EXPERIMENTAL BIOLOGY

Effect of GABAergic and Adrenergic Agents on Activity of Na^+/K^+ Pump and Cl^--Cotransport in Somatic Muscle Cells of Earthworm *Lumbricus Terrestris*

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GABA, baclofen, epinephrine, and norepinephrine hyperpolarized the membrane of earthworm somatic cells. This effect was prevented by furosemide, removal of Cl^- from the medium, or activation of Na^+/K^+ pump by 3-fold increase external potassium concentration. It was hypothesized that GABA, baclofen, epinephrine, and norepinephrine stimulate Na^+/K^+ transport via specific receptor inputs, but their effect on resting potential can be realized only under conditions of working Cl^- symport.

Key Words: *γ-aminobutyric acid*; *norepinephrine; ionic pumps; muscle cell membrane; earthworm*

GABA and norepinephrine via intracellular mechanisms can increase resting membrane potential (RMP) [1,4,5] and activate electrogenic ionic pumps in the membrane of somatic muscle cells of earthworm, namely Na^+/K^+-pump and functionally coupled symport Cl^- [7]. Potential created by ionic pumps appreciably contributes into the integral RMP [3]. It remains unknown what is the final target for the action of GABA- and adrenergic systems: Na^+/K^+-ATPase or chlorine transport. Our aim was to clarify this uncertainty.

MATERIALS AND METHODS

The experiments were carried out on surface longitudinal bundles of muscle cells from the inner side of musculocutaneous sack of earthworm *Lumbricus Terrestris* during fall-winter period. Fresh isolated preparations of longitudinally cut and free from coelomic organs fragments of the musculocutaneous sacks (10-15 segments long) were placed in a bath for electrophysiological examination in modified Dreves—Pax solution containing (in mM): 163.0 Na^+, 4.0 K^+, 6.0 Ca^{2+}, 93.0 Cl^-, 43.0 SO_4^{2-}, 2.0 $tris^+$, and 167.0 sucrose; osmolarity, 478,0 mosmol/liter, ionic strength, 229.0; pH 7.3-7.4 at room temperature [3]. Potassium ions were replaced with the corresponding amount of sodium ions, the osmolarity remained constant. Cl^- ions were replaced with NO_3^- and SO_4^{2-} ions.

Glass microelectrodes filled with 2.5 mM KCl (tip resistance 7-15 MΩ) were used to measure RMP of muscle cells. RMP was measured 5-10 min after replacement of the initial medium with the test solutions with modified ionic composition or containing pharmacological agents. Ouabain (1×10^{-4} M, Serva), furosemide (1×10^{-4} M, Sigma), GABA (1×10^{-4} M, Sigma), baclofen (1×10^{-4} M, Sigma), bicucul-

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line (1×10^{-4} M, Sigma), norepinephrine (1×10^{-5} M, Sigma), and propranolol (1×10^{-5} M) were used.

RESULTS

GABA and baclofen increased RMP of muscle cells, the latter agent being more effective (Table 1, Fig. 1). This phenomenon is related to predominant activation of B-type receptors [5,6] followed by Ca^{2+} entry into the cell and activation of electrogenic ionic pumps via calmodulin-like intracellular systems [4,5]. In addition to Na^+/K^+ antiport, the membrane of earthworm muscle cells possesses active Cl^--symport and the work of both transport mechanisms directly affects the integral value of RMP contributing to it so-called "pump potential" [3,7]. It remains unclear what is the target of additional activation: Na^+/K^+-ATPase or chlorine transport. Elimination of chloride transport by removal of Cl^- from the solution or by addition of furosemide equally depolarized the muscle membrane (Table 1, Fig. 1). The action of furosemide is comparable to that of ouabain, an inhibitor of Na^+/K^+-ATPase. GABA cannot increase RMP in the presence of furosemide and ouabain [2,5]. Furosemide or Cl^--free solution abolished the hyperpolarizing effect of baclofen on muscle membrane (Table 1, Fig. 1). It can be hypothesized that activity of Cl^- symport is a prerequisite for GABA and baclofen potency to hyperpolarize the muscle membrane. The contribution of pump potential into RMP created by Cl^- symport is low compared to the ability of GABA and baclofen to increase RMP [5]. This value is relatively constant and does not directly depend on the degree of Na^+/K^+-ATPase activation. Activity of Na^+/K^+-pump depends on concentration of K^+ in the solution and attains maximum at 12 mM [3]. In the presence of ouabain and 12 mM K^+ (3-fold increase) RMP is 33.4±1.0 mV, while in potassium-rich and ouabain-free solution it is 46.3±1.1 mV ($p<0.001$). Under these conditions, GABA or baclofen had no effect on RMP (Table 1, Fig. 2). Thus, when the power of Na^+/K^+-pump is maximum, GABA or baclofen cannot affect its work.

Epinephrine and to a greater extent norepinephrine also hyperpolarize the earthworm muscle membrane (Table 1) [1]. The intracellular mecha-

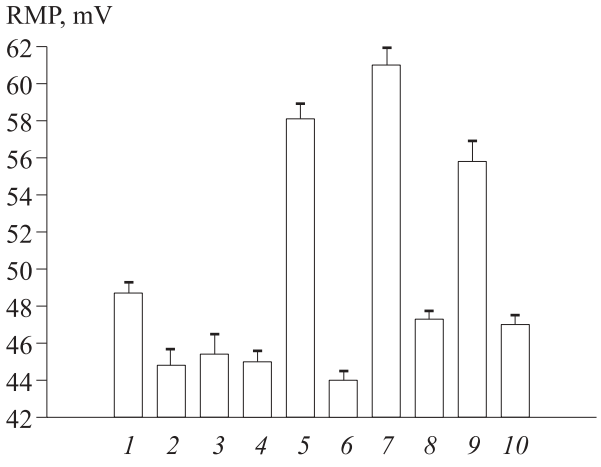

Fig. 1. Effect GABA, baclofen, epinephrine, their combination with furosemide, furosemide, ouabain, and Cl^--free solution on resting membrane potential (RMP) of earthworm somatic muscle cells. *1*) control; *2*) ouabain; *3*) $[Cl^-]=0$; *4*) furosemide; *5*) GABA; *6*) GABA+furosemide; *7*) baclofen; *8*) baclofen+furosemide; *9*) norepinephrine; *10*) norepinephrine+furosemide.

