PHYSIOLOGY

Effects of Norepinephrine and Epinephrine on Resting Membrane Potential in Body Wall Muscle Cells of *Lumbricus Terrestris* Eearthworm

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Norepinephrine and to a lesser extent epinephrine increased the resting membrane potential of earthworm body wall muscle cells. Ouabain, phentolamine, propranolol, and replacement of Ca^{2+} with Mg^{2+} in the incubation medium abolished this effect. External 3'5'-cAMP in high concentration, dibutyryl cAMP, and dibutyryl cGMP did not induced hyperpolarization of muscle cell membranes. It was concluded that norepinephrine and epinephrine increased the resting membrane potential in earthworm body wall muscle cells via activation of Na^+, K^+ pump. This process involves Ca^{2+} and sarcolemmal adrenergic structures, similar to α-adrenoceptors in vertebrate, but not the cyclic nucleotide systems.

Key Words: *earthworm; muscle cells; resting membrane potential; adrenoceptors; ionic pump*

Resting membrane potential (RMP) of earthworm somatic muscle cells is determined by potassium and chloride diffusion potentials and by electrogenic activity of ionic pumps [3,12]. In contrast to vertebrate muscles, the latter contributes considerably to RMP in the earthworm [12]. It was found that norepinephrine can hyperpolarize earthworm muscle cell membrane, presumably though activation of ionic pumps [3]. However, the mechanisms coupling the interaction of agonists with adrenoreactive membrane structures in muscle cells and the system activating ionic pumps remain unknown.

Here we studied membrane mechanisms underlying norepinephrine- and epinephrine-induced activation of ionic pumps in earthworm body wall muscle cells.

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MATERIALS AND METHODS

Experiments were carried out on superficial longitudinal fiber bundles of the skin-muscle sac (SMS) of *Lumbricus terrestris* earthworm in October-April. Freshly prepared SMS fragments (10-15 segments) free from coelomic organs were incubated in a bath for electrophysiological recording at room temperature in a medium containing (mmol/liter) [8]: 164 Na^+, 4 K^+, 6 Ca^{2+}, 43 SO_4^{2-}; 93 Cl^-, 2 $Tris^+$, 167 sucrose (pH 7.3-7.4). RMP of muscle cells was measured using glass microelectrodes filled with KCl solution (2.5 mmol/liter, 7-15 mW tip resistance) and standard measuring devices. The electrodes were inserted into the cells under visual control. RMP was measured before and 5-10 min after drug application or replacement of bathing solution with a modified medium. The drugs used were norepinephrine (10^{-5} mol/liter), epinephrine (10^{-5} mol/liter, both from Sigma), ouabain (10^{-4}

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mol/liter, Serva), phentolamine (10^{-5} mol/liter, propranolol (10^{-5} mol/liter), 3,5-cAMP (10^{-4} mol/liter), dibutyryl-cAMP (10^{-5} mol/liter), dibutyryl cGMP (10^{-6} mol/liter, all from Sigma). In some experiments Ca^{2+} in the incubation medium was replaced with equimolar Mg^{2+}.

