ELECTROPHYSIOLOGICAL STUDY OF TRANSMITTER RELEASE IN FROG NEUROMUSCULAR SYNAPSES UNDER COLCHICINE BLOCKADE OF AXOPLASMIC TRANSPORT

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The appearance of denervation-like changes in the membrane of frog muscle fibers after disturbance of axoplasmic transport (AT) in the nerve supplying the membrane [1] by colchicine is accompanied by a decrease in heterogeneity of the myoneural synapses in the sartorius muscle as reflected in the quantum composition (m) of the end-plate potential (EPP) [2]. It can be tentatively suggested that substances transported to the muscle by AT not only help to keep the muscle membrane in a differentiated state [1], but also determine the character of release of acetylcholine (ACh) in the neuromuscular junction. At the same time, we know that synaptic ACh and the firing pattern of the nerve [9] can play a definite role in the mechanism of neurotrophic control of the muscle fiber.

To test the hypothesis of regulation of the functional state of the presynaptic membrane of the motoneuron soma, and also to explain the possible importance of a change in ACh secretion for the development of denervation-like changes in the muscle membrane, it was decided to study the character of ACh release by nerve endings after disturbance of AT in nerve fibers.

The object of the present investigation was to study the character of evoked and spontaneous ACh release in the neuromuscular junction after disturbance of AT in the motor nerve by means of colchicine, at times when denervation-like changes in the muscle fiber membrane are already clearly defined [1].

EXPERIMENTAL METHOD

Experiments were carried out on a preparation of sciatic nerve and sartorius muscle from frogs *(Rana ridibunda)* in the fall and winter. To disturb AT, a segment of the nerve supplying the sartorius muscle was treated with 30 mM colchicine solution (from Sigma, USA) by the method described previously [1]. Muscles of frogs in which the nerve was treated with Ringer's solution without colchicine were used as the control. All animals were kept in a terrarium with running water at room temperature.

EPP and miniature EPP (MEPP) were recorded by the standard microelectrode technique. The mean values of the frequency and amplitude of MEPP were calculated after 100-150 consecutive MEPP had been recorded. The value of m was determined by the direct method [4] during stimulation of the nerve at a frequency of 0.5 Hz. The absolute refractory phase (ARP) of the nerve endings was taken to be the longest interval between stimulating pulses for which the second stimulus just did not evoke an EPP [5]. During the experiment, the muscle was kept in a bath containing running Ringer's solution of the following composition (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8 in bicarbonate buffer, pH 7.2. To record EPP uncomplicated by the action potential and contraction of the muscle [4], some Ca^{++} ions (1.05 mM) in the solution were replaced by Mg^{++} ions (2 mM).

EXPERIMENTAL RESULTS

On the 2nd and 7th days after application of colchicine to the nerve supplying the sartorius muscle, m of EPP and the frequency of MEPP did not differ from the control values (Table 1).

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TABLE 1. MEPP Frequency, EPP Quantum Composition, and ARP of Nerve Endings in Fibers of Frog Sartorius Muscle in Control and at Different Times after Application of Colchicine to Motor Nerve (M±m)

Parameter studied	Control (application of Ringer's solution)	Time after application of colchicine, days		
		2	7	14
Frequency of MEPP, spikes/sec	0,26±0,05 (23)	0,36±0,11 (21)	0,37±0,10 (24)	0,41±0,09 (19)
Quantum composition of EPP	3,8±1,0 (34)	3,6±1,0 (16)	2,8±0,7 (23)	2,0±0,5 (32)
ARP, msec	1,1±0,1 (11)	1,0±0,1 (10)	1,1±0,1 (10)	2,8±0,6* (11)

Legend. Number of observations in parentheses; *P < 0.05 compared with control (van der Waerden's method).

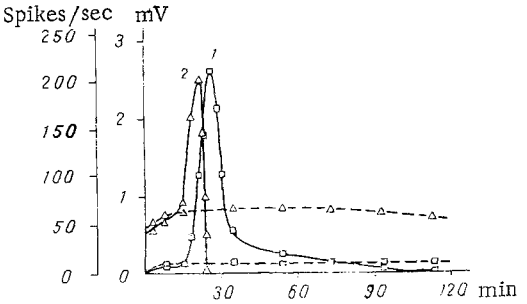

Fig. 1. Time course of MEPP frequency (1) and EPP amplitude (2) during nerve stimulation with a frequency of 5 Hz. Continuous line — in experiment, on 14th day after application of colchicine; broken line — control. Abscissa, time (in min); ordinate: frequency of MEPP (in spikes/sec) and amplitude of EPP (in mV).

