J. Membrane Biol. 101, 165–172 (1988)

The Journal of
Membrane Biology

Ca^{2+}-Activated K^+ Conductance of Human Red Cell Membranes Exhibits Two Different Types of Voltage Dependence

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Summary. The voltage dependence for outward-going current of the Ca-activated K^+ conductance ($g_K(Ca)$) of the human red cell membrane has been examined over a wide range of membrane potentials (V_m) at constant values of $[K^+]_{ex}$, $[K^+]_i$ and pH_i, the intact cells being preloaded to different concentrations of ionized calcium. Outward-current conductances were calculated from initial net effluxes of K^+ and the corresponding ($V_m - E_K$) values. The basic conductance, defined as the outward-current conductance at ($V_m - E_K$) ≥ 20 mV and $[K^+]_{ex}$ ≥ 3 mM (B. Vestergaard-Bogind, P. Stampe and P. Christophersen, *J. Membrane Biol.* **95**:121–130, 1987) was found to be a function of cellular ionized Ca. At all degrees of Ca activation $g_K(Ca)$ was an apparently linear function of voltage (V_m range −40 to +70 mV), the absolute level as well as the slope decreasing with decreasing activation. In a simple two-state model the constant voltage dependence can, at the different degrees of Ca activation, be accounted for by a Boltzmann-type equilibrium function with an equivalent valence of ~0.4, assuming chemical equilibrium at V_m = 0 mV. Alternatively, the phenomenon might be explained by a voltage-dependent block of the outward current by an intracellular ion. Superimposed upon the basic conductance is the apparently independent inward-rectifying steep voltage function with an equivalent valence of ~5 and chemical equilibrium at the given E_K value.

Key Words Ca^{2+}-activated K^+ conductance · human red cell membrane · function of cellular calcium · voltage dependence

Introduction

The Ca^{2+}-activated K^+ channel of the human red cell membrane has been shown to exhibit a moderate inward rectification of the single-channel conductance (Grygorczyk & Schwarz, 1983). Recently, it was demonstrated that, in addition, an inward rectification in the proper sense (*cf.* Hille, 1984) of the Ca^{2+}-activated conductance, $g_K(Ca)$, exists (Vestergaard-Bogind *et al.*, 1987). ATP-depleted red cells, containing 145 mmol K^+ per liter cell water and preloaded with ionized calcium to maximal activation of the K^+ channels, were suspended in salt solutions with different K^+ concentrations ($[K^+]_{ex}$). During outward-going currents $g_K(Ca)$ increased exponentially from a basic value of ~45 μS/cm² (extrapolated value at V_m = 0 mV, *see below*) towards a zero-current conductance of about 165 μS/cm² as V_m approached the E_K value in question.

The conductances at zero current were determined (at $[K^+]_{ex}$ = 30 to 150 mM) by measuring the equilibrium isotope flux at $V_m = E_K$. Inserting the single-file flux-ratio exponent, n = 2.7, previously reported (Vestergaard-Bogind, Stampe & Christophersen, 1985) in the equation of Hodgkin and Keynes (1955), $g_K(Ca)$ was then calculated.

At ($V_m - E_K$) ≥ 20 mV it was found that $g_K(Ca)$ increased by about 35% as $[K^+]_{ex}$ increased from about 0.1 to ~3 mM. At the latter concentration the specific effect of extracellular K ions apparently saturated. At $[K^+]_{ex} \approx 3$ mM $g_K(Ca)$ was found to decrease constantly with increasing V_m within the range of −90 to −4 mV, the slope of the line being 0.25 μS/(cm² · mV). Moreover $g_K(Ca)$ determined at $[K^+]_{ex}$ values > 3 mM and at ($V_m - E_K$) ≥ 20 mV always resulted in values which fitted the line giving $g_K(Ca)$ at $[K^+]_{ex} \sim 3$ mM as a function of V_m, confirming that the specific effect of extracellular K ions had saturated at $[K^+]_{ex} \approx 3$ mM. The linear function was, therefore, defined as the basic conductance. The absolute level of this conductance will of course be a function of the cellular pH (pH_i) (Stampe & Vestergaard-Bogind, 1985) and the cellular concentration of ionized calcium, which in the above-mentioned case was saturating with respect to activation of the K^+ conductance.

In the present paper we report the results of a study of the basic conductance at lower concentrations of ionized calcium.