RESULTS

In the control medium, RMP of muscle cells of earthworm SMS superficial longitudinal bundle was -48.7± 0.6 mV, which agrees with published data [3,4,7]. In the presence of norepinephrine RMP increased by on average 7 mV ($p<0.001$, see Table 1). Ouabain abolished the hyperopolarizing effect of norepinephrine and decreased RMP of muscle cells ($p<0.01$), which agrees with our previous findings [3]. These data suggest that the norepinephrine-induced increase in RMP of earthworm muscle cells is associated with activation of ouabain-sensitive ionic pumps, first of all Na^+, K^+-pump [6]. Since RMP increased within several minutes, this effect of norepinephrine could be attributed to potentiation of the electrogenic component to the integral RMP, rather than to changes in ionic gradients for potential-forming ions [6]. α-Adrenoblocker phentolamine [5] and β-adrenoblocker propranolol [5] had no effect on RMP, but abolished the hyperpolarizing effect of norepinephrine (Table 1). It can be assumed that activation of ionic pumps in earthworm muscle cells is mediated by adrenosensitive membrane structures, similar to α- and/or β-adrenoceptors in vertebrates [5]. In vertebrates, intracellular effects caused by stimulation of α-adrenoceptors (*e.g.* activation Na^+,K^+-ATPase) in the majority of cells including skeletal muscle fibers are mediated by the guanylate cyclase-cGMP system, whereas those caused by stimulation of β-adrenoceptors — by adenylate cyclase-cAMP system [5,9-11]. Addition of cAMP in high concentrations [2] or dibutyryl cAMP, a membrane-permeable analog of cAMP, had no effects on muscle cell RMP (Table 1). Dibutyryl cGMP also exerted no hyperpolarizing effect (Table 1). These findings contradict the hypothesized scheme of the RMP increase: norepinephrine→adrenoreactive structures→intracellular second messengers (cAMP and /or cGMP)→ionic pump→ RMP, as it occurs in vertebrate skeletal muscles [1]. Epinephrine also hyperpolarized the membrane of earthworm muscle cells ($p<0.05$, Table 1), but the absolute RMP increase was almost 3-fold lower than after norepinephrine. The effects of epinephrine are mediated primarily via β-adrenoceptors, while those of norepinephrine — via α-adrenoceptors [5,9-11]. β-Adrenoceptor blocker propranolol abolished the effect of epinephrine on muscle cell RMP (Table 1). These results suggest that earthworm muscle cell membranes contain primarily adrenosensitive structures similar to vertebrate α-adrenoceptors, whereas the density of β-receptors seems to be much lower. It is known that calcium ions (both entering through the membrane and released from intracellular stores) and Ca-accepting structures are involved in the realization of intracellular events caused by stimulation of adrenoceptors [5,11]. Replacement of the bathing solution with a medium containing Mg^{2+} instead of Ca^{2+} induced membrane depolarization ($p<0.001$, Table 1). This effect was similar to that of ouabain. This can be explained by inactivation of electrogenic ionic pumps operating solely in the presence of Na^+, K^+ and Cl^- [12]. It can be hypothesized that Ca^{2+} is also essential for their activity. Addition of norepinephrine to Ca^{2+}-free medium produced no membrane hyperpolarization in contrast to its effect in the standard medium, *i.e.* in the presence of Ca^{2+} (Table 1). These data indicate that Ca^{2+} is necessary for both the basal activity of earthworm muscle membrane pumps and its stimulation by norepinephrine or epinephrine.

In conclusion, norepinephrine and epinephrine induced hyperpolarization of earthworm SMS membranes by enhancing ionic pump activity (primarily Na^+,K^+ pump) only in the presence of Ca^{2+} in the bathing medium. The effect of catecholamines was realized via adrenosensitive structures of cell sarcolemma, similar to α- and, to a lesser extent, β-adrenoceptors in vertebrates, without involving cAMP and cGMP as

TABLE 1. Effects of Epinephrine, Norepinephrine, Phentolamine, Propranolol, Ouabain, Dibutyryl cAMP, 3'5'-cAMP, Dibutyryl cGMP, and Medium Containing Mg^{2+} Instead of Ca^{2+} on RMP in Muscle Cell of SMS Fibers in *Lumbricus terrestris* Earthworm (*M±m*, *n*=72)

Experiments	RMP, mV	Number of measurements
Standard medium	-48.7±0.6	400
+norepinephrine	-55.8±1.2	100
+phentolamine	-49.5±1.2	80
+norepinephrine	-48.3±1.0	80
+propranolol	-49.1±1.1	80
+norepinephrine	-46.4±1.1	80
+3'5'-cAMP	-50.8±1.3	80
+dibutyryl cAMP	-47.1±1.0	80
+dibutyryl cGMP	-50.1±1.2	80
+ouabain	-40.8±1.0	80
+norepinephrine	-38.6±1.0	80
+epinephrine	-51.2±0.9	80
+propranolol	-49.3±1.1	80
Medium containing Mg^{2+} instead of Ca^{2+}	-33.3±0.8	80
+norepinephrine	-31.3±0.7	80

intracellular second messengers. It should be noted that there is no convincing evidence of the presence of catecholamines in the earthworm internal medium.

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