EXPERIMENTAL BIOLOGY

Effects of Some Transmitters on Resting Membrane Potential of Somatic Cell in *Lumbricus terrestris* Muscle Wall

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Serotonin, glutamate, glycine, ATP, and muscarine had no effect on resting membrane potential of muscle cell in earthworm *Lumbricus terrestris*. Nicotine depolarizes and GABA hyperpolarizes the muscle membrane. Removal of K+, Cl- and addition of ouabaine and strychnine to the solution abolished the effect of GABA. The authors conclude that the *Lumbricus terrestris* myocyte membrane contains nicotine receptors and GABAergic receptors sensitive to strychnine. Stimulation of these receptors activates sarcolemmal ionic pumps and causes membrane hyperpolarization.

Key Words: *transmitters; potential at rest; muscle cells; Lumbricus terrestris*

Cholinomimetics depolarize the somatic muscle cell membrane of the *Lumbricus terrestris* musculocutaneous sac similarly as in vertebrate skeletal muscles [3,4]. This fact is an evidence of cholinergic transmission of excitation in the annelid neuromuscular system [4]. It is known that *Lumbricus terrestris* are characterized by polyneuronal (mediated by different transmitters) multiterminal innervation of somatic muscles [4], that is, the *Lumbricus terrestris* muscle cells are sensitive not only to acetylcholine (AC), but also to other agents acting as transmitters. However, the available data are scanty and extremely contradictory. We studied the pharmacological type of the muscle membrane AC receptors and the effects of some transmitters on resting membrane potential (RMP) in the *Lumbricus terrestris* muscle cells.

MATERIALS AND METHODS

Experiments were carried out on the *Lumbricus terrestris* surface muscle cells of longitudinal bundles of the inner side of the musculocutaneous sac. Fresh preparations of longitudinally dissected fragments of the musculocutaneous sac (10-15 segments) free from celomic organs were placed in cuvettes for electrophysiological studies into modified Drewes—Pax solution [6] of the following ionic composition: 163.0 mmol/liter Na^+, 4.0 mmol/liter K^+, 6.0 mmol/liter Ca^{2+}, 93.0 mmol/liter Cl^-, 43.0 mmol/liter SO_4^{2-}, 2.0 mmol/liter $Tris^+$, 167 mmol/liter sucrose; osmolarity 478.0 mosmol/liter, ionic strength 229.0 mmol/liter, pH 7.2-7.4, at 20°C. In potassium-free solution K^+ was replaced by an equimolar amount of Na^+, and in chlorine-free solution Cl^- was replaced by equimolar amount of NO_3^- [2].

Muscle cell RMP was measured using glass microelectrodes filled with 2.5 mol/liter KCl with the tip resistance of 10-15 mΩ. RMP was measured before and 5-10 min after solution replacement or addition of drugs. The following drugs were used: nicotine (1×10^{-5} mol/liter, Serva), muscarine (1×10^{-5} mol/liter, Reanal), serotonin (1×10^{-4} mol/liter, Sigma), glutamate (1×10^{-4} mol/liter, Sigma), glycine (2×10^{-4} mol/liter, Sigma), GABA (1×10^{-4} mol/liter, Sigma), ATP (1×10^{-4} mol/liter, Sigma), ouabaine (1×10^{-5} mol/liter, Serva), and strychnine (1×10^{-5} mol/liter, Sigma).

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RESULTS

We previously showed that AC and its analog carbacholine depolarized the *Lumbricus terrestris* somatic muscle cell membrane in a dose-dependent manner [3]. On the other hand, AC receptor blockers, such as d-tubocurarine, α-bungarotoxin, atropine, and benzohexonium, do not cancel the depolarizing effect of cholinomimetics [3]. We therefore hypothesized that the *Lumbricus terrestris* muscle cell AC receptors cannot be referred to any of the known classical families of vertebrate AC receptors [3,10]. Addition of muscarine to the solution did not lead to changes in the muscle cell RMP, while nicotine caused a decrease of the potential at rest (Table 1). Hence, atypical AC receptors of the *Lumbricus terrestris* somatic muscles, similarly as the vertebrate skeletal muscle receptors, can be referred to the same group of the nicotine type receptors.