Abbreviations

CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disul-

fonic acid; HEPPS, N-2-hydroxyethyl-piperazine-N'-3 propanesulfonic acid; MES, 2-(N-morpholino) ethanesulfonic acid; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid; EGTA, ethyleneglycol bis (β-aminoethylether)-N,N'-tetraacetic acid.

Materials and Methods

Cells

Cells from freshly drawn blood from healthy human donors were depleted of ATP and 2,3-diphosphoglycerate as previously described (Vestergaard-Bogind & Stampe, 1984). In experiments where all chloride was replaced by nitrate, the cells were washed and Ca-loaded in nitrate salt solutions. The cells were loaded with Ca to given concentrations of ionized Ca using 0.5 to 1 μmol of ionophore A23187 per liter cells (for details *see* Vestergaard-Bogind et al., 1987).

Phthalate Method

Cellular contents of K^+, Na^+, and ^{45}Ca and extracellular concentrations of K^+ were determined by the phthalate method as previously described (Vestergaard-Bogind et al., 1985).

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 μM of the protonophore CCCP, which mediates a fast electrochemical equilibration of protons across the cell membranes (for details *see* Vestergaard-Bogind et al., 1987).

Experimental Procedure

In all experiments packed cells loaded with appropriate Ca were transferred into buffer-free salt solution [3 mM KCl, 0 to 153 mM NaCl, isotonic conditions maintained by addition of sucrose (264 mM sucrose taken as isotonic)], containing 20 μM CCCP, thermostatted at 37°C and vigorously stirred. Since the cells were transferred into the salt solutions with open K^+ channels the efflux started instantaneously (for further details *see* Vestergaard-Bogind et al., 1987).

Calculations

The net efflux of K^+ in mmol per liter cells per hr was calculated from the initial, constant decrease in cellular K^+ content by linear regression. The correlation coefficient, $r \geq 0.97$. The value of the Nernst potential for K ions at the peak of hyperpolarization was calculated from the extracellular K^+ concentration at that time and the initially determined intracellular concentrations. The membrane potential (V_m) was calculated from the CCCP-mediated new electrochemical equilibrium of protons across the cell membranes. A pH difference ($pH_{ex} - pH_i$) of one unit was taken to be equivalent to a membrane potential of 61.5 mV (inside negative).

The Ca^{2+}-activated K^+ conductance, $g_K(Ca)$ was calculated from the equation of Hodgkin and Huxley (1952*a*)

$$I_K = J_K \cdot F = (V_m - E_K) \cdot g_K(Ca). \quad (1)$$

Here I_K is the current of K ions across the membrane, that is $J_K \cdot F$, where J_K is the net flux of K ions in μmol/(cm^2 · sec) and F is the Faraday constant. In the calculation of J_K per cm^2 of membrane it was assumed that the area of one liter of cells equals 1.75×10^7 cm^2. The conductance is accordingly obtained in μS/cm^2.

Results

In ATP-depleted human red blood cells, preloaded with calcium to a cellular concentration of Ca^{2+} sufficient to activate the K^+ channel to a significant degree, only two important conductance pathways are present. Besides the Ca^{2+}-activated K^+ conductance there is an anion conductance, which under physiological conditions is based on net flux of chloride ions.

At zero net current across the cell membranes, that is at the peak of hyper- or depolarizations (*cf.* Fig. 1), V_m is therefore given by the equation:

$$V_m = \frac{E_{An} \cdot g_{An} + E_K \cdot g_K(Ca)}{g_{An} + g_K(Ca)} \quad (2)$$

where E_{An} is the Nernst equilibrium potential of the anion and g_{An} the corresponding conductance. As mentioned, it has previously been demonstrated that at constant cellular pH (~7.1) and concentration of ionized calcium (~30 μM), $g_K(Ca)$ apparently saturated with respect to activation by extracellular K^+ ions at a concentration of ~3 mM (Vestergaard-Bogind et al., 1987). In the present experiments the $[K^+]_{ex}$ value was ~3.6 mM at the peak of hyper- or depolarizations. With ATP-depleted cells with a normal K^+ concentration of ~145 mmol/liter cells water the E_K value was therefore constantly about −98 mV.

