EXPERIMENTAL BIOLOGY

Functional Heterogeneity of the "Transporter" of Electrogenic Ionic Pump of the *Lumbricus Terrestris* Somatic Myocyte Membrane

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Potential created by electrogenic ionic pumps under conditions of maximum activation in a warm standard ionic medium with K^+ after preincubation in cold potassium-free solution has two components: a higher ouabaine-insensitive "stationary" component, and a lower "regulatory" component sensitive to ouabaine, furosemide, and removal of Cl^- or Ca^{2+} from the medium. Functional heterogeneity of electrogenic ionic pumps is hypothesized implying the existence of two components: "stationary" (not regulated extracellularly) and "regulatory" (controlled and directly related to active Cl^- transfer).

Key Words: *resting potential; Na^+,K^+-ATPase; chlorine simport; muscle cells; Lumbricus terrestris*

The work of electrogenic ionic pumps creates a "potential" essential for the integral value of the membrane resting potential (MRP) of the *Lumbricus terrestris* somatic muscle cells [1]. Active Na^+,K^+-countertransport is functionally related to secondary active simport of Cl^-, also directly modulating the MRP value [5]. The Na^+,K^+-countertransport "transporter" is an integral protein consisting of several isomeric forms of subunits differing by the molecular weight and primarily by the functional significance for ionic transfer [3].

We studied the structure and functions of "transporters" of electrogenic ionic pumps of the earthworm somatic muscles.

MATERIALS AND METHODS

Experiments were carried out on surface myocytes of longitudinal bundles of the inner side of the *Lumbricus terrestris* musculocutaneous sac in winter. Fresh preparations of longitudinal fragments of the musculocutaneous sac (10-15 segments long) free of celomic organs were placed for 6 h into a Petri dish with modified Drewes—Pax solution, with K^+ substituted for an equimolar quantity of Na^+ at 5-7°C. The preparation was then transferred into a cuvette for electrophysiological studies with a solution containing 163 mmol/liter Na^+, 4.0 mmol/liter K^+, 6.0 mmol/liter Ca^{2+}, 93 mmol/liter Cl^-, 43 mmol/liter SO_4^{2-}, 2 mmol/liter Tris, 167 mmol/liter sucrose (osmolality 478.0 mosmol/liter, ionic strength 229 mmol/liter, pH 7.2-7.4, 20-22°C) [1]. Ca^{2+} in the solution was substituted for Mg^{2+}, Cl^- for NO_3^-. All substitutions were carried out so that to retain constant osmolarity and ionic strength of solution [1]. The test drugs were added to the solution after the preparation was placed into a cuvette for studies. The experiment consisted of two series: 1) measurements in cold potassium-free solution and 2) measurements 5-10 min after replacement of the solution with warm standard (normal) solution, modi-

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fied or containing the test preparations. MRP was measured with glass electrodes filled with 2.5 mol/liter KCl with the tip resistance of 7-15 MΩ by standard electrophysiological technology. Ouabaine (10^{-6}-10^{-4} mol/liter; Sigma), baclophene (10^{-4} mol/liter; Sigma), GABA (10^{-4} mol/liter; Sigma), and furosemide (10^{-4} mol/liter) were used.

RESULTS

The mean MRP of surface muscle cells on the inner side of the earthworm musculocutaneous sac was 50 mV [1,4]. Long-term exposure of the muscle strip in potassium-free medium at low temperature sharply reduced MRP at the expense of inactivation of electrogenic ionic pumps, changes in electrochemical gradients of potential-forming ions, and primarily accumulation of Na^+ in the cell cytoplasm. Transfer of the muscle preparation into warm solution containing Na^+,K^+-ATPase activator ions led to a sharp intensification of the ionic pump work and significantly increased the contribution of the "pumping" potential into MRP [1].

The mean MRP of muscle cells in different experimental series after 6-h exposure in cold potassium-free solution varied from 18 to 21 mV and did not differ statistically (Table 1). Five to ten minutes after transfer into warm solution of normal ionic composition, MRP sharply increased (more than 2.5 times; Table 1), which is in line with previous data [1]. Ouabaine in concentrations of 10^{-6} and 10^{-5} mol/liter did not modify the pattern of MRP increase and its values did not differ from those without ouabaine (Table 1). Increasing ouabaine concentration to 10^{-4} mol/liter did not prevent the increase of MRP in warm solution, but in this case MRP was by 5-6 mV below the control (*i.e.* solution without the test preparation, Table 1). This ouabaine concentration significantly reduced MRP (by 5-6 mV) in fresh preparations initially incubated in normal solution at ambient temperature [1]. Hence, there are good grounds to assume that low ouabaine concentrations cannot eliminate the electrogenic component of ionogenic pumps, amplified during their activation. On the other hand, high ouabaine concentration was partly effective. Two components were distinguished in the emerging "pumping" potential: a greater (about 80%) component, insensitive to ouabaine, and a lesser one (20%) sensitive to ouabaine (abolished by it). The integral Na^+,K^+-countertransport "transporter" protein consists of at least 2 subunits differing by molecular weight and sensitivity to ouabaine and, presumably, functional role [3]. The presence of two components in the "pumping" potential created by ionic pump work can imply the existence of two functionally and most likely structural subunits of ionic "transporter" in the earthworm muscle cell membrane: sensitive and insensitive to ouabaine.

