Role of Cell Signaling Systems in the Regulation of Ca^{2+}-Activated Potassium Channels in Erythrocytes

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The effect of inhibitors of calmodulin and protein kinase C, complex $[AlF_4]^-$, and potassium channel blockers on the parameters of calcium-induced hyperpolarization in human erythrocytes is studied by recording proton distribution between the cytoplasm and incubation medium in the presence of a protonophore. It is shown that the calmodulin inhibitor dis-C_3-5, the protein kinase C inhibitor staurosporine, and the blocker of high-conductance calcium-activated potassium channels considerably suppress the calcium-induced hyperpolarization in erythrocytes. High concentration of $[AlF_4]^-$ in the incubation medium inhibits hyperpolarization.

Key Words: *erythrocytes; calcium-activated potassium channels*

It has been established in the early 1970s that Gardosh effect, i.e., potassium leakage from ATP-depleted erythrocytes, results from opening of calcium-dependent potassium channels — K(Ca)-channels [8]. High-selective K(Ca) channels were then found in nerve endings, smooth and skeletal muscles, and different types of the epithelial tissue [1]. In some cases high- and low-conductance K(Ca) channels can be identified [10]. The first type includes K(Ca) channels of electroexcitable and epithelial cells, the second group consists of erythrocyte K(Ca) channel. It has been shown that high-conductance K(Ca) channels are sensitive to charybdotoxin [7,11] and low-conductance channels can be blocked by apamin [6].

Since direct study of low-conductance channels by patch-clamp technique is difficult due to their weak dependence on the membrane potential, indirect methods are of great interest. One of such approaches is the monitoring of the membrane potential in erythrocytes from pH changes in the medium in the presence of a protonophore [3].

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Previous studies have showed that erythrocyte K(Ca) channels are regulated by cell backbone proteins [4,12] and calmodulin [2]; however, the role of protein kinases in this regulation is poorly understood.

GTP-binding proteins (G-proteins) coupling external stimuli and cell effector systems are a key element of cell regulation systems. The role of G-proteins in the regulation of Ca-activating potassium conductivity in the erythrocyte membrane has not been established.

The aim of the present study was to examine the effect of potassium channel blockers, calmodulin and protein kinase C inhibitors, and $[AlF_4]^-$ complex on calcium-induced hyperpolarization in erythrocytes.

MATERIALS AND METHODS

The following reagents were used: glucose and sucrose (Reakhim), carbonyl-m-chlorophenylhydrazone, dis-C_3-5 (diisopropylthiodicarbocyanine iodide), A23187, staurosporine, apamin, and charybdotoxin (Calbiochem).

Erythrocytes were isolated from fresh donor blood and blood from outbred rats as described previously [3]. The dynamics of erythrocyte membrane potential was recorded by the method [9] with our modifications [3].

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In the presence of a protonophore proton distribution depends on the membrane potential (E_m): $E_m = RT/F(pH_i - pH_o)$, where pH_i and pH_o are the pH of cytoplasm and incubation medium, respectively. When the incubation medium has low buffer capacity (in our experiments it was 100-fold lower than buffer capacity of the cytoplasm), changes in pH_i can be ignored and its quasistationary level can be determined by cell hemolysis in the presence of a detergent.

The experiments were performed according to the following scheme. Packed erythrocytes (100 μl) were added to 1.9 ml incubation medium containing 150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 50 μM $CaCl_2$, and 10 mM glucose and after a 5- or 15-min incubation at 37°C and constant stirring, 20 μM protonophore was introduced into the suspension. Two minutes later 3 μM A23187 was added and, finally, Triton X-100 (0.2%) was added for determination of intracellular pH.

pH was recorded using an S904 electrode (Beckman) and a pH-121 pH-meter (Russia).

The amplitude of hyperpolarization (ΔE, mV) and the rates of hyperpolarization (V_1, mmol OH/liter×min) and restoration of the membrane potential (V_2, mmol/liter×min) were determined as described previously [3].

RESULTS

The use of specific blockers is an experimental approach for studying structural and functional features of ionic channels.

We used tetraethylammonium (TEA), charybdotoxin, and apamin, blockers of low- and high-conductance K(Ca) channels, respectively.

It was shown that K(Ca) channels in human erythrocytes are insensitive to the high-conductance channel blocker TEA and the low-conductance channel blocker apamin. In rat erythrocytes apamin (10 nM) markedly reduced the amplitude of hyperpolarization (Fig. 1).

