INCREASED AMPLITUDE OF POSTSYNAPTIC POTENTIALS IN THE FROG NEUROMUSCULAR SYNAPSE DURING COMBINED DIRECT AND INDIRECT STIMULATION OF THE MUSCLE FIBER

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The possibility of existence of feedback between the muscle fiber and neuromuscular synapse was subjected to direct experimental investigation. Activity of the motor end-plate in an isolated preparation of the sciatic nerve and sartorius muscle of Rana temporaria was recorded intracellularly during partial blocking of synaptic transmission by low-frequency (1/sec) indirect stimulation for 7-10 min. Simultaneous direct and indirect stimulation of the muscle fibers by a volley of stimuli was followed after an interval of 50-500 msec by a testing stimulus applied to the nerve. An effect of an increase in amplitude of the testing end-plate potential (EPP) compared with the amplitude of the corresponding EPP evoked after application of a volley of stimuli to the nerve only, was observed in several experiments. The presence and magnitude of this effect were found to depend on the depth of the block in the experimental preparation and on the intensity of potentiation of the control EPPs.

The possibility of a modulating or activating feedback from a skeletal muscle to the neuromuscular synapse and, in particular, to the motor nerve and its ending has been discussed by several writers [1-9]. One way of obtaining direct evidence that such feedback exists is by creating experimental conditions for the function of the neuromuscular synapse under which generation of end-plate potentials (EPPs) and muscle action potentials (APs) would be uncoupled so that it would be possible to observe in turn either isolated EPPs or combined EPPs with muscle APs, simulating normal unblocked transmission.

This approach to the problem was used in the investigation described below.

EXPERIMENTAL METHOD

EPPs and APs of a muscle fiber were recorded intracellularly by means of "floating" glass microelectrodes [2] with a tip about 1 μ in diameter from an isolated preparation of the sartorius muscle and sciatic nerve of the frog Rana temporaria. The electrical activity was led to a dc amplifier and photographed from the screen of a type S1-18 cathode-ray oscilloscope. The nerve was stimulated by square pulses (0.5-1.0 msec, 30 or 50 Hz) through silver electrodes situated 20-30 mm from the muscle. For direct stimulation, Ag—AgCl electrodes 1.5-2 mm in diameter were applied to the surface of the muscle 10 mm from the recording point. The muscle contracted under isometric conditions. The threshold strength of the stimulus for direct stimulation was 0.3-0.5 V. Stimulation above threshold strength was used. The two-channel EST-10A and the ESL-1 stimulators were used. Neuromuscular transmission was blocked by stimulating the nerve for 7-10 min with single pulses at 1 Hz. The depth of the block was considered to be

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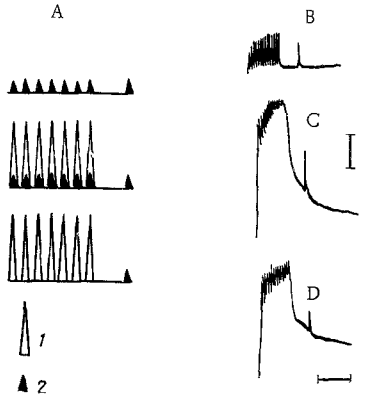

Fig. 1

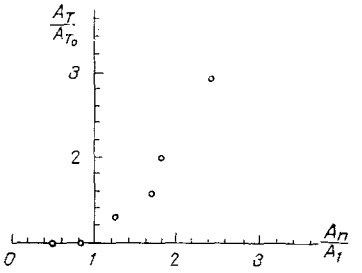

Fig. 2

Fig. 1. Experiments with a combination of direct (1) and indirect (2) stimulation of the muscle: A) scheme of experimental method; (B-D) results of one experiment; B) control series of EPPs with delayed testing EPP; C) simultaneous direct and indirect stimulation of muscle, testing EPP appreciably greater than in the control; D) control series of muscle APs with delayed testing EPP. Calibration 10 mV, time marker 0.5 sec.

