Role of Potassium and Chlorine Channels in the Regulation of Thymocyte Volume in Rats

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Regulatory decrease in thymocyte volume under conditions of osmotic stress was abolished by potassium and chlorine channel blockers. Osmotic stress-activated chlorine channels belong to 2 pharmacological types. The maxi-anion channel is sensitive to Gd^{3+}. The volume-sensitive outwardly rectifying chlorine channel is inhibited with glybenclamide and phloretin.

Key Words: *lymphocytes; osmotic stress; ion channels*

Lymphocytes are often exposed to osmotic stress (*e.g.*, during passage through renal capillaries around the nephron or transport in small vessels adjacent to the pulmonary or intestinal epithelium). Lymphocytes regulate their volume under hyposmotic conditions. They swell rapidly, but shrink slowly in the follow-up period. These changes are mediated by the system for regulatory volume decrease [4]. Immature lymphocytes of the thymus (thymocytes) rapidly proliferate and are subjected to a complex process of positive or negative selection. Non-reactive and autoreactive cells are removed and undergo apoptosis. Proliferation and apoptosis require an effective system for the regulation of cell volume. Ion channels that are activated during osmotic cell swelling play a key role in these processes [5,10]. The regulatory system of thymocytes was described previously [1,3,4,7]. However, ionic mechanisms of this process are poorly understood. Published data show that thymocyte swelling is followed by activation of the system for electroneutral co-transport of potassium and chlorine ions [3,13]. Water channels play an important role in the regulation of thymocyte volume. Blockade of these channels is followed by inhibition of regulatory volume decrease under conditions of hypotonic stress [1,7].

Here we studied the effect of potassium and chlorine channel blockers on the regulation of thymocyte volume under conditions of hyposmotic stress.

MATERIALS AND METHODS

Normal Ringer's solution contained 140 mM NaCl, 6 mM KCl, 15 mM HEPES, 1 mM $CaCl_2$ and 1.5 mM $MgCl_2$ (pH 7.4, 300±2 mOsm/kg H_2O). Hypotonic solution (148 mOsm/kg H_2O) was prepared by dilution of normal Ringer's solution with a buffer containing 15 mM HEPES, 1 mM $CaCl_2$, and 1.5 mM $MgCl_2$ (pH 7.4, 34±2 mOsm/kg H_2O) in the 3:4 ratio. Experiments were performed on thymocytes from 6-8-week-old Wistar rats. Thymocytes were isolated routinely at room temperature [2]. Cell viability was estimated in the test of trypan blue exclusion. The final suspension containing not more than 5% dead cells was stored in Ringer's solution with 5 mM glucose for 3-5 h. Changes in thymocyte volume were estimated from light transmission of the suspension in transmitted light at 610 nm. The study was conducted with the cell suspension (final concentration 10^7 cells/ml) on a MKMF-1 microcolorimeter at 25°C [1,6,7,11]. The

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recording of variations in cell volume was scanned and digitized with Graph Digitizer 2.15 software. Experiments were performed with $GdCl_3$, glybenclamide, phloretin, and HEPES (Sigma). Other reagents were manufactured in Russia. Concentrated matrix solutions of blockers and inhibitors were prepared in dimethylsulfoxide (DMSO). Potassium channel blockers, barium ions, and tetraethylammonium (TEA) ions were used in a wide range of concentrations. The final concentration of DMSO did not exceed 0.1%. The solvent in this concentration had little effect on recorded parameters. We used aqueous solutions of $BaCl_2$ and $GdCl_3$. Osmotic pressure of solutions was measured on an OM802 osmometer (Vogel). Regulatory volume decrease was calculated as described elsewhere [7].

RESULTS

Complete inhibition of cell volume regulation in high-potassium media indicates that potassium ions play a key role in this process [1,7]. $BaCl_2$ in concentrations of 1 and 10 mM significantly inhibited the phase of thymocyte shrinkage after hypotonic stimulation (Fig. 1, *a*, *b*). TEA ions were more effective than barium ions and in a concentration of 5 mM completely abolished the regulatory volume decrease (Fig. 1, *c*, *d*). Hence, potassium ions are eliminated through selective potassium channels during hypotonic stimulation. Our results contradict the hypothesis on the key role of coupled K^+/Cl^- in this process [3,13].

Stilbene derivatives serve as Cl^- transport inhibitors. We showed that SITS (200 μM) has a strong inhibitory effect on thymocyte volume regulation (Fig. 2, *a*, *b*). SITS blocks not only anion channels, but also several transporters (*e.g.*, Cl^-/bicarbonate exchanger). However, there are no highly specific blockers of Cl^- channels. Therefore, our experiments were performed with inhibitors of various Cl^- channels. Gd^{3+} ions were potent in inhibiting the regulation of thymocyte volume (Fig. 2, *c*, *d*). This ca-

