DIFFERENCES BETWEEN MECHANISM OF DEPRESSANT ACTION OF TETRODOTOXIN AND PROCAINE ON FROG SKELETAL MUSCLE FIBERS

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Numerous investigations conducted on nerve and muscle fibers have shown that procaine and other local anesthetics block the increase (activation) of sodium conductance of the cell membrane during its depolarization. Most investigators consider that procaine, like the venom of the Japanese fish fugu – tetrodotoxin [11, 13, 16], depresses the "maximal sodium conductance" (g_{Na}), i. e., it reduces the number of sodium channels in the membrane accessible for activation or inactivation under the action of the depolarizing stimulus [6, 11, 17]. In contrast to this, Weidmann [18], B. I. Khodorov and V. I. Belyaev [5], and E. G. Vornovitskii and B. I. Khodorov [3] concluded that the depressant action of procaine in Purkinje fibers and medullated nerve and skeletal muscle fibers is due mainly to inactivation of the sodium system rather than to changes in the value of g_{Na}.

In the present investigation, to continue the study of this problem, the effect of procaine and tetrodotoxin on electrical activity of the muscle fiber was compared. The comparative analysis of the action of these agents was carried out in ordinary conditions and in the presence of an increased Ca ion concentration in the medium or of hyperpolarization of the fiber membrane by a constant current.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscles of the grass frog. Two glass microelectrodes, filled with 3M KCl solution, were introduced separately into the muscle fiber so that the distance between their points was about 50 μ. One electrode was used for recording the membrane potential, the other for transmitting the polarizing and stimulating currents. The potentials for recording were fed into a 2-channel electrometric dc amplifier with input capacitance compensation. The solutions of procaine and tetrodotoxin were made up in Ringer's solution of the following composition (in g/liter): NaCl 6.5, KCl 0.1, $CaCl_2$ 0.2, $NaHCO_3$ 0.2. The experimental method is described fully elsewhere [2, 3].

EXPERIMENTAL RESULTS

In Ringer's solution of normal composition the resting potential (RP) of the investigated muscle fibers was -81.3 ± 2.6 mV, the overshoot of the action potential (AP) was $+30.7 \pm 3.5$ mV, and the critical potential (the potential of the inner aspect of the membrane during transformation of the local response into AP) was 50.3 ± 3.5 mV.

Tetrodotoxin, in concentrations of $10^{-8} - 5 \cdot 10^{-8}$ g/ml, caused only a small decrease in amplitude of the AP; when the concentration of toxin was increased to 10^{-7} g/ml, depression of the AP became very obvious. For instance, after the muscle had been in a solution of 10^{-7} g/ml for 30 min, generation of the AP was suppressed in most muscle fibers (Fig. 1, B and C). In those fibers in which AP were still generated (Fig. 1, D), they were marked by low amplitude (overshoot absent or not exceeding 3-4 mV), a gentle rise of the ascending phase, and an increased critical potential (-33.5 ± 11.1 mV).

The RP of the muscle fibers was usually unchanged by the action of tetrodotoxin, and only if the muscle was kept in the experimental solution for a long time (more than 1 h) was slight membrane depolarization observed (77.8 ± 4.7 mV).

The changes in electrical activity of the muscle fibers observed in these experiments under the influence of tetrodotoxin were indistinguishable in principle from those observed in analogous conditions

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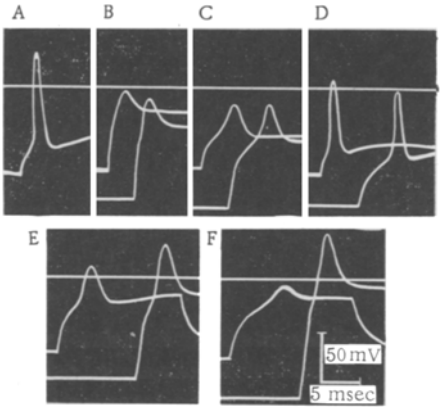

Fig. 1. Effect of hyperpolarization of the membrane on frog skeletal muscle fibers treated with tetrodotoxin. A—AP of muscle fiber in Ringer's solution of normal composition; B-D—responses of muscle fibers placed in tetrodotoxin solution in concentration of 10^{-7} g/ml to stimuli applied before and during hyperpolarization of the membrane by a constant current; E-F—the same after addition of 36 mmole $CaCl_2$ to the tetrodotoxin solution.