It is known that serotonin, glutamate, glycine, and ATP are excitation transmitters in vertebrates and invertebrates [5,7]. ATP can serve as a cotransmitter released together with the main neurotransmitter, e.g., AC, and is capable of modulating the functional characteristics of the postsynaptic membrane [8]. Addition of such substances as serotonin, glutamate, glycine, and ATP into solution in independent experimental series did not modulate the *Lumbricus terrestris* muscle cell RMP (Table 1). Presumably, muscle cells of the musculocutaneous sac do not contain receptors for serotonin, glutamate, glycine, and ATP, at least in quantities sufficient for modulating RMP. On the other hand, addition of GABA to the solution caused hyperpolarization of the muscle membrane (Table 1). It was proven that somatic muscle cells of *Lumbricus terrestris* possess double innervation: stimulatory (depolarizing) mediated by AC and inhibitory (hyperpolarizing) mediated by GABA [4]. The GABA-induced increase in RMP can be realized via two different mechanisms: first, modulation of ionic membrane permeability through activation of ionic channels allowing potassium outward current or chlorine entry; second, increase in membrane potential created ionic pumps [1,2,9]. We previously showed that resting potential of earthworm muscle cells is an integral indicator of K^+ and Cl^- diffuse potentials and the potential created by active Na^+-K^+ pump and secondary active chlorine co-transport [2]. The contribution of the latter component to RMP was considerable [2,9]. The experiments showed that RMP decreased in chlorine-free solution, which confirmed the previous data [2], and in this case GABA lost the capacity to hyperpolarize the muscle membrane (Table 1). It is known that the equilibrium potential for Cl^- in earthworm muscle is not equal to RMP, and the gradient is created by active Cl^- transport [2]. Hence, the muscle membrane presumably contains GABAergic receptors conjugated with chlorine channels. Activation of GABAergic receptors induces chlorine ionic inflow by the gradient, which leads to RMP increase. On the other hand, we proved that ionic pumps were active and modulated (the pump potential) RMP only in the presence of all potential-forming ions in the solution including Cl^- [2]. Hence, the absence of GABA hyperpolarizing effect on muscle cell RMP in chlorine-free solution can be due to inactivation of amperogenic ionic pumps. In order to verify this hypothesis, we studied the effect of GABA on RMP after blockade of ionic pump with ouabaine [1,2].

Experiments showed that inactivation of ionic pumps with ouabaine decreased RMP (Table 1), which is in line with previous reports [1,2]. GABA treatment after ouabaine did not induce membrane hyperpolarization (Table 1), and therefore activation of ionic pump, rather than Cl^- entry, was responsible for GABA-induced increase in transmembrane potential. Removal of K^+ from the solution is also known to inhibit ionic

TABLE 1. Effects of Muscarine, Nicotine, Serotonin, Glycine, Glutamate, ATP, GABA, Ouabaine, Strychnine, Potassium-Free and Chlorine-Free Solutions on the Somatic Muscle Cell RMP of a *Lumbricus terrestris* Musculocutaneous Sac (*M*±*m*)

Experiment conditions	RMP, mV	Number of fibers
Control	48.7±1.0	120
Muscarine 1×10^{-5} mol/liter	48.8±1.1	120
Nicotine 1×10^{-5} mol/liter	44.9±0.9*	120
Serotonin 1×10^{-4} mol/liter	46.0±1.1	120
Glutamate 1×10^{-4} mol/liter	48.4±1.1	120
Glycine 2×10^{-4} mol/liter	47.1±0.8	120
ATP 1×10^{-4} mol/liter	46.4±1.3	120
GABA 1×10^{-4} mol/liter	52.1±1.0*	120
Ouabaine 1×10^{-5} mol/liter	45.3±1.1*	120
Ouabaine 1×10^{-4} mol/liter+ GABA 1×10^{-4} mol/liter	44.6±1.1*	120
Solution without K^+	49.7±0.9	120
Solution without K^++ GABA 1×10^{-4} mol/liter	47.1±1.0	120
Solution without Cl^-	45.4±1.1*	120
Solution without Cl^-+ GABA 1×10^{-4} mol/liter	44.6±1.1*	120
Strychnine 1×10^{-4} mol/liter	48.9±0.8	240
Strychnine 1×10^{-4} mol/liter+ GABA 1×10^{-4} mol/liter	48.6±0.9	120

Note. Muscles from 4-8 animals were used in each experimental series. *$p<0.05$ compared to the control.

pumps, but potassium-free solution does not modulate RMP because of specific features of the *Lumbricus terrestris* muscle cell resting membrane potential [2]. In our experiments the absence of potassium ions in the solution did not modulate RMP (Table 1). Application of GABA to muscle in a K^+-free medium did not modulate resting potential (Table 1). It should be emphasized that inactivation of membrane ionic pumps of *Lumbricus terrestris* muscle cells abolished the hyperpolarizing effect of GABA. Strychnine added to bathing solution did not modulate RMP (Table 1). On the other hand, in the presence of strychnine GABA lost the capacity to increase the transmembrane potential (Table 1). Strychnine is a blocker of glycine-dependent chlorine channels and $GABA_A$ receptors also associated with chlorine channels. It seems that GABA-induced activation of ionic pump is mediated by receptors sensitive to strychnine blocking and, probably, by transmembrane transport of Cl^-.

Several conclusions can be formulated from these findings. The sensitivity of *Lumbricus terrestris* muscle cells to cholinergic receptor agonists can be explained by the presence of atypical nicotinic AC receptors. On the other hand, the number of structures sensitive to serotonin, glycine, glutamate, and ATP modulating RMP in muscle cells seems to be insufficient. At the same time GABA caused hyperpolarization of muscle cell membrane. This effect is realized via membrane receptors sensitive to the blocking effect of strychnine and via activation of ionic pumps playing an important role in the formation of RMP in *Lumbricus terrestris* somatic cells.

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