Effect of Oxotremorine on Resting Membrane Potential and Cell Volume in Skeletal Muscle Fibers in Rats after *in Vivo* Blockade of NO-Synthase

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Denervation of rat phrenic muscle or block of NO-synthase *in vivo* increased the cross-section area of muscle fibers and decreased membrane resting potential. Oxotremorine prevented the development of denervation-induced or denervation-like (*i.e.* induced by NO-synthase blockade) membrane depolarization and increase of the cross-sectional area of muscle fibers. Pirenzepine abolished the effects of oxotremorine. It was concluded that non-quantal acetylcholine can be involved in the regulation of skeletal muscle fiber volume via activation of M_1 muscarinic receptors followed by NO synthesis.

Key Words: *skeletal muscle; neuromuscular synapse; cell volume; M_1 muscarinic receptors; NO-synthase*

We previously showed that non-quantal acetylcholine (Ach) affects M_1 muscarinic receptors in skeletal muscle fibers and triggers Ca^{2+}-dependent synthesis of NO in the sarcoplasm [6,7]. NO molecules can produce a retrograde effect on nerve terminal via modulation of guanylate cyclase producing cAMP. Disturbances in anterograde (non-quantal Ach) and retrograde (NO) intercellular signaling after denervation induce some changes in skeletal muscle fibers [1,4,8].

It is known that resting membrane potential (RMP) decreases during the very first hours after denervation [4], which is related to activation of Na^+,K^+,Cl^--cotransport in muscle fibers [2,5,8]. In intact fibers this transport system is inactive due to inhibitory influences from the motoneuron [1,7,8]. This effect of motor innervation is partially mediated by non-quantal synaptic Ach [7]. It is also important that apart from participation in generation of membrane potential, Na^+,K^+,Cl^--cotransport plays a role in regulatory restoration of cell volume in skeletal muscle fibers in a high-osmolarity medium [1,2].

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Our aim was to study the role of non-quantal Ach in neural regulation of fiber volume of skeletal muscles.

MATERIALS AND METHODS

Experiments were carried out on male random-bred albino rats weighing 150-200 g. Phrenic muscle was isolated and cut into fragments. The fragments were placed in Petri dishes containing 12 ml medium 199; pH was adjusted (7.2-7.4 sec) with HEPES (Sigma). The dishes were kept for 3 h in a thermostat at 37°C in a humid atmosphere (95% O_2 and 5% CO_2). RMP was recorded with routine microelectrode technique immediately after muscle isolation (innervated muscles) or after 3-h incubation (denervated muscles).

Immediately after isolation or incubation, the muscle fragments were processed by a routine histological technique. Cross sections were photographed with a Leica DMLS/MPS-32 microscope. The cross-section area S_{sec} was measured using original software and used for evaluation of changes in fiber volume [9].

The experimental rats were subcutaneously injected with L-NAME, an NO-synthase blocker (50 mg/kg). The injections were made every 12 h for 4 days. The specificity of L-NAME effect was evalua-

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ted using a dextrorotatory isomer D-NAME, which is ineffective towards NO-synthase.

The effect of non-quantal Ach was simulated with muscarinic agonist oxotremorine (5×10^{-8} M). We previously showed that the effect of non-quantal Ach on RMP is mediated via M_1 receptors on muscle fibers [7]. Probably, the blockade of these receptors should impede the effect of muscarinic receptor agonist not only on RMP, but also on the cell volume of incubated muscles. For verification of this hypothesis, pirenzepine (5×10^{-6} M), a specific blocker of M_1 receptors, was added to the culture medium.

The results were analyzed statistically using Student's t test at $p<0.05$.

RESULTS

After 3-hour incubation, RMP of muscle fragments isolated from intact rats significantly decreased ($p<0.001$, Fig. 1, *a*). This denervation-induced drop of RMP is caused by activation of Na^+,K^+,Cl^--cotransport in the muscle membrane [7,8] and water entry into the cells [1,2]. In addition, muscle denervation increased cell volume ($p<0.001$). Oxotremorine decelerated the drop of RMP and decreased S_{sec}.

Pirenzepine markedly weakened the effect of cholinomimetic oxotremorine: it decreased RMP and increased S_{sec} ($p<0.001$, Fig. 1, *b*). Therefore, in intact rats RMP returned to the control value, while S_{sec} markedly increased (by 22.7 arb. units), but did not returned to the control level. It should be concluded that oxotremorine acts via M_1 receptors of muscle fibers thereby preventing the development of denervation-induced depolarization [7] and the increase in muscle fiber volume.