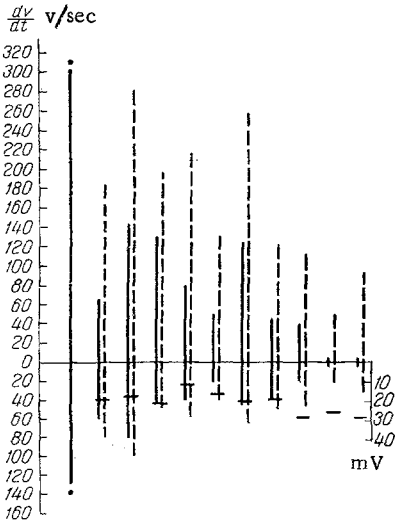

Fig. 2

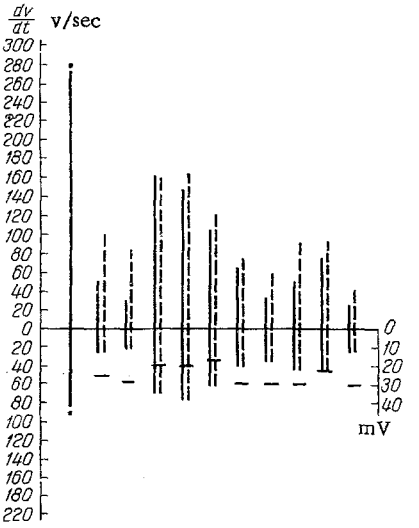

Fig. 3

Fig. 2. Restoration of maximal velocities (dV/dt) of rise and fall of AP of muscle fibers treated with procaine under the influence of hyperpolarization of the membrane. The first vertical line and the dot above it indicate the mean value and standard deviation of maximal velocities of rise (above the zero line) and fall (below the zero line) of AP in Ringer's solution of normal composition. The subsequent continuous vertical lines denote values of maximal velocities of changes in membrane potential of muscle fibers kept in 0.03% procaine solution. Broken lines represent maximal velocities of rise and fall of AP of the same muscle fibers under the influence of membrane hyperpolarization by a constant current. The short horizontal lines represent changes (in mV) in RP during action of hyperpolarizing current (right hand scale).

Fig. 3. Effect of hyperpolarization of membrane on maximal values of RP and fall of AP in muscle fibers treated with tetrodotoxin in concentration of 10^{-7} g/ml. Legend as in Fig. 2.

by Narahashi [14]. They were also similar to the effects produced on muscle fibers by procaine in concentrations of $3 \cdot 10^{-4}$ - $5 \cdot 10^{-4}$ g/ml [2, 3, 10].

The authors have previously shown that a sharp decrease in the steepness of rise and in the amplitude of the AP under the influence of procaine may be abolished or considerably reduced by hyperpolarization of the muscle fiber membrane by a constant current (Fig. 2) or by an increase in the Ca ion concentration in the medium [1-3]. These results were confirmed in the present investigation. Different results were obtained in experiments in which muscle fibers treated with tetrodotoxin were subjected to the action of a hyperpolarizing current. In this case even powerful hyperpolarization of the membrane did not restore AP generation (Fig. 1, A-D). In those cases when AP generation was not completely suppressed, the hyperpolarizing current caused only a small increase in the steepness of rise and in the amplitude of the AP (see Figs. 1 and 3).