TABLE 1. Effect of GABA, Baclofen, Epinephrine, Norepinephrine, Ouabain, Furosemide, Bicuculline, Propranolol, K^+-Rich Saline, Cl^--Free Saline on RMP of Somatic Muscle Cells in Earthworm ($M\pm m$)

Experimental conditions	RMP, mV	*n*
Control	48.7±0.6	400
GABA, 1×10^{-4} M	58.1±0.8	80***
Baclofen, 1×10^{-4} M	61.0±0.9	80***
Furosemide, 1×10^{-4} M	45.0±0.6	80*
GABA (1×10^{-5} M)+ furosemide (1×10^{-4} M)	44.0±0.5	80*
Baclofen (1×10^{-4} M)+ furosemide (1×10^{-4} M)	47.3±0.4	80
$[Cl^-]=0$ mM	45.4±1.1	100*
Baclofen (1×10^{-4} M)+ $[Cl^-]=0$ mM	46.0±1.2	80
$[K^+]=12$ mM	46.3±1.1	120
$[K^+]=12$ mM+GABA (1×10^{-4} M)	46.8±0.9	80
$[K^+]=12$ mM+baclofen (1×10^{-4} M)	45.9±0.7	80
Ouabain, 1×10^{-4} M	44.8±0.9	100*
$[K^+]=12$ mM+ouabain (1×10^{-4} M)	33.6±0.9	80***
Epinephrine, 1×10^{-5} M	52.2±0.9	140**
Norepinephrine, 1×10^{-5} M	55.8±1.1	80***
Epinephrine (1×10^{-5} M)+ furosemide (1×10^{-4} M)	49.8±0.8	80
Norepinephrine, 1×10^{-5} M+ furosemide (1×10^{-4} M)	47.0±0.5	80
Propranolol (1×10^{-5} M)	50.1±1.2	80
Propranolol (1×10^{-5} M)+ baclofen (1×10^{-5} M)	50.1±1.2	80
Bicuculline, 1×10^{-4} M	43.0±1.1	80**
Bicuculline (1×10^{-4} M)+ norepinephrine (1×10^{-5} M)	53.0±1.2	80***

Note. In each experiment, the muscles were taken from not less than 4 animals. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control.

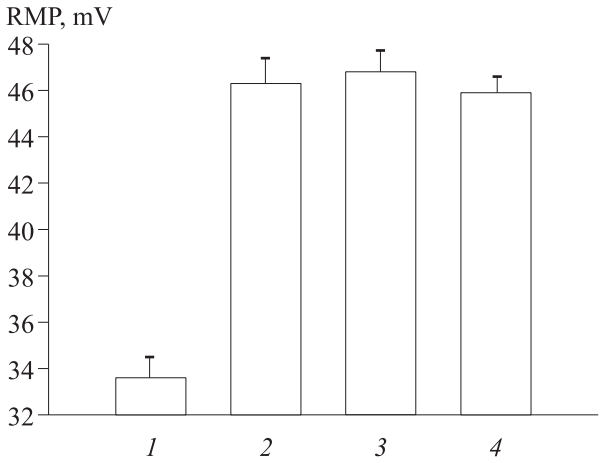

Fig. 2. Effect ouabain, GABA, and baclofen on RMP of earthworm somatic muscle cells in K^+-rich solution with $[K^+]$=12 mM. *1*) ouabain; *2*) control; *3*) GABA; *4*) baclofen.

nisms mediating the effect of catecholamines on ionic pumps and RMP are virtually the same as those mediating GABA effect [4,5] except their own specific adrenergic receptor input [1,4]. Similar to GABA and baclofen, furosemide abolished the effect of epinephrine and norepinephrine on RMP (Table 1, Fig. 1). Removal of any potential-forming ion from the solution inactivates ionic pumps in earthworm somatic muscle cells [3,7]. Bicuculline, a wide-spectrum blocker of GABAergic receptors, decreases RMP of muscle cells (Table 1) [5]. In the presence of this agent, norepinephrine hyperpolarizes the muscle membrane, which attests to specificity of adrenergic receptor input. At the same time, β-adrenergic blocker propranolol produces no action on RMP, but removes the effect of norepinephrine [1] and baclofen (Table 1). Probably, the adrenergic and GABAergic RMP tuning mechanisms mediated via a common pathway have a link vulnerable to the action of adrenoblockers (*e.g.* propranolol). However, this possibility needs further evaluation.

Thus, GABA, baclofen, epinephrine, and norepinephrine can hyperpolarize muscle membrane only during working electrogenic Na^+/K^+-pump and active Cl^--symport. Probably, Na^+/K^+-ATPase is the target in GABA- and adrenergic mechanisms, because during its maximum activation the corresponding agents lose the ability to affect RMP. Activity of Cl^--symport is a prerequisite to chemical modulation of RMP. It can be hypothesized that inactivation of this symport induces stoichiometric rearrangements of ionic transfer by the transport systems, which lose electrogenic potency. It decreases the contribution of pump potential into the integral RMP and results in a possibility to quickly affect it via activation of ionic pump electrogenesis.

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