Similar to intact rats, 3-hour incubation of muscle fibers of control rats produced denervation-induced drop of RMP ($p<0.001$, Fig. 1, *a*) and an increase in S_{sec} (Fig. 1, *b*). Addition of oxotremorine into culture medium hyperpolarized muscle fibers ($p<0.001$) and decreased S_{sec} ($p<0.001$). In these experiments, S_{sec} did not differ from the corresponding values in intact rats, while RMP was slightly lower than in intact rats. Pirenzepine prevented the effects of muscarinic receptor agonist on RMP and S_{sec} ($p<0.001$). It should be concluded that activation of M1 receptors with oxotremorine after treatment with D-NAME prevented the drop of RMP and increase in cell volume. These effects of muscarinic receptor agonist were similar to those observed on intact muscles.

In experimental rats, RMP of freshly isolated muscle fibers was significantly smaller than RMP in intact rats ($p<0.001$, Fig. 1, *a*). Incubation of these muscles for 3 h produced no changes in RMP. In experimental rats with blocked NO-synthase, S_{sec} of freshly isolated fibers was larger than that of intact rats ($p<0.001$). Similar to intact rats, S_{sec} in experimental rats increased by 25 arb. units ($p<0.001$) after a 3-h denervation. Probably, the fall in RMP and the increase in cell volume of freshly isolated muscle fibers are caused by initial activation of Cl^- transport (similar to that produced by denervation) resulting from disturbance of NO synthesis *in vivo*. The presence of oxotremorine in the incubation medium hyperpolarized the incubated muscle fibers with inactive NO-synthase ($p<0.001$) and decreased S_{sec} to 35.2 arb. units ($p<0.001$, Fig. 1, *b*). Pirenzepine prevented these effects of oxotremorine and normalized RMP and S_{sec}. Therefore, activation of M_1 receptors with oxotremorine against the background of *in vivo* NO-synthase blockade hyperpolarizes the membrane and prevents denervation-induced increase in cell volume. This effect was less pronounced compared to the some effect on intact muscles, but significantly differed from the

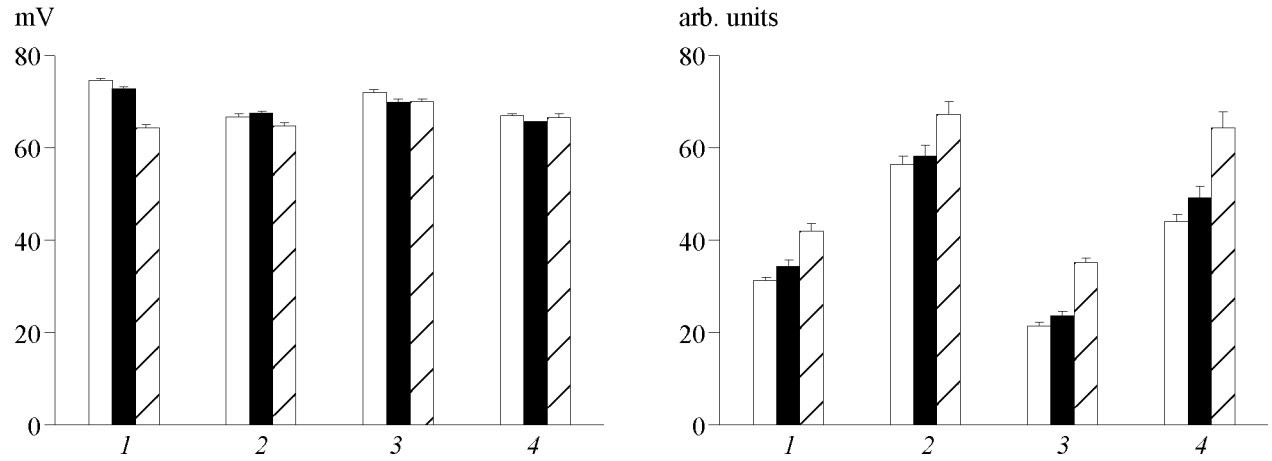

Fig. 1. Membrane resting potential (*a*) and transversal cross-section area (*b*) of innervated muscle fibers (*1*) and muscle fibers incubated for 3 h in 199 medium (*2*) or in the medium with oxotremorine (*3*) and pirenzepine (*4*). The light, solid, and hatched bars mark the intact, control, and experimental rats.

analogous effect in rats treated with L-NAME, which attests to specific modulation of NO-synthase with L-NAME.

Our findings suggest that oxotremorine affects RMP and the volume of muscle fibers via M_1 receptors, when it is used in concentrations mimicking the action of non-quantal Ach. Probably, non-quantal Ach participates in the regulation of morphofunctional properties of muscles via modulation of M_1 receptors of muscle fibers. Activation of these receptors stimulates Ca^{2+}-dependent NO synthesis, which indirectly inhibits activity of Na^+,K^+,Cl^--cotransport in muscle fibers. Disturbances of intercellular signaling caused by denervation or blockade of NO-synthase *in vivo* increase transport of Cl^-, which results in a drop of RMP and increase in cell volume.

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