Effect of Insulin on Ca^{2+}-Dependent Hyperpolarization in Erythrocytes from Healthy Donors and Patients with Type 2 Diabetes Mellitus Accompanied by Arterial Hypertension

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Insulin decreased A23187-induced hyperpolarization of the erythrocyte membrane in healthy donors. These data indicate that insulin plays a role in the regulation of Ca^{2+}-activated potassium channels in human erythrocytes. However, insulin had little effect on hyperpolarization response of cells induced by artificial ascorbate—phenazine methosulfate donor-acceptor system. Addition of insulin to cell suspension from patients with type 2 diabetes mellitus did not modulate hyperpolarization of the erythrocyte membrane induced by A23187 or ascorbate-phenazine methosulfate, which reflects impairment of regulatory mechanisms for Ca^{2+}-activated potassium channels in erythrocytes.

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

Insulin regulates various physiologically important functions of cells in insulin-dependent tissues, including the rate of glucose utilization, intracellular metabolism of glucose, and transcriptional and translational synthesis of proteins [7,14]. Insulin also modulates the permeability of cells to ions [8,11]. The development of insulin resistance is a major stage in the pathogenesis of diabetes mellitus (DM), arterial hypertension (AH), and other associated diseases [1,12,15].

It was hypothesized that apart from calcium ions, redox agents are involved in the regulation of Ca^{2+}-activated potassium channels in erythrocytes. These compounds probably have a specific site on the cell membrane [2,3,9].

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Here we studied the effect of insulin on erythrocyte membrane permeability to potassium ions induced by calcium ionophore A23187 and redox system of ascorbate and phenazine methosulfate (PMS). We examined healthy donors and patients with type 2 DM (DM2) accompanied by AH.

MATERIALS AND METHODS

Erythrocytes were isolated from 6 patients with DM2 and AH and 6 healthy donors to study the hyperpolarization response (HR) of cells. These groups were comparable by sex and age.

The cells were isolated and erythrocyte membrane potential was determined by changes in medium pH in the presence of protonophore carbonyl cyanide-m-chlorophenyl hydrazone (ClCCP) [5,6]. pH was measured using a HI 1332 combination pH-sensitive electrode (HANNA Instruments) and

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TYP N517 pH-meter. The amplitude of erythrocyte hyperpolarization (ΔE) was calculated as follows:

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

where pH_1 is the initial pH value of the erythrocyte incubation medium; and pH_2 is pH of the incubation medium during erythrocyte hyperpolarization. This parameter served as an integral criterion for function of intracellular Ca^{2+}-dependent potassium channels [5].

HR of erythrocytes was initiated by adding 0.25 ml packed erythrocytes to 4.75 ml isosmotic incubation medium (320 mosm) containing 150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, and 10 μM calcium. Incubation was performed at 37°C and constant agitation for 5 min. The protonophore ClCCP was added to a final concentration of 20 μM. Calcium ionophore A23187 (0.5 μM) was added after 2 min. Redox-induced hyperpolarization of the erythrocyte membrane was induced by consecutive addition of 10 mM ascorbate, 20 μM ClCCP (after 5-min incubation), and 0.1 mM PMS (after 2-min incubation) to the incubation medium.

To study the effect of insulin on membrane permeability to potassium ions, erythrocytes were incubated in the presence of 0.3 nM insulin.

The significance of intergroup differences was estimated by statistical analyses for dependent and independent groups (Statistics 5.0 software).

RESULTS

Our previous studies showed that addition of calcium ionophore A23187 or artificial ascorbate—PMS donor-acceptor system to the incubation medium produced similar changes in the membrane potential of human erythrocytes [4].

The amplitude of A23187-induced HR in erythrocytes from healthy donors in the absence of insulin was -27.59±2.69 mV. Incubation of cells with insulin for 1 and 5 min decreased this parameter to -19.72±3.39 ($p<0.05$) and -23.87±1.79 mV, respectively.

