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PHYSIOLOGY

Presynaptic Nicotinic Cholinoreceptors Modulate Velocity of the Action Potential Propagation along the Motor Nerve Endings at a High-Frequency Synaptic Activity

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Abstract—Experiments on frog neuromuscular junctions have demonstrated that asynchrony of the acetylcholine quantal release forming the multi-quantal evoked response at high-frequency synaptic activity is caused, in particular, by a decrease in velocity of the action potential propagation along the non-myelinated nerve endings, which is mediated by activation of the α7 and α4β4 nicotinic cholinoreceptors.

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In the neuromuscular synapses of vertebrates, high-frequency rhythmic stimulation (HFRS) of the motor nerve is known to cause quantitative changes in acetylcholine (ACh) quantal release, which are accompanied by an increase in asynchrony of individual quantal secretion [1–4]. Individual acetylcholine quanta form the multi-quantal endplate potentials, and their secretion asynchrony reduces the amplitude of the consecutive postsynaptic responses. Clarifying the mechanisms of changes in the time course of mediator secretion is obviously important for understanding the nature of synaptic plasticity and reliable intercellular signaling. In the neuromuscular junctions of amphibians, with their long motor nerve endings (hundreds of microns) and a low velocity of the action potential propagation, asynchrony of ACh quantal release is caused not only by stochastic operation of ACh active zone, but also by the fact that secretion process starts non-simultaneously in the proximal and distal areas of a nerve ending [4–6]. It is known that, when operating in the high-frequency mode, the myoneural junction is under the conditions when ACh is accumulated in perisynaptic space [7], and there are reasons to believe that the delayed kinetics of quantal secretion is a result of presynaptic cholinoreceptor activation by an endogenous mediator. Indeed, we have demonstrated that inactivation of M1-cholinoreceptors prevents asynchrony of the individual quanta secretion under HFRS of the motor nerve [8]. ACh and exogenous analogues are known to modulate the shape of action potentials and the velocity of action potential propagation along the nerve fibers [9].

Here, we have studied whether the velocity of action potential propagation along the frog motor nerve endings is changed in response to activation of the nicotinic and muscarinic receptors upon rhythmic activation of synapses.

Experiments were conducted on isolated neuromuscular preparations of m. cutaneous pectoris from a frog (*Rana ridibunda*). An isolated muscle with a nerve fragment was placed into a 3.0-mL chamber and superfused with Ringer solution of the following composition (mmol/L): NaCl, 113.0; KCl, 2.5; $NaHCO_3$, 3.0; $CaCl_2$, 1.8; pH 7.3–7.4. The experiments were performed at 20.0 ± 0.3°C. The recording complex included a high-speed analog-to-digital converter and a computer station to ensure high-precision measurements of synaptic signals and analysis of their amplitude and time parameters. Rectangular supramaximal stimuli of 0.1-ms duration were applied to the motor nerve at a frequency of 0.5 and 100 pulses/s. The electrophysiological experiments were conducted at the "physiological" level of calcium ions; therefore, the muscle contractions arising in response to nerve stimulation were blocked by the lateral dissection of muscle fibers [10, 11]. Using two extracellular electrodes, the action currents were simultaneously recorded in the proximal and distal parts of the nerve ending. Time intervals between the sodium component peaks of the proximal and distal spikes were measured (Fig. 1). The velocity of the action potential propagation was determined as the ratio of the distance between microelectrode tips to a specified time interval.

The velocity of the action potential propagation during HFRS of the motor nerve was estimated individually for each signal pair (from the proximal and distal parts) in a pulse burst. The time course of prop-

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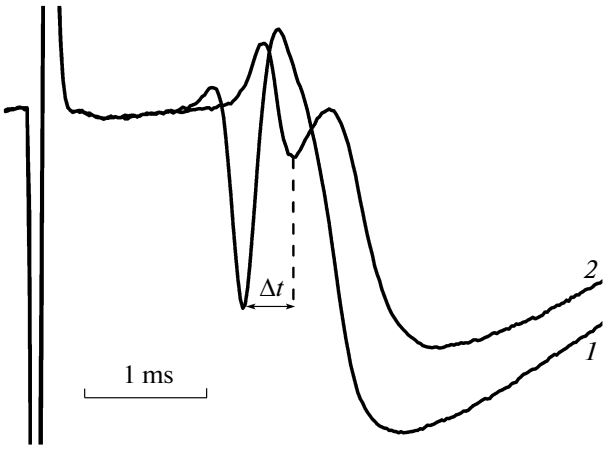

Fig. 1. An example of simultaneous recording of action potentials on a single nerve terminal with two microelectrodes (*1* and *2*) situated at a distance of 210 μm from one another. Δ*t*, time of the action potential propagation along the nerve ending between the microelectrodes *1* and *2*.

