PHYSIOLOGY

Ionic Selectivity of Chloride Ion Symport in Mechanisms Controlling Resting Potential and Osmotic Homeostasis in Earthworm Somatic Muscle Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 5, pp. 484-486, May, 2008
Original article submitted January 31, 2008

Replacement of Cl^- for Br^- in bathing solution did not reduce resting potential and had no effect on modulation of transmembrane potential in hyper- and hypoosmotic solutions. Under these conditions, baclofen, an agonist of GABAergic B-type receptors, failed to activate Na^+/K^+-pump in earthworm somatic muscle cells. It was hypothesized that the contribution of Cl^- symport to osmotic homeostasis is not highly selective in respect to replacement of Cl^- to Br^- ions, whereas in case of activation of electrogenic ion pumps, this replacement is equivalent to removal of Cl^- ions from the bathing solution.

Key Words: *muscle cells; ionic pump; chloride symport; earthworm*

In addition to Na^+/K^+-pump, active symport of chloride ions exists in muscle cells (MC) of earthworm musculocutaneous sac and is involved in the regulation and maintenance of resting membrane potential (RMP) [1,6]. The contribution of electrogenic ionic pumps into the integral value of resting membrane potential is high and reflects simultaneous work of two systems: Na^+/K^+-antiport and chloride symport [2,6]. We previously found that these systems possess two different membrane "transporters" and independent activation mechanisms [5], at the same time, these systems are closely related and inactivation of one system turns off its counterpart [3,5]. However, the selectivity of Cl^- transfer in relation to the entire system of active transport of potential-generating ions remains unclear.

Our aim was to study the contribution of active anion symport into RMP and osmotic regulation of somatic MC under conditions of replacement of chlorides for bromides.

MATERIALS AND METHODS

Experiments were carried out during autumn-winter period on MC of longitudinal fascicles from the inner side of musculocutaneous sac of Lumbricus terrestris earthworm. Freshly isolated fragments of musculocutaneous sack consisting of 10-15 segments were cut longitudinally, cleaned from celomic organs, and placed at room temperature into the bath with physiological solution containing (in mM): 163 Na^+, 4.0 K^+, 93 Cl^-, 43 SO_4^{2-}, 2 $Tris^+$, 167 sucrose, osmolarity 478 mosmol/liter, ionic strength 229 mM [2]. Osmolarity of the solution was changed with sucrose. In hyperosmotic solutions (HyperOS) the concentration of sucrose was doubled to 334 mM, while in hypoosmotic solutions (HypoOS) it was decreased to 84 mM. Chloride ions were replaced with an equivalent amount of bromide ions.

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0007-4888/08/1455-0549

RMP of MC was measured before and 10-15 min after changing the solutions or adding the test agents using glass microelectrodes filled with 2.5 M KCl (tip resistance 7-10 MΩ) connected to a standard amplifier. The experiments used furosemide (10^{-4} M, Sigma) and baclofen (10^{-4} M, Sigma).

RESULTS

Removal of Cl^- ions from bathing solution or application of furosemide, a blocker of Cl^- transfer, depolarized muscle cell membrane of earthworm similarly to inactivation of Na^+/K^+-pump with ouabain [3]. In our experiments, replacement of chlorides for bromides did not decrease RMP of somatic MC (Table 1). Thus, in resting state this procedure is of little importance for the genesis of RMP in MC.

We previously showed that GABA and baclofen (a selective agonist to B-receptors) can significantly increase RMP [1,4], the latter being even more efficient than GABA [4]. Considering this feature, we chose baclofen as a membranotropic agent with a pronounced hyperpolarizing effect on somatic MC. This effect of baclofen can be eliminated by furosemide (Table 1). Similarly, baclofen was inefficient after replacement of chloride with bromide ions in the bathing solution (Table 1). Thus, in this case the replacement of chlorides for bromides was equivalent to removal of Cl^- ions from the solution [3,5] or exposure of the cells to furosemide, a blocker of Cl^- transfer.

Replacement of initial solution for HyperOS increased the transmembrane potential (Table 1). Furosemide eliminated this effect. In contrast, HypoOS depolarized the plasmalemma of MC (Table 1). Replacement of chlorides for bromides did not prevent the hyperpolarizing effect of HyperOS (Table 1), while furosemide (similar to the experiments with chlorides) prevented hyperpolarization. When chloride ions were replaced for bromides ones, HypoOS also depolarized the muscle membrane (Table 1). We previously showed that active Cl^--symport is involved in the regulation of intracellular osmotic homeostasis, while changes in extracellular osmotic pressure modulate activity of Cl^- symport, thus affecting RMP of MC [3]. Under resting conditions, replacement of chlorides for bromides did not depolarize the muscle plasmalemma both in the absence of Cl^- ions or in the presence of furosemide, but baclofen under the same conditions could not hyperpolarize the membrane, which indicates impossibility for Br^- ions to compensate for the absence of Cl^- ions. It can be hypothesized that replacement of Cl^- for Br^- ions is not so far crucial under resting conditions and relatively moderate activity of Na^+/K^+-pump, but in case of pump mobilization this replacement becomes nonequivalent, so RMP does not increase.

The control mechanisms of intracellular osmotic homeostasis involving Cl^- symport were insensitive to replacement of Cl^- for Br^- ions. In experiments with HyperOS and HypoOS, we observed similar changes in RMP either in chloride or bromide solutions. In both cases, furosemide eliminated this effect, which attests to the involvement of the system of secondary chloride (anion) symport into this process. Thus, the response of RMP in MC is specific and reflects changes in the character of active anion transfer.

TABLE 1. Effect of Cl^- for Br^- Replacement, Baclofen, Furosemide, HyperOS, and HypoOS on RMP of Earthworm Somatic Muscle Cells ($M\pm m$)

Experimental conditions	RMP, mV	N
Control	49.0±0.6	600
Replacement Cl^- for Br^-	50.1±1.1	80
Baclofen (10^{-4} M)	62.0±0.9	100*
Baclofen (10^{-4} M)+furosemide (10^{-4} M)	47.6±0.7	80
Baclofen (10^{-4} M) and Cl^- for Br^- replacement	51.1±1.1	80
HyperOS, sucrose 334 mM	57.0±0.9	140*
+furosemide (10^{-4} M)	51.1±1.1	100*
Cl^- replaced with Br^-	56.7±0.9	120*
Cl^- replaced with Br^-+furosemide (10^{-4} M)	48.3±1.2	80
HypoOS, sucrose 84 mM	45.7±0.9	80*
Cl^- replaced with Br^-	46.0±0.9	100*

Note. In each series of experiments, we used muscles isolated from no less than 5 animals. *$p<0.01$ compared to the control.

The contribution of active chloride symport into the regulation of osmotic homeostasis in the cell is characterized by low ionic selectivity and is tolerant to replacement of Cl^- for Br^- ions. Alternatively, the used method could be not so much specific to resolve these differences. In any case, changes produced by this replacement could not be especially dramatic. In contrast, there are other features in the processes where Na^+/K^+-ATPase plays the leading role. In the resting state or during low activity of ionic pump, there were no significant differences in RMP in both chloride or bromide solutions, while during pronounced activation of Na^+/K^+-pump, the replacement of Cl^- for Br^- ions was not equivalent, which resulted in a decrease of electrogenicity of the pump. Probably, the transmembrane counterflows of Na^+, K^+, and Cl^- (in case of the replacement of Cl^- for Br^-) largely loose their asymmetry.

The study was supported by the Russian Foundation for Basic Research (grant No. 06-04-48458).

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