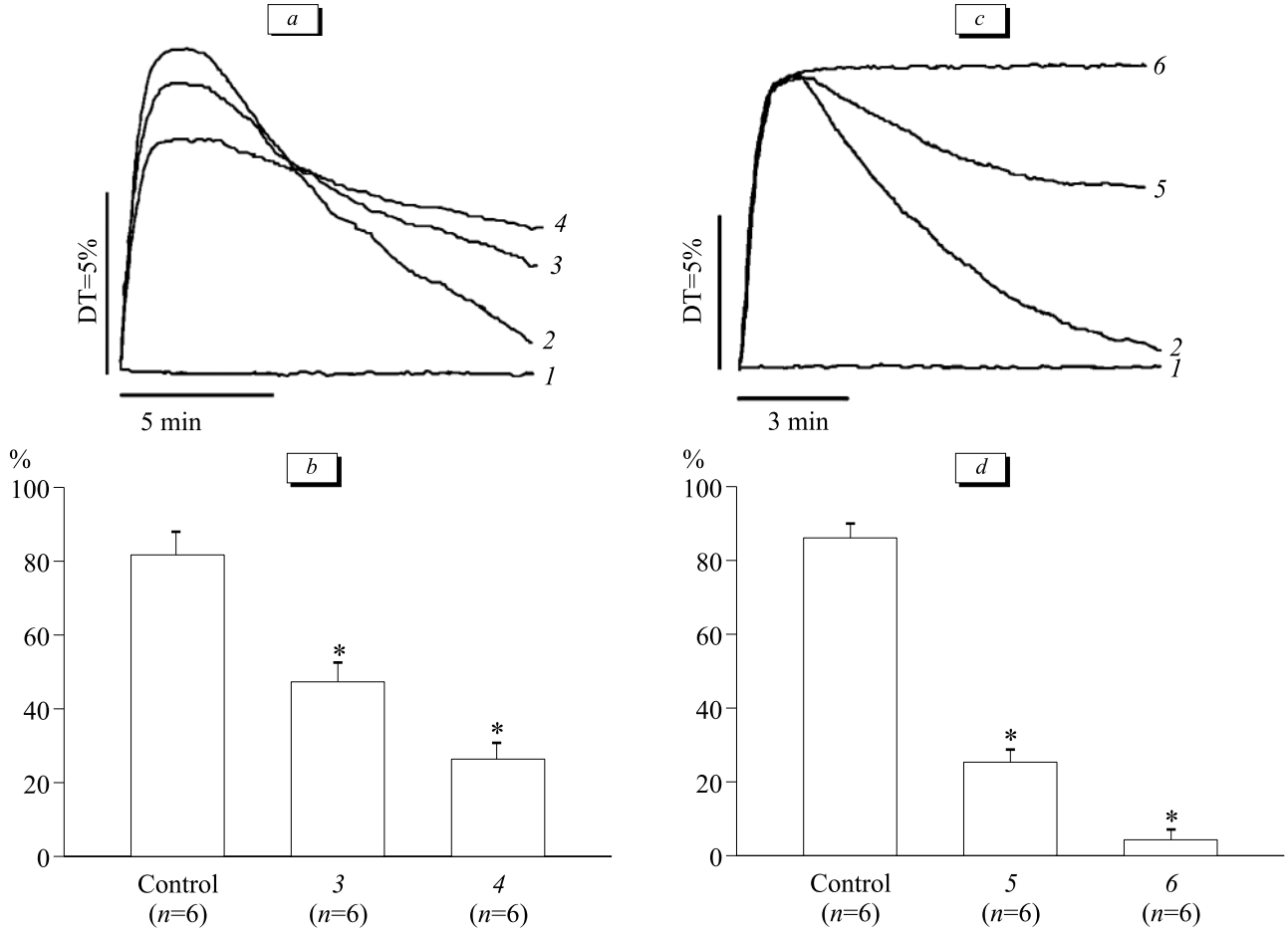

Fig 1. Effect of potassium channel blockers on thymocyte volume regulation. (*a*, *c*) Light transmission of the thymocyte suspension in an isotonic medium (*1*), control hypotonic medium (*2*), and hypotonic medium with barium ions (1 mM, *3*; 10 mM, *4*) and TEA ions (1 mM, *5*; 5 mM, *6*). Regulatory decrease in thymocyte volume after 15-min incubation in a hypotonic medium containing barium ions (*b*) and TEA ions (*d*). Here and in Fig. 2: *$p<0.05$ compared to the control. *n*, number of experiments.

