was measured by means of a two-channel dc amplifier with an input capacitance compensator. The strength of the current passing through the object at the moment of stimulation was measured from the fall in potential across a resistance of 15 kΩ connected in the circuit in series with the object. To measure the input impedance of the fibers, rectangular pulses of hyperpolarizing current were used. The composition of the Ringer's solution (in millimoles) was: NaCl 111.1, KCl 1.3, $NaHCO_3$ 1.4, $CaCl_2$ 1.8. The concentration of Ca ions varied in the course of the investigation from 0.18 to 27 mmole. Novocain solutions of concentrations of $0.3\text{-}7.5 \times 10^{-4}$ g/ml were used. The concentration of NaCl in the solutions was not changed.

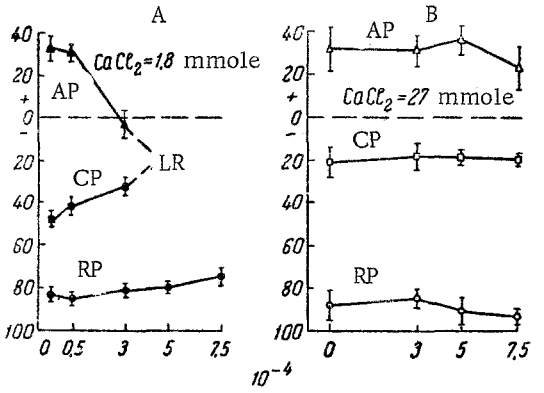

Fig. 1. Effect of Novocain on magnitudes of the resting potential (RP), the action potential (AP), and the critical potential (CP) of the skeletal muscle fiber. A) With a normal concentration of Ca ions in the Ringer's solution (1.8 mmole); B) with 27 mmole $CaCl_2$. Along the axis of abscissas) concentration of Novocain (in g/ml); along the ordinates) potentials (in mV). The vertical lines denote the standard deviations. LR) Local response.

EXPERIMENTAL RESULTS AND DISCUSSION

In Ringer's solution for cold-blooded animals the resting potential (RP) of the muscle fibers was 83.32 ± 3.46 mV (70),* the action potential (AP) was 116.5 ± 4.5 mV (44), and the critical potential (CP), measured as the magnitude of the membrane potential at which the AP appeared, was 47.15 ± 3.53 mV (60). The input impedance of the fibers was 352.15 ± 47.67 kΩ (41). The scatter of the values of the input impedance in the various experiments was fairly considerable: from 200 kΩ to 1 MΩ.

Before commencing the study of the effect of changes in the concentration of $[Ca]_n$ on the action of Novocain, the

* Here and in future the number of fibers tested is placed in parentheses.

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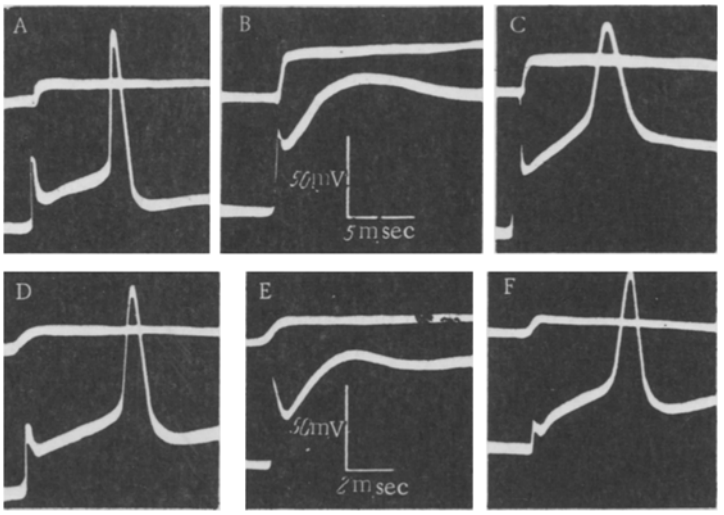