In order to obtain positive values of V_m, E_{An} was shifted to positive values by substituting sucrose for extracellular salt. The effect of this substitution was enhanced by replacement of extracellular as well as cellular chloride with nitrate ions. In this way g_{An} was increased by a factor of ~2.5 (Vestergaard-Bogind et al., 1985) compared to the situation with chloride as the conducting anion, where g_{An} would have a value of ~20 μS/cm^2 (Bennekou, 1984). In addition, the depleted cells were only loaded with calcium to given concentrations of ion-

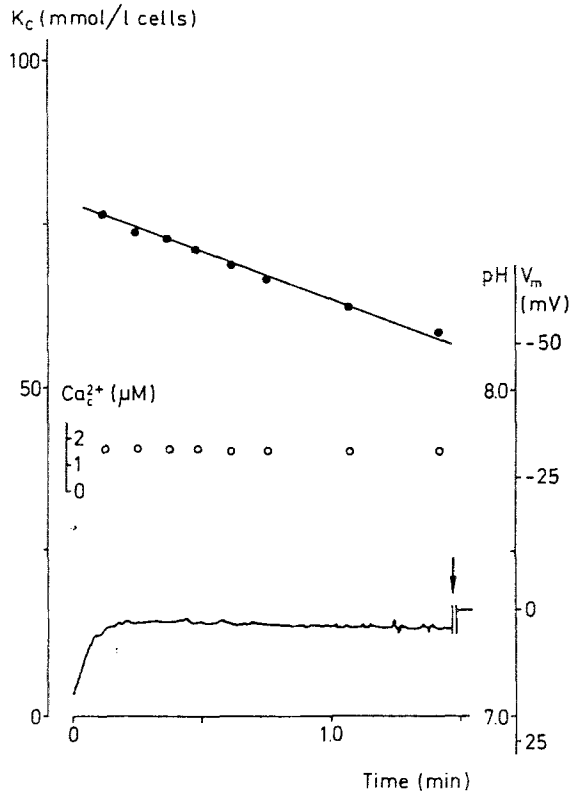

Fig. 1. Results of a typical experiment with K^+ net efflux from ATP-depleted cells preloaded with Ca to a cellular concentration of ionized calcium of 1.6 μM. The abscissa is time in minutes and the ordinates are cellular content of K^+ in mmol per liter cells [K_c, (●)], cellular concentration of ionized calcium in μM [Ca_c^{2+}, (○)], pH (—), and membrane potential [V_m, (same curve as pH)] in mV. The arrow indicates the determination of cellular pH by addition of Triton X-100. The membrane potential is calculated from the difference between the extracellular and the cellular pH (*see* section on Calculations). The net efflux of K^+ is calculated from the slope of the curve showing K_c. Note that the change in Ca_c^{2+} during the experiment is negligible

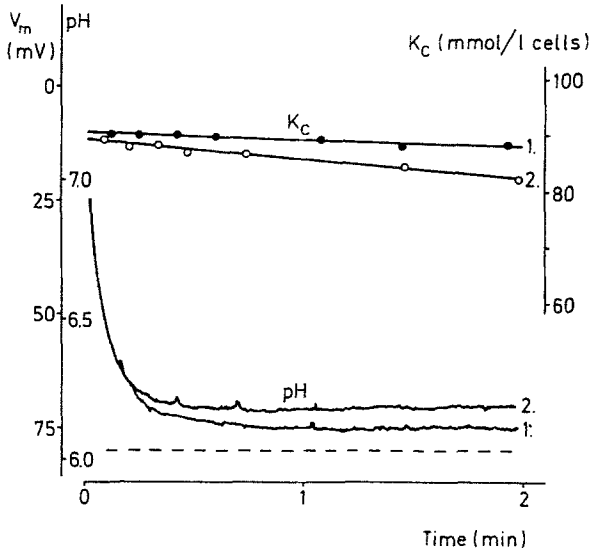

Fig. 2. Results of two experiments illustrating the effect of low ionic strength (3 mM KNO_3, 2 mM $NaNO_3$, 256 mM sucrose) upon K^+ net efflux from cells preloaded (2) with calcium (Ca^{2+} ~ 30 μM) and cells not preloaded (1) with Ca. The abscissa is time in minutes and the ordinates are cellular content of K^+ in mmol per liter cells (K_c), membrane potential (V_m) in mV and pH_{ex}. The broken line indicates the equilibrium potential of the anion E_{NO_3}

ized calcium which were submaximal with respect to activation of the K^+ conductance, the basic conductance hereby being reduced from the above-mentioned maximum value of ~45 μS/cm² at V_m = 0 mV.

