# EFFECT OF EXOGENOUS ACETYLCHOLINE ON IONIC CURRENTS OF THE FROG AND MOUSE MOTOR NERVE ENDING

**A. L. Zefirov, D. M. Shakir'yanova, I. A. Khalilov, and E. E. Nikol'skii**

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Besides its own mediator function acetylcholine (ACh) has a modulating action on neuromuscular transmission [7]. It has also been shown that ACh and its mimetics cause inhibition of induced mediator release from motor nerve endings [4]. It is considered that the modulating action of ACh is connected with its effect on the actual process of mediator release from motor nerve endings. At the same time it can be postulated that the presynaptic effects of ACh may be mediated by its effect on ionic currents of the nerve ending, changes in which may lead to modification of the shape of the presynaptic action potential (AP), and may thus act on mediator release from nerve endings. In the investigation described below the action of exogenous ACh on ionic currents of nerve endings was investigated on frog and mouse synapses.

## EXPERIMENTAL METHOD

The first series of experiments was undertaken on nerve-muscle preparations of the cutaneothoracic muscle of frogs (*Rana ridibunda*). The preparation was superfused continuously with Ringer's solution of the following composition (in mmoles/liter): NaCl – 118.0; KCl – 2.5; $CaCl_2$ – 0.45; $MgCl_2$ – 2.0; $NaHCO_3$ – 2.4, pH 7.4, at a temperature of 20°C; the rate of perfusion was 5.0 ml/sec. The second series of experiments was undertaken on nerve-muscle preparations of the mouse hemidiaphragm. For superfusion in this case Krebs' solution was used: NaCl – 154.0; KCl – 5.0; $NaH_2PO_4$ – 1.0; $MgCl_2$ – 6.0; $CaCl_2$ – 0.4; $NaHCO_3$ – 1.8 pH 7.2, at 24°C. Evoked electrical responses of the nerve ending and end-plate currents (EPC) were recorded extracellularly by means of microelectrodes filled with 2 M NaCl, with a resistance of 2-5 MΩ. The microelectrode was applied to different parts of the nerve ending under visual control (×400) [1]. The area of the terminal in warm-blooded animals was identified from the shape of the recorded responses [5]. These responses were amplified and averaged by means of an automated system based on a microcomputer (20-30 realizations, signal measurement interval 20 μsec). The quantum composition of EPC was calculated by the equation $m = \log N/N_1$, where N is the number of stimulations (120 pulses), and $N_1$ the number of stimulations not inducing release. The numerical results were subjected to statistical analysis by Student's t test.

## EXPERIMENTAL RESULTS

**Frog Nerve-Muscle Preparation.** A three-phase response of the nerve ending, recorded in the proximal parts of the frog nerve terminal, consisted of a first and third positive phase and a second, high-amplitude negative phase (sodium current). Addition of ACh to the superfusion solution in concentrations of 0.1-0.6 mmole/liter led to a con-

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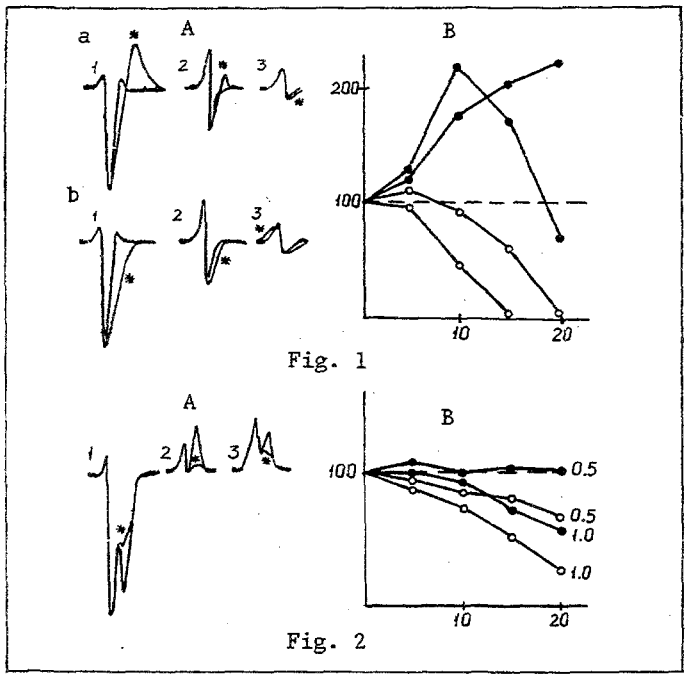


Fig. 1




Fig. 2

Fig. 1. Effect of exogenous ACh on ionic currents of nerve ending and mediator secretion in frogs. A) Ionic currents under normal conditions and after action of ACh for 20 min (asterisk) in a concentration of 0.5 mmole/liter (a) and 1 mmole/liter (b) in proximal (1), central (2), and distal (3) parts of terminal; B) dynamics of changes in phase 3 of response of nerve ending in proximal part (filled circles) and quantum composition of EPC (empty circles) during action of ACh (concentrations in mmoles/liter indicated alongside graphs). Abscissa, duration of action of ACh (in min); ordinate, changes in response (in % of control).

