Volume-Dependent Regulation of Ca^{2+}-Activated Potassium Channels in Erythrocytes from Healthy Donors and Patients with Type II Diabetes Mellitus Aggravated by Arterial Hypertension

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Increase in intracellular Ca^{2+} concentration caused by calcium ionophore A23187 or ascorbate+phenazine methosulphate electron donor system added to erythrocyte suspension induced similar shifts in erythrocyte membrane potential. These processes are most likely mediated by Ca^{2+}-activated potassium channels. Changes in the osmolarity of the incubation medium produced opposite effects on membrane hyperpolarization induced by A23187 or ascorbate+phenazine methosulphate in erythrocyte isolated from healthy donors, which attests to the existence of different mechanisms of regulation of Ca^{2+}-activated potassium channels. There was no difference in the volume-dependent changes of potassium permeability in cells from patients with type II diabetes mellitus combined with arterial hypertension induced by application A23187 or electron-donor system.

Key Words: *erythrocytes; Ca^{2+}-activated potassium channels*; *type II diabetes mellitus*; *redox agents*

Ca^{2+}-activated potassium channels are regulated via different pathways. Ca^{2+}-activated potassium permeability depends on the state of cytoskeleton proteins, in particular, spectrin [4]. It is known that Ca^{2+}-activated potassium channels participate in processes modifying erythrocyte shape [3]. In addition, activity of this ion-transporting system can be controlled by redox agents such as ascorbate+phenazine methosulphate (PMS) artificial electron donor system [1,2,5].

Patients with type II diabetes mellitus (DM II) demonstrate pronounced changes in morphofunctional status of erythrocytes: increased intracellular Ca^{2+} concentration, disturbed metabolism of redox agents (glutathione, ascorbate, NADH, and NADPH), and defects of cytoskeleton proteins [6]. These changes can modify Ca^{2+}-activated potassium permeability of the erythrocyte membrane.

Our aim was to compare volume-dependent mechanisms of regulation of Ca^{2+}-activated potassium channels in erythrocytes of healthy donors and patients with DM II associated with arterial hypertension (AH).

MATERIALS AND METHODS

Erythrocytes of healthy donors (n=13) and patients with DM II+AH (n=12) were used to study membrane hyperpolarization of erythrocytes induced by application of A23187 calcium ionophore. The experiments with ascorbate+phenazine methosulphate artificial electron donor system were performed on erythrocytes of healthy volunteers (n=7) and patients with DM II+AH

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(*n*=7). The examined groups were sex- and age-matched.

Preparation of erythrocyte suspension and recording of membrane potential by the changes in pH of incubation medium in the presence of protonophore carbonyl cyanide m-chlorophenylhydrazone (ClCCP) were described elsewhere [3,4].

Recording of pH was performed with a combined pH-sensitive electrode HI 1332 (HANNA Instruments) and a TYP N517 pH-meter.

Erythrocyte hyperpolarization (ΔE) was used as an integral characteristic of the state of Ca^{2+}-dependent K^+ channels. It was calculated according to the following formula:

$$\Delta E = RT/F\ (pH_1 - pH_2),$$

where pH_1 and pH_2 are pH of incubation medium before and after application of calcium ionophore, respectively [3]. To induce membrane hyperpolarization, packed erythrocytes (0.25 ml) were added to 4.75 ml incubation medium (150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, and 10 μM $CaCl_2$). The suspension was incubated for 5 min at 37°C under continuous stirring. Then protonophore ClCCP was added to a final concentration of 20 μM, and Ca^{2+}-ionophore A23187 was added 2 min later in a concentration of 0.5 μM [3,4]. Redox-induced hyperpolarization of erythrocyte membrane was induced by consecutive addition of ascorbate (10 mM), ClCCP (20 μM, after 5 min), and PMS (0.1 mM, after 2 min) [2]. At the end of experiment, Triton X-100 was added to erythrocyte suspension to a final concentration of 0.2% in order to determine intracellular pH.

Changes in erythrocyte volume were caused by varying osmolarity of the incubation medium. Addition of sucrose in corresponding concentrations to the isosmotic solution (320 arb. units) induced cell shrinkage in hyperosmotic medium (420 and 520 arb. units). When concentration of NaCl was decreased (hyposmotic medium, 200 arb. units) erythrocyte volume increased.