Charybdotoxin, a blocker of high-conductance K(Ca) channel, effectively suppressed hyperpolarization in both human and rat erythrocytes. This effect was dose-dependent (Fig. 2). The concentration-response curves for the amplitude of hyperpolarization were S-shaped, which can be attributed to cooperative binding of the inhibitor to the channels.

These experiments demonstrated some peculiarities of erythrocyte K(Ca) channels: they are low-conductance channels that can be inhibited by the high-conductance channel blocker charybdotoxin. K(Ca) channels in rat erythrocytes are sensitive to both apamin and charybdotoxin.

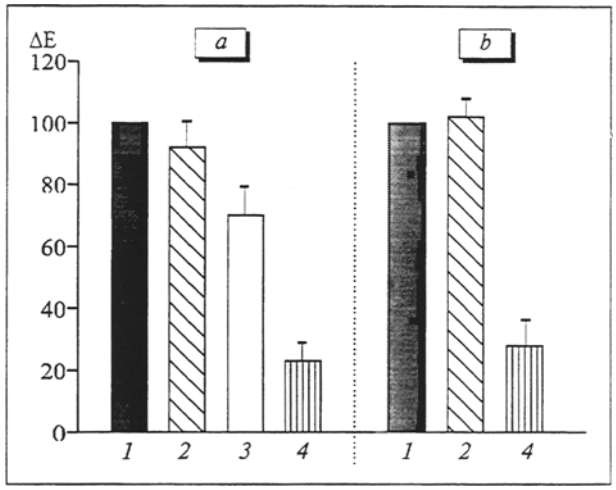

Fig. 1. Effect of K(Ca) channel blockers on amplitude of Ca^{2+}-induced hyperpolarization in rat (*a*) and human (*b*) erythrocytes. *1*) control, *2*) 20 mM TEA, *3*) 10 nM apamin, *4*) 10 nM charybdotoxin.

The presence of Ca-binding proteins is the common structural feature of K(Ca) channels [13]. The role of these proteins in the functioning of K(Ca) channels can be elucidated by using calmodulin inhibitors, for instance dis-C_3-5. It has been shown that dis-C_3-5 binds to the Ca-calmodulin complex and prevents its interaction with effector proteins [5]. The addition of dis-C_3-5 to the incubation medium lowers all parameters of Ca-induced hyperpolarization.

These data confirm the assumption that calmodulin-like Ca-binding protein is a structural or regulatory component of K(Ca) channels in the erythrocyte membrane.

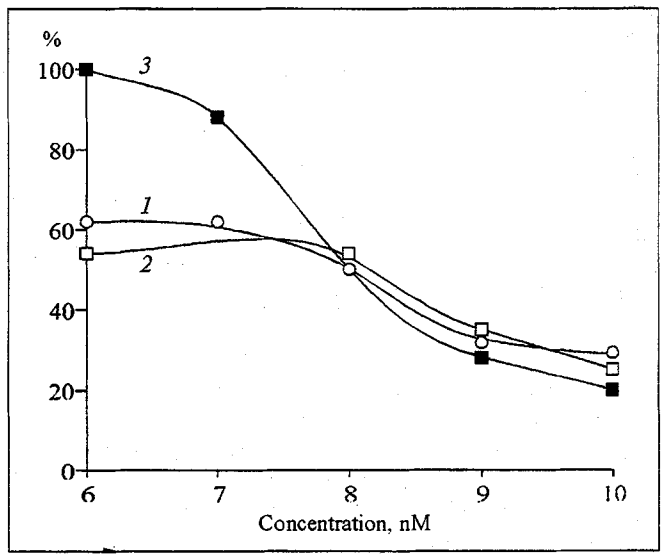

Fig. 2. Ca^{2+}-induced hyperpolarization in human erythrocytes as a function of charybdotoxin concentration. *1*) amplitude, *2*) rate of restoration of membrane potential, *3*) rate of hyperpolarization development.

Activation of G_i-like GTP-binding regulatory proteins can be achieved with $[AlF_4]^-$ complex. Our experiments showed that the addition of $[AlF_4]^-$ in the incubation medium depressed the amplitude and attenuated the development of hyperpolarization and restoration of the membrane potential.

Thus, it can be concluded that GTP-binding proteins are involved into the regulation of K(Ca) channels in erythrocytes.

The data on the participation of protein kinase C in the regulation of K(Ca) channels are contradictory. In our experiments, staurosporine, a protein kinase C inhibitor, suppressed the Ca-induced hyperpolarization of cell membrane, which attested to the involvement of protein kinase C into the regulation of K(Ca) channels in erythrocytes.

Thus, our experiments showed that the regulation of calcium-activated potassium channels in erythrocytes is mediated through GTP-binding proteins and protein kinase C.

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