The lowest concentration of extracellular salt used in the experiments was 3 mM (3 mM KCl or KNO_3), corresponding to an E_{An} value of +94 mV. Net effluxes of KCl from human erythrocytes suspended in media with low ionic strength have recently been described by Jones and Knauf (1985). At ~5 mM extracellular salt these fluxes were half-maximal (~135 mmol K^+ net efflux per liter cells per hr) and at 15 mM extracellular salt their magnitude was insignificant. It is not clear whether all or only a fraction of these fluxes are electrogenic. The best way to estimate the importance of the interference from this effect of low ionic strength proved to be a comparison of the net efflux- and V_m values determined in experiments where cells with activated K^+ channels and cells with nonactivated K^+ channels were suspended in identical salt-sucrose media (*see* Fig. 2).

The results of an experiment, in which a moderate depolarization of the cells took place, is shown in Fig. 1. The salt concentration was 30 mM (3 mM KNO_3, 27 mM $NaNO_3$, 213 mM sucrose) and the cellular concentration of ionized Ca was 1.6 μM. V_m adjusted to +3 mV, corresponding to a driving force ($V_m - E_K$) of ~105 mV. The net efflux of K^+ was quite large, namely 1000 mmol/(liter cells · hr). In Fig. 2 an experiment with cells transferred to a salt solution containing only 5 mM salt (3 mM KCl, 2 mM NaCl, 256 mM sucrose) is represented. In this experiment V_m adjusted to a value of +65 mV, corresponding to a ($V_m - E_K$) value of about 170 mV. In spite of the very large driving force the net efflux of K^+ was only 200 mmol/(liter cells · hr). As seen from the Figure, the control experiment with depleted cells, which were not preloaded with calcium, showed that about half of the K^+ net efflux could be ascribed to the "leak" of KCl induced by the low ionic strength of the extracellular phase. Assuming that the leak fluxes are electrogenic they correspond to g values of 1 and 2 μS/cm² at extracellular salt concentrations of 5 and 3 mM, respectively. This magnitude is insignificant in all experi-

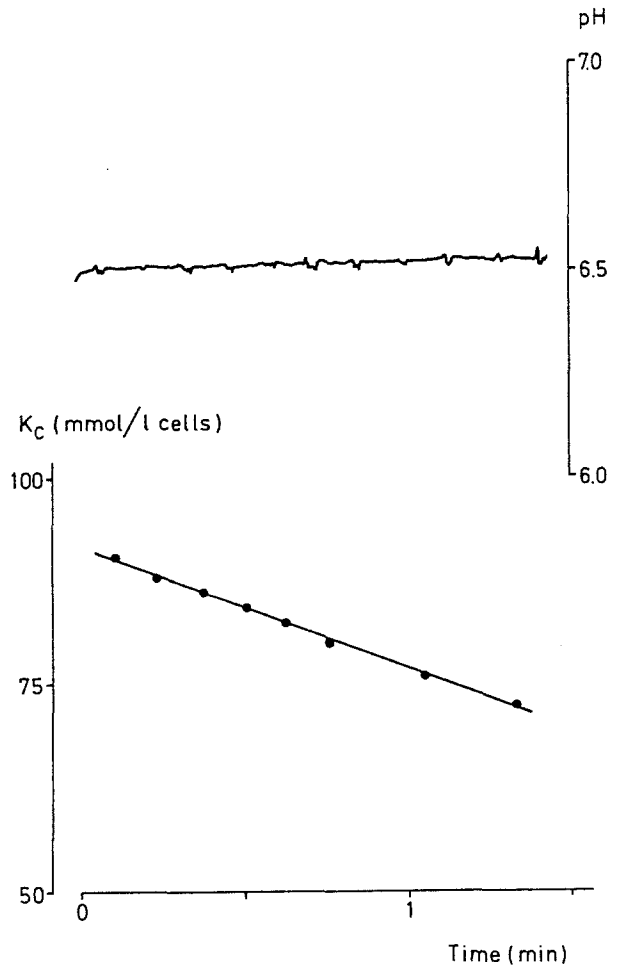

Fig. 3. Results of a control experiment at low extracellular pH and a cellular pH of 7.2. To avoid breakdown of the pH gradient the cells were treated with DIDS and no CCCP was present. The extracellular phase was buffered with 2 mM MES. The abscissa is time in minutes. The ordinates are cellular content of potassium (K_c) in mmol per liter cells and pH_{ex}. Note the constant extracellular pH

ments apart from the two at $V_m = +71$ and $+77$ mV at the lowest level of ionized Ca (*compare* Fig. 6). No correction has been applied to these values.

