THE EFFECT OF ELECTRICAL POLARIZATION OF THE MOTOR NERVE ENDINGS ON THE TRANSMISSION THROUGH THEM OF SINGLE IMPULSES

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 6, No. 11,
pp. 11-14, November, 1963
Original article submitted June 17, 1963

A study of the functional features of presynaptic terminations is highly important in connection with determining the mechanism of neuro-muscular transmission. The results obtained by many authors leads to the conclusion that the function of these endings is determined by the degree of polarization of their membrane. Depolarization leads to an increased frequency of spontaneous discharges of the miniature end-plate potentials and to a reduction of the end-plate potentials evoked by nervous impulses [3, 7, 9]. Hyperpolarization is associated with a reduction of the discharge frequency of miniature potentials at the end-plates and to an increase of the synchronized potentials of the end-plates. However, these results do not appear to be complete, because owing to the inadequacy of the method of electrical polarization, a high current strength could not be used, and a block of conduction in the nerve fibers themselves could easily be produced.

By now a new method of producing polarization has been worked out; it enables considerable changes in the nerve endings themselves to be produced without any block of conduction in the nerve fiber [6, 8]. This method has been successfully used to study functional changes evoked by electrical polarization of the presynaptic endings of the rat diaphragm.

An extremely interesting application of this method is to apply it to a determination of the functional features of nerve endings in the classical frog nerve-muscle preparation.

EXPERIMENTAL METHOD

For the experiments we used a nerve-muscle preparation of the frog sartorius. In some cases immobilization was induced by curarization ($1.5\text{-}2.0 \cdot 10^{-6}$ D-tubocurarine), and in others by an increase of magnesium ion concentration (11 mM) of the solution surrounding the muscle. Stimulation was by square-waves delivered through a radio-frequency lead. The end-plate potentials (EPP) were led off from the synaptic region of the muscle fiber by intracellular microelectrodes having a resistance from 5 to 15 megs. The polarizing current was applied through two silver chlorided electrodes, one of which was connected through an agar bridge to the nerve and the other was placed in a micropipette filled with agar-Ringer (resistance not greater than 3 megs) (Fig. 1). The micropipette was not further than 100 μ from the end plate. This method provided wide variation in the duration and extent of the polarization of the motor nerve endings without leading to any block of the impulses in the nerve fibers.

EXPERIMENTAL RESULTS

In our experiments we were able to use polarizing currents from 5 to 20 μA for hyperpolarization or depolarization of the motor-nerve endings. It was only with a current of this strength that a block occurred in the nerve fibers as shown by a complete disappearance of the EPP.

With hyperpolarization of the motor nerve endings (which [illegible]) a current too small to block [illegible] conduction, the amplitude of the EPP [illegible] times. This increase was not easily observed

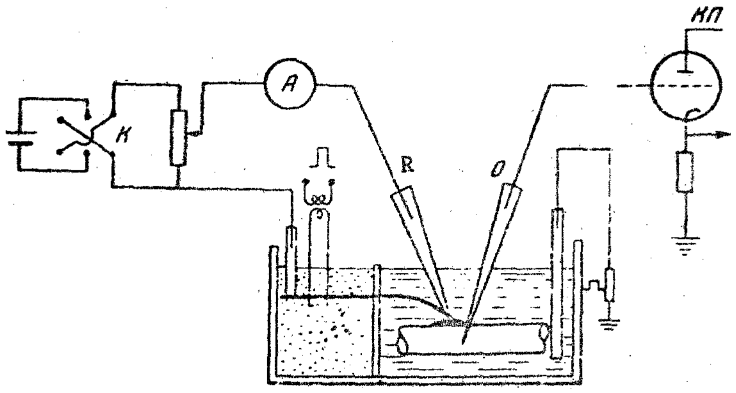

Fig. 1. Diagram showing the method of polarization of the presynaptic terminations of the neuromuscular junction in the frog. The muscle lies in the right-hand part of the chamber which is filled with Ringer. The EPPs were led off by means of an intracellular electrode (O). The polarizing current was applied between a micropipette (P) lying by the end-plate and an electrode in contact with the nerve immersed in vaseline (in the left part of the chamber). The polarizing current was measured by a microammeter (A).

