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NONQUANTUM ACETYLCHOLINE RELEASE IN THE FROG MYONEURAL JUNCTION AFTER DISTURBANCE OF AXOPLASMIC TRANSPORT BY COLCHICINE

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UDC 612.815.2:612.816].014.46:615.277.3:547.944.6

KEY WORDS: nonquantum acetylcholine release; axoplasmic transport; neurotrophic control; muscle fiber.

Disturbance of axoplasmic transport (AT) by colchicine (Co) in a frog motor nerve lowers the resting membrane potential (MP) of muscle fibers, the potassium conductance of the membrane becomes greater than the chloride conductance, and extrasynaptic sensitivity to acetylcholine (ACh) appears, just as after denervation of the muscle [2-4]. However, in the first case transmission of excitation from nerve to muscle is preserved [1, 2]. Similar denervation-like changes in the membrane of skeletal muscles develop after AT blockage in mammals also [5, 11]. These observations suggest that the properties of the membrane of the innervated skeletal muscle fiber are determined by substances transported to the muscle by AT [2-5, 11].

However, the opinion also is held that synaptic ACh may also play the role of factor responsible for neurotrophic control of the muscle membrane [7]. A special role in this process, it will be noted, has recently been ascribed to ACh [7] liberated by nerve endings in nonquantum form [6, 10]. It was accordingly interesting to study the effect of AT blockade on nonquantum ACh release, and the investigation described below was carried out for that purpose.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscle of the frog Rana ridibunda in the fall and winter, using a standard microelectrode technique. AT in the nerve supplying the muscle was blocked with 10 mM solution of Co (from Merck, West Germany) by the method described previously [2]. During the experiment the muscle was kept in continuously flowing Ringer's solution of the following composition (in mM): NaCl 118, KCl 0,* $CaCl_2$ 1.8, in phosphate buffer, pH 7.3, at 10 ± 0.5°C. Before the experiment the muscle was kept for 30 min in Ringer's solution containing 5×10^{-6} M armin,† an irreversible acetylcholinesterase inhibitor, and was then rinsed for 20 min. MP was recorded alternately in the synaptic and extrasynaptic zones of several

*As in Russian original – Publisher.
†Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

Department of Biology and Biophysics, Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A.D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 18-20, October, 1982. Original article submitted April 15, 1982.

muscle fibers. Miniature end-plate (EP) potentials with a duration of the ascending phase of about 1 msec served as the criterion that the microelectrode was actually in the EP membrane. Before addition of 1×10^{-5} to 5×10^{-5} g/ml D-tubocurarine chloride (D-TB, from Orion), the microelectrode was left in the synaptic region of the muscle fiber to record MP continuously. The initial reference point for measuring the amplitude of the hyperpolarizing response (HR) was the MP of the muscle fiber at the moment of complete disappearance of the miniature EP potentials. MP was then again measured alternately in the synaptic and extrasynaptic zones of the muscle fibers.

The input resistance of the muscle membrane was measured with two microelectrodes by determining the voltage drop on the membrane [8]. The zone and value of maximal sensitivity of the muscle fibers to ACh were studied by iontophoretic application of the mediator from a micropipet [8].

EXPERIMENTAL RESULTS

Hyperpolarization of the EP membrane in response to application of D-TB can be recorded only in the presence of irreversible inhibition of acetylcholinesterase, but in this case also the value of HR was extremely small [6], making a comparative analysis of HR difficult. Inactivation of Na,K-ATPase of the presynaptic membrane is known to increase nonquantum ACh release [10], and HR of the EP membrane obtained under those conditions become amenable to statistical analysis. Accordingly all experiments were carried out after preliminary inhibition of acetylcholinesterase and of Na,K-ATPase [9].

The experiments showed that HR in fibers of the intact muscle under these conditions was 0.9 ± 0.4 mV ($n = 11$, where n is the number of fibers studied) relative to the initial MP of -104.6 ± 3.2 mV ($n = 11$). HR of the EP membrane of the frog muscle fibers recorded in these experiments were close to values obtained previously under similar experimental conditions [10].