At positive V_m values and with CCCP-mediated electrochemical equilibration of protons as V_m indicator a decrease in pH_{ex} at a constant pH_c takes place (*compare* Fig. 2). It has previously been shown (Stampe & Vestergaard-Bogind, 1985) that the net efflux of K^+ is not affected by the increase in extracellular pH resulting from the CCCP-mediated redistribution of protons in case of a hyperpolarization. It was now necessary to control the possible influence of a decreasing pH_{ex} on $g_K(Ca)$.

The protocol of these control experiments was as follows. ATP-depleted cells with all chloride replaced by nitrate were loaded with calcium ($Ca_c^{2+} \sim$ 30 μM) and at the same time treated with DIDS. By the DIDS treatment the anion-exchange mechanism was inhibited strongly (Cabantchik & Rothstein, 1974) and an imposed pH difference between the intra- and extracellular phases was now sufficiently stable for minutes (*compare* Fig. 3). As in the depolarization experiments, nitrate was used in the control experiments as the conducting anion. In addition to comparability a reasonably large net efflux could now be obtained since the residual anion conductance of DIDS-treated cell membranes was larger than in a corresponding chloride experiment. In these control experiments the cells were loaded to maximal Ca^{2+} activation of the channels in order to get as large fluxes as possible. Since CCCP would degrade an imposed pH difference across the membranes even more effectively than the anion exchange mechanism, the control experiments were performed in the absence of CCCP, and accordingly V_m and $g_K(Ca)$ could not be calculated.

Net effluxes of K^+ were determined in experiments where cells with open K^+ channels were transferred to salt solutions containing 3 mM KNO_3, 150 mM $NaNO_3$ and 2 mM of an appropriate buffer (MES, HEPES, HEPPS) titrated to the desired pH. With 100 μl cells, in 3000 μl buffered salt solution the buffering capacity of the extra- and intracellular compartments were of the same magnitude and a shift in pH_{ex} should reflect a reverse shift in pH_c of almost identical magnitude. The experiment represented in Fig. 3 shows that the change in pH_{ex}, and accordingly pH_c, was insignificant. In Fig. 4 the net efflux of K^+, J_K, is plotted as a function of pH_{ex} within the range of 8.0 to 6.0 pH. As the Figure shows, the net flux increased significantly with decreasing pH_{ex}. However, as shown in the same Figure, an identical increase in K^+ net efflux with decreasing pH_{ex} was found in a series of experiments where valinomycin was the electrogenic carrier of K ions. In these experiments, where depleted cells with all chloride replaced by nitrate ions were used, the cells were treated with DIDS, but not preloaded with Ca. Valinomycin was added in the appropriate amount to a stock suspension of the cells in a K^+-equilibrium nitrate solution; the cells were centrifuged and the packed cells were stored on ice. As usual, samples of 100 μl packed cells were used in each experiment. Since the valinomycin-mediated K^+ transport would be expected to be little affected by pH_{ex} and since it is most unlikely that the electrogenic valinomycin-mediated K^+ transport and the net efflux through the Ca^{2+}-activated K^+ channels should have exactly identical pH_{ex} dependencies, then the conclusion of the combined results represented in Fig. 4 was that g_{NO_3}, but not $g_K(Ca)$ is a function of pH_{ex} within the range of 6.0 to 8.0 pH.