Fig. 2. Changes in the electrical activity of the muscle fibers during the action of Novocain with an excess or a deficit of $[Ca]_n$. A) AP in a medium with a normal concentration of Ca ions; B) action of $5 \cdot 10^{-4}$ g Novocain/ml; C) combined action of $5 \cdot 10^{-4}$ g Novocain/ml and 27 mmole $CaCl_2$; D) AP in "normal" saline medium with $0.3 \cdot 10^{-4}$ g Novocain/ml; E) muscle in a solution with $0.3 \cdot 10^{-4}$ g Novocain/ml and 0.18 mmole $CaCl_2$; F) AP in Ringer's solution with 0.18 mmole $CaCl_2$. The top line corresponds to the zero line for the intracellular microelectrode; the stimulating current applied to the fiber through the second microelectrode is also shown on it. The bottom line shows the potential recorded by the microelectrode.

action of Novocain and Ca ions on the electrical activity of the muscle fibers was investigated separately.

When the Ringer's solution of "normal" composition was changed for a solution containing Novocain or for Ringer's solution with a changed concentration of Ca ions, the muscle was kept in this solution for 30 min before measurement of the electrical parameters began.

Effect of Novocain on electrical parameters of the muscle fibers in the normal Ringer's solution. During the action of Novocain in concentrations of $0.5 \cdot 10^{-4}$ g/ml, a slight increase in the RP took place, to 85.56 ± 3.17 mV (25). This value differed significantly from the value of the RP in Ringer's solution of "normal" composition ($P < 0.002$). The amplitude of the AP remained unchanged and was 116.5 ± 3.5 mV (25), while the CP fell significantly to reach 41.24 ± 4.84 mV (32; $P < 0.001$; Fig. 1A).

An increase in the concentration of Novocain to $5 \cdot 10^{-4}$ g/ml led to a decrease in the RP to 76.66 ± 3.87 mV (34; $P < 0.001$) and to total suppression of the AP (see Fig. 1A). The results obtained with respect to the effect of Novocain in a concentration of $2 \cdot 10^{-4}$ g/ml on the parameters of the electrical activity of the frog's muscle fibers are in agreement with those obtained by other investigators [9].

Changes in electrical activity in the presence of an excess of deficit of $[Ca]_n$. An increase in the concentration of $CaCl_2$ to 27 mmoles caused an increase in the RP from 83.32 ± 3.45 mV (70) to 88.33 ± 7.46 mV (25). The difference is significant ($P < 0.002$). The amplitude of the AP in a solution with an increased concentration of $CaCl_2$ was not appreciably changed (Fig. 1B), while the CP was significantly lowered – to 21.33 ± 7.15 (29); $P < 0.001$. The input impedance of the fibers was unchanged.

A decrease in the concentration of $CaCl_2$ in the Ringer's solution to 0.9 mmole led to a lowering of the RP to 76.38 ± 4.78 (65), $P < 0.001$. No statistically significant difference was observed between the "overshoots" of the AP in 1.8 and 0.9 mmole $CaCl_2$. In Ringer's solution with 0.9 mmole $CaCl_2$ the CP was significantly lowered – to 32.9 ± 5.84 mV (65). The subsequent decrease in the $CaCl_2$ concentration to 0.18 mmole caused spontaneous activity of the fibers, the values of the RP were essentially unchanged, but a decrease in the "overshoots" of the AP

was observed. The CP measured in the fibers which did not contract spontaneously was lowered to 28 ± 5.7 mV (11); $P < 0.001$. No significant difference was found between the values of the input impedance of the muscle fibers in the presence of a normal and a lowered concentration of $CaCl_2$ in the solution.

Other investigators [4, 5] have observed similar changes in the electrical activity of the muscle fibers with a decrease in the $CaCl_2$ concentration in the Ringer's solution.