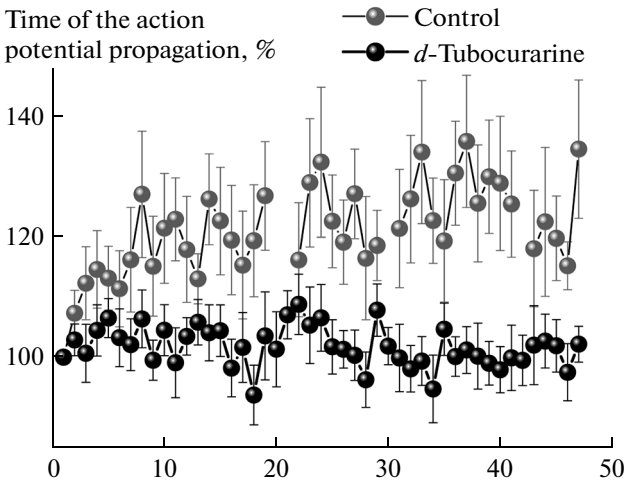

Fig. 2. Relative changes in the time (Δ*t*) of the action potential propagation along the nerve ending during the high-frequency rhythmic synaptic activity (100 pulses per second) in control and in the presence of nicotinic cholinoreceptors antagonist d-tubocurarine (1 μmol/L). X axis, the pulse serial number. $M \pm m$, $n = 35$.

agation was expressed as percentage of time interval for the first signal pair within a pulse burst.

The following drugs were used to block nicotinic cholinoreceptors: the nonselective nicotinic cholinoreceptors antagonist d-tubocurarine (1 μmol/L) and the selective blockers of α4β4, α4β2, and α7 subtypes mecamylamine (10 μmol/L), DHβE (1 μmol/L), and methyllicaconitine (MLA, 0.01 μmol/L). Muscarinic receptors were inactivated with the nonselective blocker atropine (1 μmol/L). All reagents were from Sigma (United States).

In control experiments with low-frequency nerve stimulation (0.5 pulses per second), the velocity of the action potential propagation between the proximal and distal parts of a nerve ending averaged 0.42 ± 0.04 m/s. At the stimulation frequency of 100 pulses per second, the time of action potential propagation increased significantly (by 24.6 ± 3.6%) by the 30th signal of the pulse burst as compared to the value for the first signal pair (Fig. 2).

In the case of low-frequency stimulation, the nonselective cholinergic antagonists (atropine and d-tubocurarine) had no effect on the nerve-ending action current or the time of action potential propagation.

During HFRS in the presence of the nonselective muscarinic antagonist atropine, the velocity of the action potential propagation along the nerve endings decreased to the same extent as in intact preparations. Since we have earlier demonstrated that secretion desynchronization during HFRS is partly prevented by blocking of the M1-subtype muscarinic cholinoreceptors [8], we believe that this effect is not related to delayed involvement of various pulses of the nerve ending into secretion process, but rather with changes in the kinetics of ACh release in individual active zones.

In contrast, application of the nonselective nicotinic cholinoreceptors antagonist d-tubocurarine prevented an increase in the time of the action potential propagation along the nerve ending during HFRS (Fig. 2), which testifies to the fact that retardation of the active potential propagation along the nerve ending during rhythmic activation of synapses is a result of the nicotinic cholinoreceptor activation by endogenous ACh. To identify the subtypes of nicotinic receptors that mediate changes in the velocity of propagation in response to HFRS, we used selective agents blocking different subtypes of the neuronal nicotinic receptors: mecamylamine (α4β4), DHβE (α4β2), and MLA (α7).

The experiments showed that, after preliminary blocking of α7 and α4β4 cholinoreceptors, there were no changes in the velocity of action potential conduction in response to HFRS. At the same time, blocking of α4β2 receptors led to a decrease in the velocity of action potential propagation as in intact preparations.

We have earlier showed [4, 8] that HFRS of the nerve leads to asynchrony of ACh quantal release, which is caused by at least two factors: a decrease in the velocity of action potential propagation along the nerve ending and changes in the kinetics of quantal secretion in individual active zones. The results of our study suggest that a decrease in the velocity of action potential propagation along the non-myelinated nerve ending is mediated by activation of the nicotinic cholinoreceptors of α7 and α4β4 subtypes, while the mus-

carinic receptors of the M1 subtype modulate exocytose directly in the active zones.

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