Fig. 2. Comparison of effect of increase in testing EPP after combined stimulation A_T/A_{T_0} with degree of potentiation (or depression) of last EPP in control volley A_n/A_1. Points represent results of six experiments with superficial block (amplitude of single EPP from 6 to 10 mV) for values of T in each experiment at which the value of A_T/A_{T_0} was maximal.

optimal if EPPs of sufficiently high amplitude appeared in response to indirect stimulation at 30-50 Hz without the appearance, meanwhile, of APs in the muscle fiber.

Simultaneous direct and indirect stimulation of the muscle by a volley of stimuli of fixed duration (500 msec) with the testing EPP, separated from the volley by an interval of time T, was used. The interval T could be changed from 0 to 500 msec. Under these circumstances APs, which were masked by EPPs superposed on them, and the testing EPP were recorded. A series of EPPs evoked by the same volley of stimuli applied to the nerve, together with the testing stimulus separated from it by the same time interval T, was used as the control (Fig. 1A).

In all experiments the shape of the EPP was verified in order to make sure that local responses of the electrically excitable membrane of the muscle fiber were not being recorded.

EXPERIMENTAL RESULTS

In the control series of EPPs a progressive potentiation of the successive EPPs was usually observed. The degree of potentiation of the testing EPP was smaller than that of the last EPP in the volley. With a decrease or increase in the interval T the amplitude of the testing EPP steadily approached that of the last EPP in the volley or the single EPP (the first in the volley). In some preparations potentiation was absent or a progressive decrease of the EPPs in the series was observed. Under these circumstances the testing EPP was smaller than the first but larger than the last EPP. In each experiment the degree of potentiation (or depression) of the EPPs arising after repetitive stimulation was estimated from the ratio A_n/A_1, where A_n and A_1 are the amplitudes of the last and first EPP in the volley, respectively.

In experiments with simultaneous direct and indirect stimulation an effect of increased amplitude of the testing EPP (A_T) compared with the testing EPP in the control (A_m) was observed in seven of 17 preparations. The maximal observed effect was 300% of the control value. The size of the effect in each individual case depended on T and reached its maximum in the interval from 50 to 20 msec. With a decrease in T the effect diminished in magnitude. The reason was evidently that during combined stimulation, by contrast with the control, the testing EPP was recorded against a background of marked and prolonged after-depolarization induced by the series of muscle spikes.

Analysis of the results shows a connection between the presence and magnitude of the observed effect, on the one hand, and the presence of potentiation and the depth of the block on the other hand. The depth of the block was estimated from the amplitude of the single (or first) EPP, for the recording was made each time directly from the region of the end-plate (this was shown by the presence of miniature EPPs and by the shape of the EPP). In every case in which the amplitude A was large enough (≥ 6 mV) so that, under those circumstances, definite potentiation was observed ($A_n/A_1 > 1$) this effect was well marked ($\geq 130\%$). With a deeper block ($A_1 \leq 3$ mV) and in the presence of potentiation the effect was absent or weak ($\leq 120\%$). If, however, not potentiation but depression of the EPP was observed in the control ($A_n/A_1 < 1$), no effect was found whatever the amplitude of the EPP. In experiments with EPPs of a high amplitude (superficial block) quantitative correlation was observed between the degree of increase of the testing EPP after combined stimulation A_T/A_{T_0} and the degree of potentiation A_n/A_1 (Fig. 2).

As an additional control the testing EPP was recorded after a series of APs evoked by direct stimulation only (Fig. 1D). In these experiments the amplitude of the testing EPP did not exceed that of the first EPP in a series of EPPs evoked by nerve stimulation (Fig. 1B). Repetitive excitation of the muscle fibers by itself thus did not lead to the effect described. This fact, together with the dependence on the depth of the block indicated above, show that, for the effect to develop, a certain degree of excitation of the structures on the neuromuscular synapse – of the postsynaptic and, possibly, the presynaptic membrane also – is required.

The second essential condition for manifestation of the effect is potentiation of the EPP during repetitive nerve stimulation in the control. This suggests that the mechanism of the observed effect may be similar to that of the potentiation found under these experimental conditions. If, however, the nature of the effect differs from the mechanism of potentiation, in any event summation of the two processes leads to an increase in amplitude of the EPP during repetitive activity of the nerve–synapse–muscle system.

The phenomenon described above suggests a "stabilizing" effect of the muscle fiber on the neuromuscular synapse. The concrete mechanism of this effect requires further investigation.

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