An increase in the Ca ion concentration in the solution (from 1.8 to 36 mM) likewise had no restorative action on the muscle fibers modified by tetrodotoxin. Only a small increase in RP and in the local responses to powerful stimulation took place. In those fibers which continued to generate low-amplitude AP in the presence of tetrodotoxin, an excess of Ca ions caused an increase in the critical potential (which was moved away from the RP) and a slight increase in amplitude and steepness of rise of the AP. Partial recovery of AP generation in muscle fibers modified by tetrodotoxin was obtained only by combined application of the hyperpolarizing current and an excess of Ca ions (Fig. 1, E and F). However, the RP of these fibers was lower than initially.

DISCUSSION OF RESULTS

The results described above demonstrate significant differences between the changes in properties of the excitable membrane of muscle fibers under the influence of tetrodotoxin and procaine. In the case of procaine treatment, hyperpolarization of the membrane restores AP generation even in those fibers which, under the influence of the local anesthetic, had completely lost their ability to give regenerative responses. The steepness of rise and, in particular, the amplitude of the AP in these circumstances were close to their initial values obtained on the same muscle before administration of the anesthetic. An excess of Ca ions in the solution also had a powerful restorative action on the amplitude of the AP of muscle fibers treated with procaine.

Since both agents used weaken or abolish sodium inactivation of the membrane [8, 12], it may naturally be concluded that the depressant action of procaine on skeletal muscle fibers is due mainly to inactivation of sodium permeability.

Essentially different relationships are observed in muscle fibers treated with tetrodotoxin. Not even the slight depression of AP produced by this agent can be abolished by hyperpolarization of the membrane or by an increase in the calcium ion concentration in the solution. Only a slight increase in the steepness of rise of the AP and a decrease of the critical potential take place. However, such changes are found in many muscle and nerve fibers in normal Ringer's solution. They are due to the fact that in surviving preparations at rest there is always slight inactivation of the sodium system [9] which can easily be removed by hyperpolarization of the membrane. It must also be remembered that if muscle fibers are kept for a long time in an experimental solution the RP falls slightly, and this naturally still further increases the initial sodium inactivation. In the case of total suppression of regenerative responses in the muscle fiber under the influence of tetrodotoxin, hyperpolarization of the membrane or an increase in the Ca ion concentration becomes completely ineffective.

It may be concluded from these facts that the mechanism of the depressant action of procaine on AP generation in muscle fibers differs from that of tetrodotoxin.

The action of procaine is evidently associated with its ability to penetrate into the lipid layers of the membrane [13, 15] and to react chemically with the phosphate groups of the lipids [4, 5, 7]. It may be considered that as a result of this reaction certain unbalanced structures in the membrane, which at rest are actively maintained by the transmembrane potential difference and by Ca ions, are disturbed (inactivated). In the absence of procaine, to maintain this structure in a state of readiness to react, a potential difference of the order of 75-80 mV is adequate. After treatment with procaine, however, to obtain AP it was necessary either to increase this transmembrane potential difference by 20-30 mV or to increase the Ca ion concentration in the medium substantially.

Unlike procaine, tetrodotoxin apparently reacts directly with the structural elements of the membrane concerned in transfer of Na ions through the membrane. The tetrodotoxin molecule contains a guanidine group, and guanidine is known to be able to replace Na ions in the solution during AP generation. The suggestion was therefore put forward that the tetrodotoxin molecule introduces its guanidine group into the sodium channel and, because of its enormous skeleton, is converted into a tightly fitting "plug". By "plugging" the sodium channels, tetrodotoxin thereby reduces the number of these channels accessible for activation during the action of the stimulus. Since neither hyperpolarization nor calcium ions can "unplug" the channels blocked by the toxin, no restoration of AP takes place under these conditions.

The fact described above may be summarized as follows. Until recently most investigators considered that the only difference between the action of procaine and tetrodotoxin on the excitable membrane was that tetrodotoxin depresses sodium permeability of the membrane in lower concentrations than procaine, and that in contrast to procaine, it does not modify the potassium permeability of the membrane [11, 14, 16]. The results of the present investigation show that these differences also extend to the mechanism of action of tetrodotoxin on the sodium permeability system: tetrodotoxin blocks sodium channels while procaine inactivates the mechanism responsible for their opening during depolarization.

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