EXPERIMENTAL BIOLOGY

Effects of Cholinergic Receptor Agonists and Antagonists on Miniature Stimulatory Postsynaptic Ionic Currents in Somatic Muscle Cells of *Lumbricus Terrestris*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 3, pp. 342-345, March, 2005
Original article submitted March 29, 2004

Miniature stimulating postsynaptic currents of *Lumbricus terrestris* somatic muscle cells were recorded. Atropine, d-tubocurarin, α-bungarotoxin, carbacholine, and proserin did not modify the amplitude and temporal parameters of miniature stimulatory postsynaptic currents, while carbacholine and nicotine depolarized the muscle membrane. Presumably, *Lumbricus terrestris* muscle cells contain acetylcholine-sensitive channel-receptor complexes not belonging to classical nicotinic or muscarinic acetylcholine receptors.

Key Words: *acetylcholine receptor; cholinolytics; cholinomimetics; muscle cells; Lumbricus terrestris*

Stimulation impulses from motor nerves to somatic muscles in *Lumbricus terrestris* are transmitted via the cholinergic mechanisms [6]. On the other hand, the data on the effects of AC receptor blockers on stimulation transmission from motor nerves to somatic muscles of annelids are contradictory [6]. We showed that wide-spectrum nicotinic and muscarinic AC receptor antagonists do not abolish membrane depolarization in *Lumbricus terrestris* muscle cell, developing under the effect of exogenous acetylcholine (AC) and carbacholine [5]. Analysis of the effects of nicotinic and muscarinic AC receptor antagonists on the postsynaptic ionic currents will help to understand the nature of postsynaptic chemosensitive ionic channels of *Lumbricus terrestris* somatic muscle cells; this analysis became the purpose of our study.

MATERIALS AND METHODS

Experiments were carried out on surface muscle cells of longitudinal bundles of the inner side of *Lumbricus terrestris* musculo-cutaneous sac. Fresh preparations of longitudinally dissected fragments (10-15 segments long) free from celomic organs were placed into a cuvette for electrophysiological studies with modified Drewes—Pax solution [4,7] of the following composition (mmol/liter): 163 Na^+; 4 K^+; 6 Ca^{2+}; 93 Cl^-; 43 SO_4^{2-}; 2 $Tris^+$; 167 sucrose; osmolarity 478 mosmol/liter, ionic strength 229 mmol/liter, pH 7.2-7.4 at room temperature.

The muscle cell membrane miniature stimulatory postsynaptic currents (MSPSC) were measured by glass microelectrodes filled with 0.5 mol/liter NaCl with 1 mΩ tip resistance. MSPSC were measured before and 10-15 min after addition of drugs into solution. The amplitude and time characteristics of MSPSC were analyzed using a PC in the real time mode. At least 100 discrete signals were processed for estimation of the mean amplitude (nA), time of increment (msec), and decrease (τ, msec) of MSPSC for a muscle cell in each experiment.

The following drugs were used: carbacholine (5×10^{-6} mol/liter; Sigma), d-tubocurarine (1×10^{-4} mol/liter; Serva), α-bungarotoxin (1×10^{-5} mol/liter;

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Sigma), atropine (1×10^{-5} mol/liter; Serva), and proserin (1×10^{-4} mol/liter; Russia).

RESULTS

Acetylcholine and its analog carbacholine in a dose-dependent manner depolarized *Lumbricus terrestris* musculocutaneous sac somatic muscle cell membrane before the development of muscle contraction [5]. On the other hand, d-tubocurarin, atropine, and benzohexonium did not abolish depolarization of muscle membrane induced by AC receptor blocker [5]. Serotonin, glycine, glutamate, ATP, and muscarine did not modify muscle cell membrane potential at rest (MPR), while epinephrine, norepinephrine, and GABA hyperpolarized muscle membrane [2,3]. Our data indicate that apart from AC and carbacholine, only nicotine causes a reduction of MPR [3]. α-Bungarotoxin, a highly specific marker of nicotinic receptors, also binds to the postsynaptic membrane of annelid somatic muscle cells [12]. Hence, presumably, *Lumbricus terrestris* musculocutaneous sac muscle cells contain AC receptors of the nicotine-like type. We showed that α-bungarotoxin, d-tubocurarin, or atropine alone did not modify the MSPSC amplitude (Table 1, Fig. 1).