It was previously shown, that the membrane of *Lumbricus terrestris* somatic muscle cells apart from Na^+,K^+-ATPase has a system of secondary active Cl^- simport closely linked to the work of the Na^+,K^+-pump [5]. Furosemide (Cl^- transfer blocker) and removal of Cl^- from the solution reduce MRP of muscle cells similarly as ouabaine [5]. Transfer of the muscle preparation loaded with Na^+, from cold potassium-free solution into warm Cl^--free solution did not abolish the increase in resting potential (Table 1). But in this case, similarly as in the presence of ouabaine in high concentration, the MRP values were somewhat lower. A similar result

TABLE 1. Effect of Warming in the Presence of K^+, Removal of Ca^{2+} or Cl^- from Solution, Addition of Ouabaine, Furosemide, Baclophene, and GABA in Different Concentrations into Extracellular Medium on the *Lumbricus terrestris* Muscle Cell MRP (mV) ($M \pm m$)

Preparation	Cold solution without K^+	Warm solution with K^+
Control (common solution without drugs)	19.0±0.9	52.1±1.1
Solution+ouabaine 10^{-6} mol/liter	19.0±1.0	51.0±0.9
10^{-5} mol/liter	18.0±1.1	49.2±1.2
10^{-4} mol/liter	20.0±1.0	46.0±0.9*
Solution without Cl^-	20.0±1.1	45.0±1.2*
Solution without Ca^{2+}	20.1±1.1	47.0±0.7*
Solution+furosemide, 10^{-4} mol/liter	19.0±0.9	48.0±1.1*
Solution+GABA, 10^{-4} mol/liter	21.0±1.2	49.9±0.9
Solution+baclophene, 10^{-4} mol/liter	20.0±1.1	52.0±1.0
Solution without Cl^-+baclophene, 10^{-4} mol/liter	20.0±1.2	46.0±1.1*

Note. Each series included 80-100 measurements. *$p<0.05$ compared to the control.

was obtained by adding furosemide into solution instead of Cl^- elimination or Mg^{2+}-Ca^{2+} substitution in solution (Table 1). The decrease in MRP in all three variants was about the same, similarly as with ouabaine in high concentration (Table 1). Baclophene (selective agonist of B-type GABA receptors) and GABA increase MRP of *Lumbricus terrestris* muscle cells at the expense of ionic pump activation and increase in the contribution of "pumping" potential in the integral value of resting potential [2]. This effect is abolished by ouabaine, furosemide, and removal of Cl^- from solution [1,5]. Addition of GABA and baclophene into warm solution after preincubation of the muscle in cold potassium-free medium did not stimulate MRP increase (Table 1). Treatment with baclophene after removal of Cl^- from the solution was also ineffective and MRP of muscle fibers was low in this case, similarly as in the absence of baclophene (Table 1). Hence, GABA and baclophene cannot modify MRP during maximum mobilization of ionic pump activity and without Cl^-.

These data indicate that the electrogenic constituent ("pumping" potential) of MRP value, created by active ionic pump, has two components: sensitive and insensitive to ouabaine. The former (about 80% summary "pumping" potential) is resistant to the absence of Cl^- and Ca^{2+} in the extracellular medium. The latter component can be eliminated by ouabaine and removal of Cl^- and Ca^{2+} from the solution. It seems that GABA and baclophene amplify the MRP by activation of the second constituent, because the above treatments eliminate their effects on MRP. Presumably, two functional constituents can be distinguished in the work of active electrogenic ionic pumps: 1) "stationary", providing basic electrogenesis processes, maintaining the needed (up to 80%) MRP value, and little regulated extracellularly, and 2) "regulatory (up to 20% summary "pumping" potential), controlled extracellularly through respective structures, specifically GABAergic receptors, with obligatory participation of Ca^{2+} [4]. Coordination with the work of secondary active Cl^- simport is realized in this structure. Hence, active transmembrane transfer of Cl^- directly depends on the "regulatory" component. All this suggests functional and, presumably, molecular heterogeneity of Na^+,K^+-ATPase as the main ionic transporter in the earthworm somatic muscle cells.

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