Fig. 2. Effect of exogenous ACh on ionic currents of nerve ending and mediator secretion in mice. A) Ionic currents under normal conditions and after action of ACh for 20 min (asterisk) in concentrations of 1.0-2.0 mmoles/liter in preterminal (1), central (2), and distal (3) parts of terminal; B) dynamics of change in amplitude of potassium currents of nerve ending (filled circles) and quantum composition of EPC (empty circles) under influence of ACh. Remainder of legend as to Fig. 1.

siderable increase in amplitude of phase 3 of the recorded response (Fig. 1A, a, 1). In higher concentrations (0.7-2.0 mmoles/liter) ACh had a biphasic action with time on the magnitude of phase 3 of the response of the nerve ending. The rapid initial increase in phase 3 of the presynaptic response was followed by its reduction and disappearance (Fig. 1A, b, 1). In all concentrations used (0.1-2.0 mmoles/liter) ACh caused widening of the response of the nerve ending. The time course of the change in amplitude of phase 3 of the recorded signal with two different ACh concentrations is illustrated in Fig. 1B.

In central parts of the terminal the evoked response of the nerve ending consisted of positive and negative phases. The effect of low ACh concentrations (0.1-0.6 mmole/liter) in this case was expressed as the appearance of a third phase in a biphasic signal (Fig. 1A, a, 2), whereas that of higher concentrations was to prolong the negative phase of the response of the nerve ending (Fig. 1A, b, 2). ACh had no appreciable action on monophasic responses recorded in distal regions of the terminal branch (Fig. 1A, 3). In all parts of the synapse, inhibition of mediator secretion was observed under the influence of ACh (Fig. 1B).

Phase 3 of the response of a nerve ending in the proximal part of the nerve terminal is known to reflect outward currents through voltage-dependent and calcium-activated potassium channels in the membrane of the nerve ending [3]. It has also been shown that the amplitude of the potassium currents falls gradually from proximal to dis-

tal parts [2]. Consequently, the effect of ACh described above was due in our experiments to its effect on potassium currents of the frog nerve ending. The absence of changes in the distal parts of the nerve terminal under the influence of ACh can evidently be explained by the low density of the potassium channels in these parts of the terminal.

**Mouse Nerve-Muscle Preparation.** In warm-blooded animals, with compact and short nerve endings, the sodium channels are concentrated in the preterminal region, whereas potassium channels are distributed over the whole extent of the terminal. Under these circumstances local currents from the last Ranvier node and preterminal can induce complete depolarization of the whole terminal, whereas excitation, by contrast with amphibians, spreads passively. Thanks to the creation of powerful local currents in motor nerve endings of mice, potassium currents can be recorded along the whole length of the terminal [6].

The response of a nerve ending recorded in the preterminal region consisted of one positive and two negative peaks, created by the flow of a passive depolarizing current, a sodium current, and potassium currents arising from more distal parts of the nerve terminal. In the central and distal parts of the terminal branch presynaptic responses consisting of two positive peaks created by passive depolarizing and potassium currents respectively were recorded [5] (Fig. 2A).

In the preterminal regions exogenous ACh in concentrations of 1.0-2.0 mmoles/liter caused inhibition of the second negative peak of the response of the nerve ending (Fig. 2A, 1). The action of the same concentrations in the central and distal parts of the terminal led to suppression of the second positive peak of the recorded signal (Fig. 2A, 2). Lower concentrations of ACh caused no significant changes in the magnitude of the ionic currents of the nerve ending. The effect of ACh on the shape of the presynaptic response, which was investigated, was accompanied by inhibition of mediator secretion from nerve terminals (Fig. 2B).

Our results thus indicate that exogenous ACh affects potassium currents of nerve endings in both frogs and mice. Particular features of the effects of ACh in cold-blooded and warm-blooded animals may be due to functional differences between the nerve endings of these animals. The possibility likewise cannot be ruled out that the absence of an increase in amplitude of the potassium currents under the influence of ACh may be the result of the lower than normal temperatures at which the experiments were conducted.

Voltage-dependent and calcium-activated potassium currents are known to play an important role in the mechanism of mediator secretion from motor nerve endings, by regulating the duration of AP and the rate of development of the repolarization phase of AP, and also the intensity and duration of the calcium inflow into the nerve terminal. All these changes may make a contribution to the mechanism of the modulating action of ACh on induced mediator secretion from vertebrate motor nerve endings.

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