The amplitude of redox-induced HR in erythrocytes from healthy donors in the absence of insulin was -29.19±1.41 mV. Incubation of cells with insulin for 1 and 5 min had little effect on this parameter (-29.00±1.34 and -29.87±71.20 mV, respectively).

Our results indicate that insulin decreased only the amplitude of A23187-induced HR in erythrocytes from healthy donors. Previous studies showed that charibdotoxin and clotrimazole (blockers of Ca^{2+}-activated potassium channels in erythrocytes) abolished membrane hyperpolarization induced by addition of the calcium ionophore A23187 to the cell suspension. It can be hypothesized that insulin plays a role in the regulation of Ca^{2+}-activated potassium channels in erythrocytes.

Changes in Ca^{2+}-dependent permeability of the erythrocyte membrane to potassium ions was observed within one or several minutes after treatment with insulin. It can be attributed to rapid extragenomic effects of insulin manifested in a change in cell membrane permeability to ions [7]. Previous studies showed that insulin increases the volume of hepatocytes due to activation of Na^+/H^+ exchange and $Na^+/K^+/2Cl^-$ cotransport [8,11]. Preincubation of erythrocytes from healthy donors with insulin for 1 min induced swelling of these cells due to activation of Na^+/H^+ exchange. It was accompanied by a decrease in the function of Ca^{2+}-activated potassium channels. This assumption is confirmed by published data that the increase in erythrocyte volume decreases Ca^{2+}-dependent permeability of the cell membrane to potassium ions [4]. It cannot be excluded that insulin initially causes a decrease in Ca^{2+}-dependent permeability of the erythrocyte membrane to potassium ions, which contributes to cell swelling. The cell volume rapidly recovered (within 1 min) due to ion transport [13]. These data explain the absence of changes after 5-min incubation of erythrocytes with insulin. Previous experiments with contraction and expansion of erythrocytes in anisosmotic solutions showed that variations in the cell volume occur over a period of less than 5 min. The cell volume remains unchanged in the follow-up period (30 min) [13].

Insulin had no effect on the redox-induced erythrocyte membrane permeability to potassium ions. These data suggest that regulation of Ca^{2+}-activated potassium channels in erythrocytes over a specified period also involves the hormone-insensitive mechanism. It cannot be excluded that increasing the period of cell incubation with insulin to tens minutes or hours will be accompanied by changes associated with the effect of this hormone on metabolism and redox state of cells.

Different effects on A23187- and redox-induced HR of erythrocytes indirectly confirm the hypothesis that there are at least 2 regulatory mechanisms for the function of Ca^{2+}-activated potassium channels in erythrocytes (Ca^{2+}-dependent and redox-dependent mechanisms) [4]. This hypothesis is based on the data that osmolarity of the incubation medium has an opposite effect on A23187- and redox-induced HR of erythrocytes.

The amplitude of redox-induced HR in erythrocytes from patients with DM2 and AH in the absence of insulin was -32.29±1.64 mV. Preincubation of cells with insulin for 1 and 5 min had little

effect on the amplitude of HR in erythrocytes (-27.02±2.28 and -29.21±1.44 mV, respectively).

The amplitude of redox-induced HR in cells from patients with DM2 and AH in the absence of insulin was -35.19±1.73 mV. After incubation of cells with insulin for 1 and 5 min, this parameter corresponded to -30.62±2.38 and -31.90±1.24 mV, respectively.

In patients with DM2 and AH, insulin had no effect on erythrocyte membrane permeability to potassium ions. It could be associated with impaired binding of this hormone to surface receptors on the erythrocyte membrane. The syndrome of insulin resistance is accompanied by a decrease in the number of receptors or reduction of affinity for insulin, which decreases hormone binding and receptor phosphorylation [10]. Moreover, this disease can be accompanied by the impairment of ion mechanisms for regulation of the cell volume.

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