Measurement of MP of the muscle fibers in the synaptic and extrasynaptic zones showed that MP of EP were a little lower than MP of the electrogenic membrane of the muscle fibers (Table 1). However, the difference was not significant. Addition of D-TB to the solution increased MP of EP significantly compared with MP of the extrasynaptic zone of the muscle fibers. MP of the electrogenic membrane was unchanged in response to the presence of D-TB in the solution (Table 1). D-TB thus changes MP only of EP of the muscle fiber and has no effect on the transmembrane potential difference of the electrogenic membrane. The input resistance of the EP membrane (450 ± 38 kΩ; $n = 6$) was the same as the input resistance of the electrogenic membrane (420 ± 20 kΩ; $n = 6$) of the same muscle fibers. Consequently, the action of D-TB on the postsynaptic membrane was not determined by the particular features of its electrical properties.

It can be tentatively suggested that ACh flows constantly from nerve endings and depolarizes the EP membrane [6, 10]. D-TB blocks ACh receptors of the postsynaptic membrane and thereby abolishes the depolarizing action of ACh, and this is expressed as hyperpolarization of the EP membrane. Consequently, the intensity of nonquantum ACh release from the nerve endings can be judged indirectly from the difference arising between MP of the postsynaptic membrane and of the electrogenic membrane in response to D-TB.

The value of HR was less during continuous recording of MP of the muscle fibers than during discrete measurements of MP in the synaptic and extrasynaptic zones. This difference was perhaps due to the fact that in the case of continuous recording of MP it is difficult to determine the true beginning of membrane hyperpolarization in the presence of D-TB because of the slow membrane depolarization taking place under these experimental conditions.

On the 12th-13th day after application of Co to the motor nerve the value of HR (continuous recording) was 0.7 ± 0.4 mV ($n = 9$), from an initial MP of -97.0 ± 2.7 mV ($n = 9$), which does not differ statistically significantly from the control values. During discrete recording of MP in the synaptic and extrasynaptic zones of the muscle fibers MP of EP and MP of the electrogenic membrane did not differ significantly after treatment of the muscle (Table 1). D-TB increased to MP of EP significantly (relative to the extrasynaptic membrane). The difference between values of MP of the synaptic and extrasynaptic membrane of the muscle fibers did not differ from that obtained on intact muscles. This suggests that blockade of AT by Co does not affect quantum ACh release from motor endings. Meanwhile MP of the muscle fibers was significantly lower ($P < 0.001$) after application of Co to the motor nerve than MP of intact fibers (Table 1). The zone of sensitivity to ACh, namely $1500 \pm 100\,\mu$ ($n = 6$), of the muscle fibers under these conditions also was significantly greater than in the control: $550 \pm 50\,\mu$ ($n = 11$). Values of maximal sensitivity to ACh were unchanged under these circumstances: 270 ± 75 mV/nC ($n = 6$) in the experiment and 234 ± 65 mV/nC ($n = 11$) in the control, so that this could not have affected the degree of hyperpolarization of the membrane in response to D-TB.

TABLE 1. Effect of Armin and D-TB on MP of Frog Sartorius Muscle Fibers in Control and on 12th-13th Day after Application of Co to Motor Nerve (M± m)

Experimental conditions	MP_s, mV	MP_{es}, mV	$MP_s - MP_{es}$, mV
Control:			
armin	−107,3±0,9 (24)	−109,7±0,8 (24)	+2,4±1,4
D-TB	−115,5±1,2* (21)	−109,1±1,4 (21)	−6,4±2,0
Co:			
armin	−97,2±0,9 (40)	−99,8±1,0 (40)	+2,6±0,5
D-TB	−107,2±1,1* (47)	−102,8±1,2 (47)	−4,4±0,5

Legend. Number of fibers tested shown in parentheses. MP_s) MP in synaptic zone, MP_{es}) MP in extrasynaptic zone. *$P < 0.05$.

Disturbance of AT in the motor nerve by Co reduces MP and causes extrasynaptic sensitivity to ACh, just like denervation of the muscle [2, 8]. Quantum ACh secretion is not disturbed under these circumstances, as was shown previously [1]. Nonquantum ACh release likewise is not affected under these conditions.

Consequently, there is reason to suppose that the appearance of denervation-like changes in the frog muscle fiber membrane after application of Co to their motor nerve is due to a deficiency of substances carried to the muscle by the axoplasmic flow, and is not connected with any disturbance of ACh secretion in either quantum or nonquantum form.

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