A

B

Fig. 2. Changes of amplitude of the EPP during and after hyperpolarization of the end-plates. A) Changes of amplitude caused by a hyperpolarizing current of strength 12 μA: 1) EPP led off intracellularly from a muscle fiber before connection of the hyperpolarizing current; 2,3,4) ditto, 1, 2, and 3 min after the start of hyperpolarization; B) changes in the EPP after switching off the hyperpolarizing current: 1) immediately after the current had been switched off; 2,3,4) after 1, 2, and 3 min, respectively.

immediately after the current had started. It developed during the prolonged application of a current of constant strength, and reached a maximum of 3 or 4 times the original value by the end of the third minute (Fig. 2, A).

Further increase of the time of polarization up to 10 min produced no appreciable change in the amplitude of the response.

[illegible] had been switched off there was a de[illegible] of the EPP [illegible] B),

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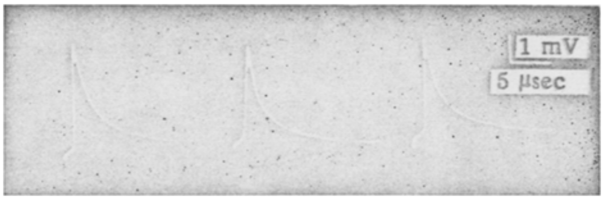

Fig. 3.

An increased amplitude of the EPP depended not only on the duration of the polarization but also on the strength of the current applied. Small currents produced either no effect, or a weak one; with increase of current strength the amplitude of the responses increased.

When solutions containing an increased magnesium content (up to 11 mM), with hyperpolarization of the motor nerve endings the same increase in the amplitude of the EPP was observed as was found in curarized preparations.

With depolarization of the nerve endings the EPP were reduced in amplitude immediately after the current was started, and remained depressed subsequently to the same degree throughout the whole period of polarization (Fig. 3). The amplitude was reduced 10–20% below the normal value, according to the current strength; with increase of current strength there was a considerable reduction in the amplitude of EPP. After the current was switched off the amplitude of the end-plate potentials immediately returned to the original value.

As in the case of hyperpolarization these changes occurred only during electrotonic polarization of the endings.

The results obtained confirmed the idea that the effectiveness of the trans-synaptic action of a nervous impulse depends to some extent on the level of electrical polarization of the nerve endings: depolarization depresses, and hyperpolarization increases the action. The critical quantity is the change of polarization of the ending itself, because when there is a distance greater than 100 μ between the end-plate and the polarizing electrode no changes occur. These results agree with those obtained previously on the nerve-muscle connection in the frog [3], and also with those obtained for other types of synapse.

Thus, hyperpolarization of the presynaptic elements brought about by an intracellular electrode inserted into the giant synapse of a squid caused an increased action potential of the presynaptic fiber and an enhanced postsynaptic potential; this effect appeared to be due to an increased amount of mediator liberated [5, 10]. The same effect was produced by hyperpolarization of the terminal branches of the afferent fibers of the spinal cord in the cat [4].

Depolarization of the presynaptic elements in all cases brought about the reverse change.

In our experiments there were in addition changes of synaptic transmission which cannot be directly related to changes of electrical polarization of the endings, and which have not been pointed out in the reports to which we have referred.

The passage of a polarizing current evoked a slow increase in the effectiveness of the termination, a change which exceeded several times those which developed in response to a short-acting current. In this respect the synaptic terminations of a frog nerve-muscle preparation correspond completely to the synaptic terminations at the neuromuscular junction of warm-blooded animals [6, 8]. Apparently the slowness of the changes accounts for this effect not having been described previously.

It is quite possible that similar properties will be found in other types of synapse, for example in central synapses, if adequate methods for producing polarization are worked out.

At the present time it is difficult to say [illegible] observed [illegible] during rapid or slow [illegible] [illegible] in the number [illegible] membrane [illegible] in hyperpolarization [illegible] correspondingly.

However, the slowly increasing changes cannot of course be related to such a mechanism. The idea has therefore arisen that hyperpolarization leads to important changes within the cytoplasm of the termination and possibly to a spread within it of the synaptic vesicles which, according to modern views [1, 2, 11] carry the mediator.

SUMMARY

A frog nerve-muscle preparation was used to study the effects of depolarization and hyperpolarization of the transmission of single impulses at nerve endings. The efficacy of synaptic transmission was determined by the value of the end-plate potential led off intracellularly from the muscle fiber.

Depolarization of the nerve endings produced a rapid reduction of up to 10-20% in the trans-synaptic action of the ending. Hyperpolarization of the endings caused the efficacy of trans-synaptic action to increase slowly for three minutes. In such cases the end-plate potential rose to 3 or 4 times the control level. These changes were observed only with a local polarization of the region of the nerve endings, and were absent when the preterminal branches of the nerve were polarized.

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