Voltage Dependence of the Ca$^{2+}$-Activated K$^+$ Conductance of Human Red Cell Membranes is Strongly Dependent on the Extracellular K$^+$ Concentration

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Summary. The conductance of the Ca$^{2+}$-activated K$^+$ channel ($g_{K(Ca)}$) of the human red cell membrane was studied as a function of membrane potential ($V_m$) and extracellular K$^+$ concentration ([K$^+$]$_e$). ATP-depleted cells, with fixed values of cellular K$^+$ (145 mM) and pH (~7.1), and preloaded with ~27 µM ionized Ca$^{2+}$ were transferred, with open K$^+$ channels, to buffer-free salt solutions with given K$^+$ concentrations. Outward-current conductances were calculated from initial net effluxes of K$^+$, corresponding $V_m$, monitored by CCCP-mediated electrochemical equilibration of protons between a buffer-free extracellular and the heavily buffered cellular phases, and Nernst equilibrium potentials of K ions ($E_K$) determined at the peak of hyperpolarization. Zero-current conductances were calculated from unidirectional effluxes of $^{36}$K at ($V_m - E_K$) = 0, using a single-file flux ratio exponent of 2.7. Within a [K$^+$]$_e$ range of 5.5 to 60 mM and at ($V_m - E_K$) = 20 mV a basic conductance, which was independent of [K$^+$]$_e$, was found. It had a small voltage dependence, varying linearly from 45 to 70 µS/cm$^2$ between 0 and -100 mV. As ($V_m - E_K$) decreased from 20 towards zero mV $g_{K(Ca)}$ increased hyperbolically from the basic value towards a zero-current value of 165 µS/cm$^2$. The zero-current conductance was not significantly dependent on [K$^+$]$_e$ (30 to 156 mM) corresponding to $V_m$ (~50 mV to 0). A further increase in $g_{K(Ca)}$ symmetrically around $E_K$ was suggested as ($V_m - E_K$) becomes positive. Increasing the extracellular K$^+$ concentration from zero and up to ~3 mM resulted in an increase in $g_{K(Ca)}$ from ~50 to ~70 µS/cm$^2$. Since the driving force ($V_m - E_K$) was larger than 20 mV within this range of [K$^+$]$_e$, this was probably a specific K$^+$ activation of $g_{K(Ca)}$. In conclusion: The Ca$^{2+}$-activated K$^+$ channel of the human red cell membrane is an inward rectifier showing the characteristic voltage dependence of this type of channel.

Key Words  
Ca$^{2+}$-activated K$^+$ channel · human erythrocytes · voltage dependence · function of [K$^+$]$_e$ · inward rectification

Introduction

During the past few years strong support has developed for the view that the Ca$^{2+}$-activated conductance of the human red cell membrane takes place via a K$^+$-specific, Ca$^{2+}$-activated ion channel. Applying the patch-clamp technique, Hamill (1981) showed that the currents across patches of Ca$^{2+}$-activated human red cell membranes occurred in discrete steps of unitary amplitude, indicating the existence of a K$^+$-specific ion channel with a single-channel conductance ($\gamma$) of ~18 pS (100 mM K$^+$, symmetrically, 19°C). In a later patch-clamp study of the human red cell membrane, Grygorczyk and Schwarz (1983) confirmed a γ value of ~20 pS (150 mM K$^+$, symmetrically, 20 to 22°C) and in addition they demonstrated that the channel showed inward rectification. The inward rectification of the K$^+$ channel of the human red cell membrane observed by Grygorczyk and Schwarz (1983) and later confirmed (Grygorczyk, Schwarz & Passow, 1984) is, however, not as pronounced as that of the inward rectifier of striated muscle membrane (Hodgkin & Horowicz, 1959) or a starfish egg cell membrane (Hagiwara & Takahashi, 1974).

The concept of a K$^+$-specific ion channel as the Ca$^{2+}$-activated conductance pathway received independent support by the demonstration that the unidirectional K$^+$ fluxes across the membranes of Ca$^{2+}$-activated human red cells did not obey the flux-ratio equation of Ussing (1949). Under net efflux conditions the ratio between the unidirectional K$^+$ fluxes followed the equation of Hodgkin and Keynes (1955) with a flux ratio exponent of ~2.7, and the conductance pathway thus exhibited single-file diffusion characteristics (Vestergaard-Bogind, Stampe & Christophersen, 1985a). The single-file exponent was found to be independent of the extracellular K$^+$ concentration within the range of 1 to 17 mM and probably up to 156 mM, independent of $V_m$ and independent of the K$^+$ conductance. Thus in terms of the single-file model the channel does not resemble the inward rectifier of striated muscle membranes, where the exponent was found to be a function of the extracellular K$^+$ concentration (Horowicz, Gage & Eisenberg, 1968; Spalding et al., 1981).

A complex relationship between the rate con-
constant of the Ca\(^{2+}\)-activated net efflux of K ions from human red cells or ghosts and the extracellular concentration of K\(^+\) and H\(^+\) has been reported by Hoffman and co-workers (for a review see Hoffman et al., 1980) and Passow and co-workers (for a review see Schwarz & Passow, 1983).

The conductance pathway being a K\(^+\)-selective ion channel showing single-filing and inward rectification, the relationships between net or unidirectional fluxes and the membrane potential, extracellular K\(^+\) concentration, etc., are not described by the constant-field regime and rate constants cannot easily be related to the K\(^+\) conductance of the membrane. In the present paper we attempt to characterize in detail \(g_K(Ca)\) at fixed values of cellular concentrations of K\(^+\) (145 mm), and ionized Ca (\(-27 \mu M\)) as a function of the extracellular concentration of K\(^+\) and the membrane potential. Since the Ca\(^{2+}\)-activated K\(^+\) conductance varies markedly with cellular pH (Stampe & Vestergaard-Bogind, 1985), all experiments were made at a cellular pH value of \(\approx 7.1\).

