Effect of Ionic Medium on Carbacholine-Induced Membrane Depolarization in *Lumbricus Terrestris* Somatic Muscle Cells

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Carbacholine depolarizes *Lumbricus terrestris* myocyte membrane. Addition of verapamil, tetrodotoxin, removal of K^+, Cl^-, and Ca^{2+}, and replacement of Ca^{2+} with Mn^{2+} in the bathing solution did not prevent the carbacholine-induced decrease in resting potential, but in a sodium-free medium carbacholine was ineffective. It was hypothesized that depolarization of the sarcolemma resulting from activation of cholinomimetic-sensitive channel-receptor complex of the *Lumbricus terrestris* somatic muscle cells is primarily determined by sodium permeability of the membrane.

Key Words: *carbacholine; sodium; muscle cells; Lumbricus terrestris*

Cholinomimetics depolarize the membrane of myocyte of the musculocutaneous sac in annelids. At the same time the cholinomimetic-sensitive channel-receptor complex of *L. terrestris* muscle cells cannot be referred to any of the known classical cholinergic receptors of skeletal muscles or peripheral neurons of vertebrates [4,9]. Here we studied the ionic nature of depolarization of the muscle membrane during its activation.

MATERIALS AND METHODS

Experiments were carried out on muscle cells of longitudinal bundles of the Celtic surface of the *L. terrestris* musculocutaneous sac (n=62). Freshly prepared longitudinal fragments of musculocutaneous sac (10-15 segments long) free from coelomic organs were put into cuvette for electrophysiological studies with modified Drewes—Pax solution containing (mmol/liter): 163.0 Na^+, 4.0 K^+, 6.0 Ca^{2+}, 93.0 Cl^-, 43.0 SO_4^{2-}, 2.0 $Tris^+$, 167.0 sucrose, 478.0 mosmol/liter osmolarity, 229 mmol/liter ionic strength, pH 7.2-7.4, t=20-22°C [2,7]. In order to maintain osmolarity after removal of Ca^{2+}, Na^+ concentration was increased to 169 mmol/liter. In some cases Ca^{2+} was replaced with an equivalent concentration of Mn^{2+} (6.0 mmol/liter).

The resting membrane potential (RMP) of muscle cells was measured using glass microelectrodes filled with 2.5 mol/liter KCl (tip resistance 10-15 MΩ) and standard equipment. RMP was recorded before and 10-15 min after replacement of solutions or addition of tests drugs. Carbacholine (5×10^{-6} mol/liter, Sigma), verapamil hydrochloride (10^{-4} mol/liter), and tetrodotoxin (10^{-5} mol/liter, Sigma) were used.

RESULTS

It was previously shown that acetylcholine and its analog carbacholine depolarized muscle cell membrane of the *L. terrestris* musculocutaneous sac in a dose-dependent manner [4] and induced muscle contracture. In the present study carbacholine in a concentration of 5×10^{-6} mol/liter effectively reduced RMP, but caused no muscle contractions (Table 1). Therefore we used this concentration of the cholinomimetic. The presence of tetrodotoxin (voltage-dependent sodium ionic channel blocker [8]) did not affect RMP of muscle cells and did not prevent carbacholine-induced

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depolarization (Table 1). Hence, the acetylcholine-sensitive channel-receptor complex of *L. terrestris* muscle cells is resistant to the blocking effect of tetrodotoxin, similarly to that in vertebrates [6]. Removal of K^+ from the solution did not change RMP of muscle cells, while removal of Na^+, Cl^-, Ca^{2+} or their replacement with Mn^{2+} led to depolarization of the muscle membrane (Table 1). The absence of hyperpolarization in the potassium-free medium and reduction of RMP in calcium-free or chlorine-free solutions can be explained by peculiarities of Na^+, K^+-pump function and Cl^- co-transport [2,3]. These ion-transporting systems are active only in the presence of these ions, while their removal causes depolarization of the muscle membrane [2,3]. In the absence of K^+, Cl^-, and Ca^{2+}, or after replacement of Ca^{2+} with calcium channel blocker Mn^{2+} [5], carbacholine-induced depolarization of muscle membrane did nit differ from that observed in a solution of normal ionic composition (Table 1).

Verapamil blocks calcium channels and reduces sensitivity of the postsynaptic membrane in skeletal muscle fibers to acetylcholine; the effects of carbacholine on acetylcholine receptors are most pronounced in denervated muscles [1]. In our experiments verapamil did not change RMP of *L. terrestris* muscle cells and did not prevent the development of carbacholine-induced depolarization (Table 1). Hence, verapamil did not reduce the sensitivity of muscle cell membrane in *L. terrestris* to the depolarizing effect of cholinomimetics (in contrast to vertebrate muscle fibers). This fact confirms the notion that cholinoreceptors in *L. terrestris* muscle cells differ from the known classical pharmacological types of peripheral acetylcholine receptors in vertebrates [4,9]. In Na^+-free medium carbacholine had no effect on RMP in muscle cell (Table 1).

Hence, carbacholine-induced depolarization of the membrane in muscle cells is determined primarily by Na^+ inward current due to increased sodium permeability. The conduction status of ionic channel of acetylcholine receptor of the *L. terrestris* somatic cell sarcolemma is resistant to the blocking effects of tetrodotoxin, verapamil, and Mn^{2+}.

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TABLE 1. Effects of Carbacholine, Tetrodotoxin, Verapamil, Removal of Na^+, K^+, Cl^-, Ca^{2+} or Replacement of Ca^{2+} with Mn^{2+} on RMP of *Lumbricus terrestris* Somatic Muscle Cells (*M*±*SEM*)

Experimental conditions	Number of observations	RMP, mV
Control	400	48.7±0.6
Carbacholine	120	41.3±0.8*
Tetrodotoxin	120	47.4±1.1
+carbacholine	120	35.0±0.8+
Na^+, 0 mmol/liter	120	43.7±0.7*
+carbacholine	120	42.5±0.9*+
K^+, 0 mmol/liter	120	51.5±1.3
+carbacholine	120	28.5±1.0*+
Cl^-, mmol/liter	120	42.9±0.7*
+carbacholine	120	34.5±0.8*+
Ca^{2+}, mmol/liter	120	41.5±0.9*+
+carbacholine	120	31.4±0.6*+
Mn^{2+}, 6 mmol/liter	114	44.7±1.0*
+carbacholine	124	24.9±0.6*+
Verapamil	120	47.5±0.7
+carbacholine	120	37.5±0.9*+

Note. $p<0.001$: *compared to the control (RMP in solution of normal ionic composition), +compared to RMP in the same solution without carbacholine.

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