# EXPERIMENTAL BIOLOGY

# Effect of Cholinergic Agonists on Resting Membrane Potential of Earthworm Body Wall Muscle Cells

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Carbachol in a concentration of $5\times10^{-8}$ mol/liter does not hyperpolarize, and in a concentration of $5\times10^{-6}$ mol/liter depolarizes the membrane of somatic muscle cell in earthworm. d-Tubocurarine, α-bungarotoxin, atropine, and hexamethonium added to the incubation medium did not abolish the carbachol-induced decrease in resting membrane potential. Each of these drugs alone had no effect on resting membrane potential in muscle cells. Presumably, the acetylcholine-sensitive receptor-channel membrane complex in earthworm muscle cell differs from acetylcholine receptor in skeletal muscle fibers and peripheral neurons of vertebrates.

**Key Words:** *earthworm; muscle cells; resting potential; acetylcholine receptor*

It is now generally accepted that acetylcholine (AC) is the major excitatory neuromuscular transmitter in annelids [4,7,11]. AC-sensitive receptor-channel complex in somatic muscle cells is classified as nicotinic-like AC receptor [10,11]. However the effect of cholinergic agonists on resting membrane potential (RMP) in earthworm body wall muscle cells is little studied [11], which determined the purpose of our investigation.

## MATERIALS AND METHODS

Experiments were carried out on muscle cell bundles from the coelomic body wall surface of *Lumbricus terrestris* in autumn-winter. Freshly isolated longitudinal body wall fragments (10-15 segments) free from coelomic organs were put into a cuvette for electrophysiological studies with Drewes—Pax solution [8] of the following composition (in mM): 163 $Na^+$; 4 $K^+$; 6 $Ca^{2+}$; 2 $Tris^+$; 93 $Cl^-$; and 43 $SO_4^{2-}$ (pH 7.2-7.3,18-20°C). Resting potential was measured with glass microelectrodes filled with 2.5 M KCl (7-15 MW). RMP was measured 5-10 min after addition of the corresponding drugs to bathing solution. AC ($5\times 10^{-8}$ and $5\times10^{-6}$ mol/liter), carbachol (CC, $5\times10^{-8}$ and $5\times10^{-6}$ mol/liter, Sigma), d-tubocurarine ($10^{-4}$ mol/liter, Sigma), α-bungarotoxin ($10^{-5}$ mol/liter, Sigma), atropine ($10^{-5}$ mol/liter, Sigma), and hexamethonium ($10^{-5}$ mol/liter) were used. The data were processed using Student's *t* test.

## RESULTS

In control preparations, RMP of earthworm muscle cell was 48.7±0.6 mV, which agree with published data [2,6]. It was previously demonstrated that cholinergic agonists, in particular CC, in low concentrations ($1\text{-}5\times10^{-8}$ mol/liter) hyperpolarized the membrane of mammalian skeletal muscle fibers by several millivolts [3,5]. This hyperpolarization results from activation of $Na^+$,$K^+$-pump [1,5]. We previously showed that electrogenic component of pumping currents greatly contribute to integral RMP of earthworm muscle cell [2]. Since AC acts as an excitatory transmitter in the neuromuscular system of annelids [6,11], it is there-

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fore logical to assume that low doses of CC, a non-hydrolyzed AC analog, also activate ionic pump and increase RMP in earthworm. CC was chosen because AC did not modify RMP (Table 1) presumably because of its rapid degradation in water. CC in a concentration $5\times10^{-8}$ mol/liter did not change RPM, while in a concentration of $5\times10^{-6}$ mol/liter it caused membrane depolarization (Table 1). Hence, in contrast to mammalian skeletal muscles, earthworm muscle cell membrane cannot be hyperpolarized by low doses of CC. Higher concentrations of CC caused total muscle contracture, thus making RPM recording impossible. However, these findings confirm that AC is the main excitatory transmitter in the neuromuscular system of earthworm [11]. Classical nicotinic receptor blockers d-tubocurarine and α-bungarotoxin [9] did not abolish the carbochol-induced depolarization of muscle cell membrane. Muscarinic receptor blocker atropine also did not prevent depolarization induced by CC (Table 1). Experiments with ganglionic blocker hexamethonium gave similar results as with nicotinic and muscarinic antagonists. Hexamethonium did not prevent the CC-induced decrease in RMP. At the same time, d-tubocurarine, α-bungarotoxin, atropine, and hexamethonium did not change RMP in muscle cell (Table 1). Therefore, membrane of body wall muscle cells in earthworm contains CC-sensitive structures, which differ from classical nicotinic or muscarinic receptors of muscle cells and from cholinergic receptors of sympathetic ganglions in vertebrates [1,9]. Our experiments revealed some peculiarities in the effect of cholinergic agonists on RMP of earthworm somatic muscle cells. CC in low concentrations cannot hyperpolarize the muscle cell membrane, while in high concentrations it decreases RMP. Qualitatively, the sensitivity of earthworm muscle cell membrane to cholinergic agonists is compatible to that of the postsynaptic membrane of skeletal muscle fibers in vertebrates [1,9]. Classical nicotinic, muscarinic, and ganglionic blockers cannot abolish CC-induced depolarization of the membrane, which attests to the presence of a peculiar AC-sensitive channel-receptor complex in the membrane of body wall muscle cells of earthworm. This complex differs from cholinergic receptors of skeletal muscle fibers and peripheral neurons in vertebrates.

**TABLE 1.** Effects of AC, CC, d-Tubocurarine, α-Bungarotoxin, Atropine, and Hexamethonium on RMP of Internal Longitudinal Muscle Bundle of *Lumbricus terrestris* (*M*±*m*)

| Drug, mol/liter | Number of observations | RMP, mV |
|---|---|---|
| Control | 400 | 48.7±0.6 |
| AC $5\times10^{-8}$ | 80 | 49.0±1.1 |
| $5\times10^{-6}$ | 80 | 47.0±1.1 |
| CC $5\times10^{-8}$ | 80 | 46.5±1.0 |
| $5\times10^{-6}$ | 80 | 41.8±0.8* |
| d-Tubocurarine $10^{-4}$ | 80 | 49.8±1.2 |
| +CC $5\times10^{-6}$ | 80 | 35.7±0.9* |
| α-Bungarotoxin $\times10^{-5}$ | 80 | 49.6±1.1 |
| +CC $5\times10^{-6}$ | 80 | 41.1±1.0* |
| Atropine $\times10^{-5}$ | 80 | 45.7±1.2 |
| +CC $5\times10^{-6}$ | 80 | 39.2±1.0* |
| Hexamethonium $\times10^{-5}$ | 80 | 49.3±1.0 |
| +CC $5\times10^{-6}$ mol/liter | 80 | 37.6±0.8* |

**Note.** *$p<0.01$ compared to the control.

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