Materials and Methods

CHEMICALS

All inorganic salts (pro analysis) were purchased from Merck. Trizma base \(^1\) and CCCP were from Sigma. The ionophore A23187 was from Calbiochem. DIDS was from Pierce Chemical Co. Di-n-butylphthalate and sucrose (Aristar) were from BDH. The test-combination for determination of hemoglobin was from Boehringer.

CELLS

Freshly drawn blood from healthy human donors was heparinized and centrifuged. Plasma and theuffy coat were aspirated, and the cells were washed twice in 5 vol high-K salt solution (90 mm KCl/66 mm NaCl/150 mm MgCl\(_2\), pH \(\approx 7.4\)) containing 50 \(\mu M\) EGTA. The cells were depleted of ATP and 2,3-diphosphoglycerate as previously described (Vestergaard-Bogind & Stampe, 1984), and washed three times in high-K salt solution.

The cells were then suspended at a cytocrit of \(\approx 20\%\) in high-K salt solution containing in addition 500 \(\mu M\) CaCl\(_2\) (with \(\text{Ca}^{4+}\)). Ionophore A23187 was added to a concentration of 0.5 \(\mu M\) per liter of cells. After 20 to 40 min of incubation at 25°C (see below) the cell suspension was centrifuged and the cells were washed once in 10 vol of high-K salt solution containing 5 \(\mu M\) of EGTA. By this incubation of ATP-depleted cells in the presence of A23187 and Ca the cells were loaded with Ca. The cellular content of Ca was determined from the cellular content of \(\text{Ca}^{4+}\), and the concentration of ionized Ca in the intracellular phase was then taken as the total content of Ca per liter cells times 0.2 (cf. Stampe & Vestergaard-Bogind, 1985).

In our experience, ionophore A23187 once added to the cells cannot be washed out. In the presence of the very low concentration of ionophore A23187 used, even more than 1 hr of incubation did not result in an equilibrium distribution of Ca\(^{2+}\) between cells and medium. A predetermined cellular concentration of ionized Ca was therefore obtained in the following way. Samples of the incubation medium were taken at intervals of \(-5\) min and the cellular content of \(\text{Ca}^{4+}\) was determined by the phthala
tate method (see later). The values obtained were plotted against time, and the time necessary to obtain the chosen cellular concentration of ionized Ca (\(-27 \mu M\)) was found by linear extrapolation. The very low concentration of ionophore was used in order to avoid ionophore-mediated changes in the cellular concentration of ionized Ca during the experimental period. In the experimental series the CCCP-mediated electrochemical equilibration of protons established proton concentration gradients across the cell membranes from 0 up to 1.5 units of pH as a result of the various degrees of hyperpolarization of the membranes. The corresponding variation in ionophore A23187-mediated equilibrium distribution ratio of ionized Ca across the cell membranes would be from 1 to 1000 (Vestergaard-Bogind & Stampe, 1984), and it would be almost impossible to adjust the Ca\(^{2+}\) activity of the various extracellular phases so that Ca\(^{2+}\) would always be in equilibrium. In all experiments the extracellular phases were free of added Ca\(^{2+}\) and 5 \(\mu M\) EGTA was present to neutralize the Ca\(^{2+}\)-contamination from the various chemicals.

PHTHALATE METHOD

Cellular contents of K\(^+\), Na\(^+\), \(^{4}K\) and \(^{4}Ca\) and extracellular concentrations of K\(^+\) were determined by the phthalate method as previously described (Vestergaard-Bogind et al., 1985a).

During an experiment 100-\(\mu l\) samples of the cell suspension (hematocrit \(\approx 3.1\%\)) were transferred to the cold phthalate tubes, and 5 sec later the tubes were centrifuged for 30 sec at 18,000 \(\times\) g. The \(-3.1\) \(\mu l\) of cells were now isolated as a pellet under the phthalate layer. The extracellular concentration of K\(^+\) was determined by flame photometry on the top phases of the phthalate tubes. The rest of the top phase and the phthalate were removed and the cell pellets were processed for scintillation counting of the \(^{4}Ca\) or \(^{4}K\) content and flame photometric determination of the K\(^+\) and Na\(^+\) contents.

MEMBRANE POTENTIAL

Changes in membrane potential (\(V_m\)) were determined according to the method of Macey, Adorante and Orme (1978). The experiments were carried out with cells suspended in buffer-free salt solution at a hematocrit of 3.1% in the presence of 20 \(\mu M\) of the protonophore CCCP, which mediates a fast electrochemical equilibration of protons across the cell membranes. Since the intracellular phase is heavily buffered, a change in \(V_m\) results in a shift in the extracellular pH to a new equilibrium value, determined by the constant intracellular proton activity and the membrane potential. At the end of an experiment Triton X-100 was added, resulting in immediate hemolysis of all cells. Since all buffering capacity was confined to the cellular phase, the pH of the hemolysate reflected the original cellular pH which remained constant during the experiment. Absolute \(V_m\) values were then

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\(^1\) Abbreviations: CCCP, carbonyl cyanide \(m\)-chlorophenylhydrazone; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; Trizma base, Tris (hydroxymethyl) aminoethane; EGTA, ethylene glycol bis (\(\beta\)-aminoethyl ether)-\(N,N'\)-tetraacetic acid.