Action of Novocain in the presence of an excess and deficit of Ca ions in the Ringer's solution. The combined action of 27 mmole $CaCl_2$ and $5\text{-}7.5 \cdot 10^{-4}$ g/ml Novocain increased the RP to 88.25 ± 5.29 mV (40) both by comparison with the normal value and by comparison with the value of the RP during the action of Novocain in a concentration of $5\text{-}7.5 \cdot 10^{-4}$ g/ml. The amplitude of the AP, lowered in the solution of Novocain $5\text{-}7.5 \cdot 10^{-4}$ g/ml, recovered to 116.38 6.52 mV (40); the magnitude of the CP was lowered (see Fig. 1B and Fig. 2A-C).

In Ringer's solution containing $3\text{-}5 \cdot 10^{-4}$ g Novocain/ml and 27 mmole $CaCl_2$ the duration of the ascending and descending phases of the AP was increased. Whereas in normal conditions the mean duration of the ascending phase was 0.5 msec (53) and of the descending phase 0.66 msec, in response to the combined action of 27 mmole $CaCl_2$ and $3\text{-}5 \cdot 10^{-4}$ g/ml Novocain the duration of the ascending phase reached 1.34 msec (40), and of the descending phase — 2.22 msec (Fig. 2A and C).

Novocain, in a concentration of $0.03 \cdot 10^{-4}$ g/ml did not change the value of the AP, but when the concentration of $CaCl_2$ in the Ringer's solution was 0.18 mmole, Novocain in a concentration of $0.3 \cdot 10^{-4}$ g/ml completely suppressed the active response of the fibers. In these circumstances the value of the RP was 79.7 mV (Fig. 2F and E).

The results of these experiments demonstrate that a lowered concentration of $[Ca]_n$ in the skeletal muscle fibers potentiates the depressant action of Novocain; conversely, an excess of Ca ions significantly weakens the effect of Novocain. This antagonism between Ca ions and Novocain is exhibited only in relation to their effect on the amplitude of the AP, for the changes in the CP under the influence of Ca ions and Novocain may take place in either direction.

In the modern view local anesthetics disturb the ability of an excitable membrane to increase its permeability to Na ions in response to stimulation [1, 9-12]. The concrete mechanism of this action of local anesthetics is not clear, but it is known that they can penetrate into the lipoid layers of the membrane and enter into chemical association with the phosphate groups of the phospholipids. Ca ions possess the same ability to become bound to the phospholipids [6]. The results obtained with respect to the antagonism between Novocain and Ca ions may therefore be naturally explained by competitive relationships between them for the same chemical groups in the membrane. This hypothesis is in good agreement with the currently widespread opinion of the role of Ca ions in the mechanism of the change in the ionic permeability of the membrane during excitation [7, 8]. Bearing in mind that in a resting state Ca ions block the sodium pores (or the "sodium carriers") of the membrane, the depressant action of Novocain may be regarded as the result of replacement of the Ca ions by Novocain in these parts of the membrane. Clearly, therefore, the lower the concentration of $[Ca]_n$, the stronger the depressant action of Novocain must be, and conversely, with an increase in the concentration of $[Ca]_n$, the effect of Novocain is considerably weakened. Taken as a whole, the results show that the principles of interaction of Novocain with Ca ions observed earlier in nerve tissue also apply to the skeletal muscle fibers.

SUMMARY

Two glass microelectrodes were used to study the action of calcium ions on the rest potential (RP), action potential (AP), and critical potential (CP) of the fibers of the frog's sartorius muscle kept in a novocain solution. An increase of $CaCl_2$ concentration from 1.8 mM to 27 mM restored the amplitude of AP suppressed by the Novocain solution of $5 \cdot 10^{-4}$ g/ml.

A combined administration of Novocain solution ($0.3 \cdot 10^{-4}$ g/ml) and $CaCl_2$ (0.18 mM) in concentrations which by themselves did not inhibit the electric activity of the fibers led to a complete suppression of AP generation.

It has been concluded that calcium and Novocain ions act as antagonists with regard to their influence on the AP amplitude and as synergists with regard to their action on CP.

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