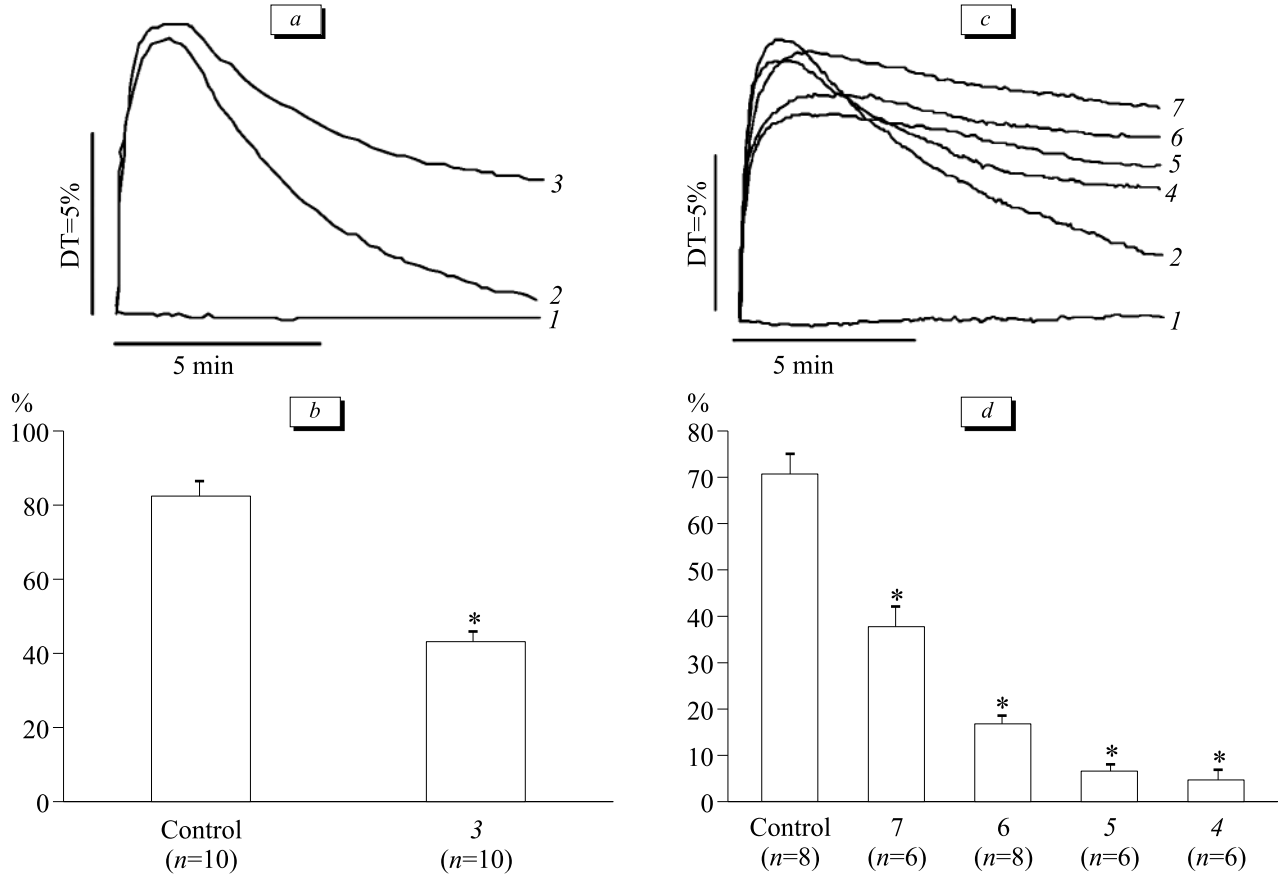

Fig. 2. Effect of an anion transport inhibitor SITS and chlorine channel blockers on thymocyte volume regulation. (*a*, *c*) Light transmission of the thymocyte suspension in an isotonic medium (*1*), control hypotonic medium (*2*), and hypotonic medium with 100 μM SITS (*3*), 100 μM $GdCl_3$ (*4*), 100 μM niflumic acid (*5*), 200 μM phloretin (*6*), and 200 μM glybenclamide (*7*). Regulatory decrease in thymocyte volume after 15-min incubation in a hypotonic medium containing SITS ions (*b*) and chlorine channel blockers (*d*).

tion completely blocks mechanosensitive nonselective cation channels and maxi-anion channels, which are expressed on T lymphocytes [8,11]. Our results indicate that high-amplitude Cl^- channels (maxi-anion channels) play a role in Cl^- transport through the membrane of osmotically swollen thymocytes.

Glybenclamide and phloretin surpassed gadolinium ions in effectiveness (Fig. 2, *c*, *d*). These inhibitors do not block the maxi-anion channel. However, they block the volume-sensitive outwardly rectifying Cl^- channel [4]. Probably, this channel plays an important role in the permeability of thymocytes for anions during hypotonic stimulation. As differentiated from glybenclamide, phloretin does not modulate cAMP-activated Cl^- channels. It can be hypothesized that this type of channels does not play a role in total anion permeability of osmotically stimulated thymocytes. Niflumic acid blocks various types of Cl^- channels, including Ca^{2+}-activated Cl^- channels and volume-sensitive outwardly rectifying Cl^- channel. This compound was most potent in inhibiting the regulation of thymocyte volume (Fig. 2, *c*, *d*).

Pharmacological study of thymocyte volume regulation in the hyposmotic medium showed that thymocytes (immature lymphocytes) have all three components of the volume regulatory system, including K^+ and Cl^- channels. Our results suggest that osmotic thymocyte swelling-activated Cl^- channels are presented by the Gd^{3+}-sensitive maxi-anion channel and volume-sensitive outwardly rectifying chlorine channel (inhibition with glybenclamide, phloretin, and niflumic acid). Both types of chlorine channels were revealed in our previous experiments with local current recording (patch clamp technique). Properties of these channels are, now studied.

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