The data were analyzed statistically using Statistics 5.0 software for independent and nonindependent groups.

RESULTS

Addition of calcium ionophore A23187 or artificial electron donor system ascorbate+PMS to isosmotic medium produced similar changes in membrane potential of erythrocytes from healthy donors and patients with DM II+AH (Fig. 1). Under these conditions, the amplitudes of hyperpolarization response in healthy donors were -38.04±0.62 mV and -37.28±1.44 mV, respectively. In patients the corresponding values were -33.62±1.13 mV and -39.77±3.55 mV.

Calcium ionophore A23187 increases intracellular Ca^{2+} concentration and induced opening of Ca^{2+}-activated potassium channels. These changes led to a release of K^+ ions from cells and hyperpolarization of erythrocyte membrane [3].

The ascorbate+PMS system is an artificial conveyor for electron transport, which induces Ca^{2+}-dependent potassium permeability by increasing affinity of Ca^{2+}-activated K^+ channels to Ca^{2+} ions [2,5]. Various reducing agents, including ascorbate, NADH, NADPH, and HS-glutathione increase affinity of Ca^{2+}-activated K^+ channels to Ca^{2+} ions in ghost erythrocytes. However, in the inside-out vesicles these agents exert their action only in the presence of PMS, because PMS and cytochrome C can bind to the membrane redox system as efficient electron carriers [5]. Indeed, in the experiments with separate addition of ascorbate or PMS to the incubation medium we observed no hyperpolarization response.

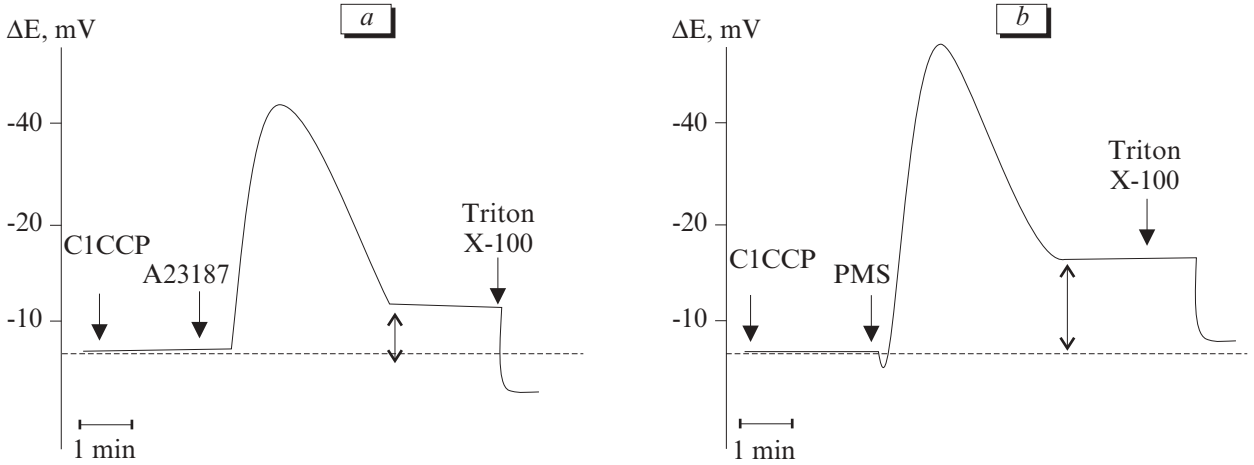

Fig. 1. Effect of calcium ionophore A23187 (*a*) or electron donor system ascorbate+phenazine methosulphate (PMS, *b*) on erythrocyte membrane potential.