Addition of d-tubocurarin or α-bungarotoxin into solution did not change the duration of MSPSC increase and decrease τ (Table 2). Atropine also did not change the time characteristics of miniature currents (Table 2). According to our data, classical nicotinic and muscarinic AC receptor blockers cannot modify MSPSC amplitude or the time characteristics of postsynaptic currents recorded in the somatic muscle cells of *Lumbricus terrestris* musculocutaneous sac. The annelid muscle tissue contains acetylcholinesterase (ACE) [6,10]. ACE inhibition by ezerine prolonged the contraction of *Lumbricus terrestris* muscle, caused by motor nerve stimulation [6]. In our experiments the duration of MSPSC increase and τ decrease did not change in the presence of proserin (Table 2), while ACE inactivation in vertebrate skeletal muscles involved the ascending and descending phases of the terminal plate currents [9]. In motor neuromuscular synapses of vertebrates desensitization of the terminal plate receptors by exogenous AC receptor agonist shortens τ of synaptic current decrease [7,8]. Addition of carbacholine into the solution washing *Lumbricus terrestris* muscle in a concentration causing membrane depolarization [5] did not change the time of MSPSC increase and decrease τ (Table 2). The absence of the effect can be due to a greater variety of the means of temporary characteristics of the postsynaptic currents' (Table 2). The latter circumstance is explained by the heterogeneity of synaptic signals by the MSPSC decrease τ: presumably, by a different quantitative ratio of the so-called "rapid" and "slow" currents in the total sample [1]. In order to minimize this characteristic, the MSPSC were recorded in one muscle cell before and after drug treatment.

Hence, the use of nicotinic and muscarinic AC receptor agonists and methods modulating the kinetics of postsynaptic currents in the vertebrate cholinergic neuromuscular synapses failed to modify the amplitude and temporal characteristics of MSPSC of *Lumbricus terrestris* somatic muscle cells under conditions of summary analysis of all recorded signals. Since nicotinic and muscarinic AC receptor agonists and ganglionic blockers do not abolish membrane depolarization developing under the effect of AC or carbacholine, we can hypothesize that postsynaptic membrane of muscle cells of *Lumbricus terrestris* musculocutaneous sac contains chemosensitive ionic

TABLE 1. Effects of α-Bungarotoxin, d-Tubocurarin, and Atropine on MSPSC Amplitude in Muscle Cells of *Lumbricus terrestris* Musculocutaneous Sac Muscle Cells (*M*±*m*)

Agent	Control, mV	Experiment, mV
α-Bungarotoxin	0.08±0.01 (4)	0.08±0.01 (4)
d-Tubocurarin	0.16±0.05 (6)	0.15±0.03 (6)
Atropine	0.25±0.06 (5)	0.19±0.03 (5)

Note. Here and in Table 2: the number of examined muscles is shown in parentheses. Control: before drug addition; experiment: 10 min after drug addition into solution.

TABLE 2. Effects of d-Tubocurarin, α-Bungarotoxin, Atropine, Proserin, and Carbacholine on the Duration of MSPSC Increase and τ of the Decrease in *Lumbricus Terrestris* Musculocutaneous Sac Muscle Cells (*M*±*m*)

Drug	Control, msec		Experiment, msec	
	front-line	decrease τ	front-line	decrease τ
d-Tubocurarin	0.40±0.06 (5)	2.0±0.6 (5)	0.60±0.09 (5)	2.5±0.3 (5)
α-Bungarotoxin	0.90±0.05 (4)	6.4±2.5 (4)	1.00±0.06 (4)	7.9±3.6 (4)
Atropine	0.80±0.07 (6)	6.6±1.6 (6)	0.90±0.08 (6)	6.2±0.6 (6)
Proserin	1.00±0.31 (7)	10.0±3.4 (7)	1.20±0.25 (7)	16.7±3.1 (7)
Carbacholine	0.80±0.06 (5)	5.4±0.5 (5)	0.80±0.14 (5)	6.7±0.6 (5)

channels stimulated by AC and nicotine, which cannot be referred to any of the known pharmacological types of the vertebrate muscle AC receptors and peripheral neurons. *Lumbricus terrestris* muscle cells with their polyaxonal multiterminal innervation [6] can contain, along with AC receptors, receptors for one more stimulatory mediator. However this last hypothesis, though cannot be completely ruled out, should be considered as hardly possible, as it is not supported experimentally [3,6].

The study was supported by the Russian Foundation for Basic Research (grants No. 03-04-48303, No. 03-04-06511), and President of the Russian Federation (grant No. NSh-1063.2003.4).

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