In synapses which remained capable of evoked and spontaneous ACh secretion, the frequency of MEPP and the m value of the EPP did not differ statistically significantly from the controls, although at the same time ARP of the nerve endings was longer (Table 1). In eight fibers tested the MEPP frequency was higher (8.3±2.1 spikes/sec, $P < 0.001$) than in the control. It can be tentatively suggested that the increase in MEPP frequency in these cases was the result of depolarization of the presynaptic membrane, as shown by the increase in ARP of the nerve endings [6]. By drawing an analogy between the postdenervation fall of the resting membrane potential in the muscle fiber [1, 9] and depolarization of the presynaptic membrane, it can be postulated that the latter, like the muscle membrane, is under the control of the motoneuron soma.

Some neuromuscular junctions which remained capable of evoked activity were stimulated with a frequency of 5 Hz. In 27 of the 47 synapses tested in this way, a sharp increase in EPP amplitude and MEPP frequency was observed (Fig. 1) on average 20 min after the beginning of stimulation, followed by an irreversible block of evoked ACh secretion. Development of the blocking of evoked mediator secretion against the background of low-frequency stimulation can be conventionally divided into four periods: 1) a sharp increase in EPP amplitude; 2) an increase in MEPP frequency by 2-2.5 orders of magnitude, appearing 3-4 min after the beginning of the increase in EPP amplitude; 3) a fall in EPP amplitude at a time of maximal MEPP frequency and irreversible blocking of evoked ACh secretion; 4) a fall in the MEPP frequency below its initial values (0.030±0.008 spikes/sec, $P < 0.01$).

The increase in evoked and spontaneous mediator liberation during low-frequency nerve stimulation may be due to an increase in the ionized Ca^{++} concentration inside the terminal [7]. The latter, in turn, may be the result either of an increase in the inward calcium current because of excessive depolarization of the presynaptic membrane, or release of Ca^{++} from intraterminal depots [7]. Evidence of the possible depolarization of the nerve endings is

given by an increase in the values of ARP. Meanwhile, removal of Ca^{++} from the solution and addition of EGTA did not abolish the sharp rise in MEPP frequency against the background of low-frequency stimulation. The increase in MEPP frequency was perhaps due not only to depolarization of the presynaptic membrane, but also to a disturbance of storage of ionized Ca^{++} in nerve endings. The increase in evoked and spontaneous ACh release from the nerve endings described above after blocking of AT in the nerve against the background of low-frequency stimulation resembles in character the pattern of action of blockers of oxidative phosphorylation on the nerve ending [7]. Just as in the case of these poisons, irreversible disturbance of ATP synthesis may have taken place.

In the control group of frogs low-frequency stimulation as a rule did not block neuromuscular transmission, preceded by an increase in EPP amplitude and MEPP frequency, except in a few of the fibers tested. This may evidently be attributable to the particular features of "winter" frogs [3].

Meanwhile, division of the nerve led to a disturbance of evoked ACh secretion by the 3rd-4th day after the operation, as was shown previously [8], and also, as our own data showed, significantly increased the number of fibers in which stimulation (5 Hz) caused the phenomenon described above to develop. There are thus grounds for considering that changes in the character of ACh secretion in neuromuscular junctions are the result of disturbance of AT by colchicine; these changes, moreover, are highly reminiscent of the pattern of disturbance of ACh secretion after division of the nerve. In the latter case, however, they develop much faster.

The experimental results indicate that the appearance of denervation-like features in muscle fibers in which evoked and spontaneous electrical activity are present after blocking of AT in the nerve supplying them [1] can hardly be connected with changes in ACh secretion. Consequently, such changes cannot be the cause of the disturbance of neurotrophic control over the muscle membrane.

It can be postulated that AT or, probably, its rapid phase [9], maintains a definite level of resting membrane potential of the presynaptic membrane, and so regulates the character of evoked and spontaneous ACh secretion. Meanwhile, the axoplasmic flow into motor presynaptic endings maintains the necessary supply of structural materials and disturbance of AT leads ultimately to destruction of the nerve ending.

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