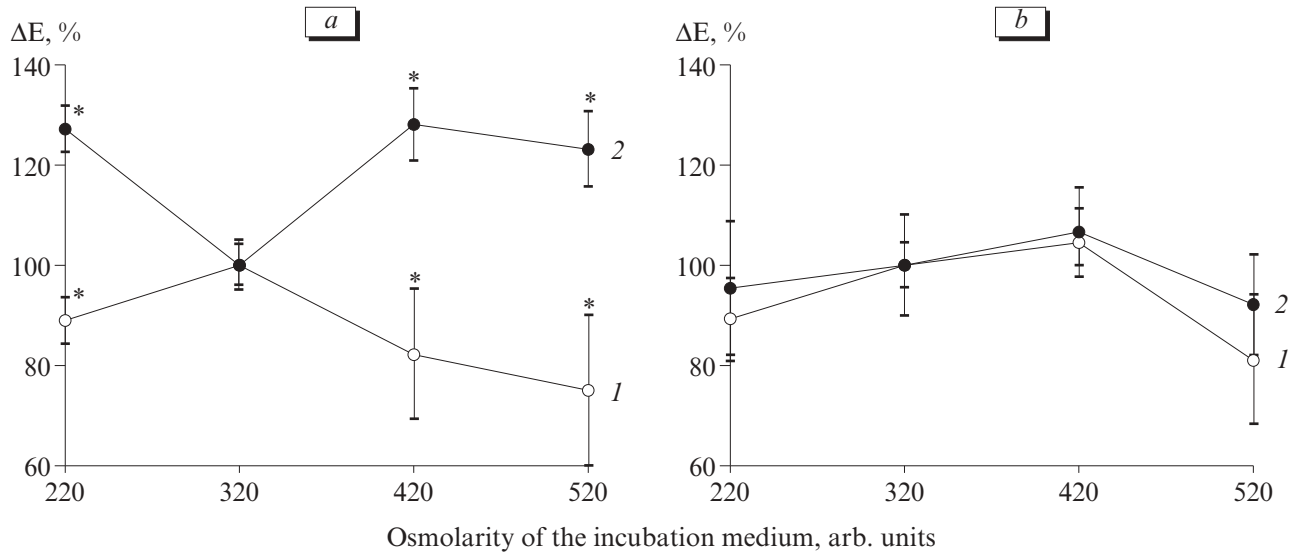

Fig. 2. Effect of osmolarity of incubation medium on hyperpolarization response of erythrocytes induced by calcium ionophore A23187 (*1*) or electron donor system ascorbate+phenazine methosulphate (PMS, *2*) in healthy donors (*a*) and in patients with type II diabetes mellitus aggravated by arterial hypertension (*b*); 100% corresponds to values recorded in isosmotic medium (320 arb. units). **p*<0.01 compared to isosmotic conditions.

Both the increase and decrease in osmolarity of the incubation medium with erythrocytes from healthy donors or patients with DM II+AH decreased hyperpolarization induced by calcium ionophore A23187. By contrast, hyperpolarization induced under similar conditions in ascorbate+PMS system was more pronounced (Fig. 2).

These data attest to the existence of two mechanisms regulating potassium permeability of the erythrocyte membrane: Ca^{2+}-dependent and redox-mediated. It cannot be excluded that these regulatory mechanisms in erythrocytes play a certain role for these cells. It is known that the release of K^+ from cells is accompanied by the loss of water. It can be hypothesized that swelling of erythrocytes *in vivo* is accompanied by activation of redox-mediated pathway of regulation of Ca^{2+}-activated K^+ channels, which elevates potassium permeability and restores cell volume. By contrast, in shrank erythrocytes Ca^{2+}-mediated pathway prevails, which decreases potassium permeability of the erythrocyte membrane. Thus, the increase and decrease of potassium permeability in erythrocyte membrane induced by different mechanisms can be considered as one of the pathways to stabilize cell volume during disturbances in osmolarity of the outer medium.

The described variations of potassium permeability were not observed in erythrocytes of patients with DM II+AH: when induced by calcium ionophore A23187 or redox agents, potassium permeability did not significantly change during cell swelling or shrinkage. This fact probably attest to some disturbances in the regulation of Ca^{2+}-activated K^+ channels in erythrocyte membrane in these patients. The causes of this phenomenon can be the changes in metabolism of NADH, NADPH, glutathione, and ascorbate due to the development of oxidative stress or certain disturbances in the state of cytoskeleton proteins observed in patients with DM II+AH [6].

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