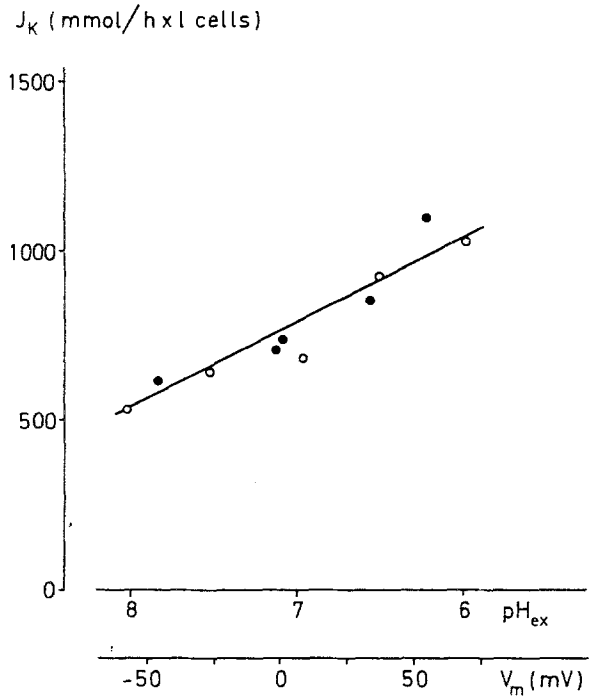

Fig. 4. K^+ net efflux (J_K) in mmol per liter cells per hour as a function of extracellular pH at a fixed value of cellular pH of 7.2. Each net efflux was determined in an experiment of the type shown in Fig. 3. (●) The net efflux of KNO_3 was induced by the addition of valinomycin (*see* text). (○) The net efflux of KNO_3 from cells, preloaded with Ca to a concentration of ionized Ca of ~30 μM, transferred to a salt solution of the given pH. For comparison with Fig. 6 a scale with corresponding V_m in mV is shown below the pH_{ex} scale

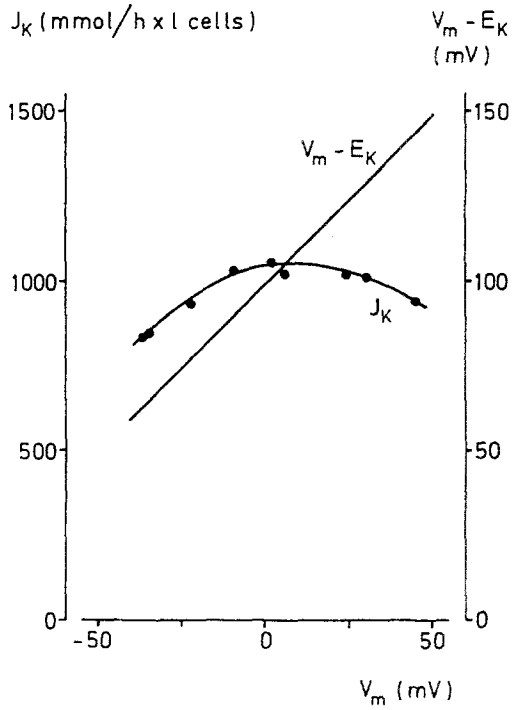

Fig. 5. K^+ net efflux (J_K) in mmol per hour per liter of cells and ($V_m - E_K$) as functions of V_m in mV. Cellular concentration of ionized Ca was ~3 μM. Each flux was determined as shown in Fig. 1, and E_K was −98 mV in all experiments

The net effluxes of K^+ determined in the main series of experiments, exemplified in Figs. 1 and 2, could accordingly be considered as a function of membrane potential exclusively, and in Fig. 5 the values of J_K and ($V_m - E_K$) are shown as a function of V_m. As seen from the Figure the net efflux decreases slightly with increasing V_m values in spite of the simultaneously large increase in the driving force. The calculated g_K(Ca) values plotted versus V_m (Fig. 6) resulted in apparently linear relationships with slopes from 0.04 to 0.17 μS/(cm^2 · mV).

Discussion

The following discussion of present and previously reported (Vestergaard-Bogind et al., 1987) results is based on the assumption that the human red cell membrane contains only a single class of Ca^{2+}-activated K^+ channels. These channels exhibit inward rectifier properties and the basic conductance as intrinsic characteristics. In favor of this assumption is the fact that after several years of patch-clamp experiments with human red cells only one type of K^+ channels has been reported (Hammil, 1981; Grygorczyk & Schwarz, 1983, 1985; Grygorczyk, Schwarz & Passow, 1984; Christophersen & Bennekou, 1987). In addition, under different experimental conditions involving large variations in V_m, $[K^+]_{ex}$, pH_c, etc., there has never been found any kinetic evidence, which could be interpreted as the result of two or more classes of channels.

The main experimental result of the present study is represented in Fig. 6. At cellular concentrations of ionized Ca corresponding to submaximal activation of the K^+ conductance (cellular ionized Ca 0.3, 1.6 and 3.0 μM), and at a fixed $[K^+]_{ex}$ value of ~3.6 mM, g_K(Ca) for outward-going currents were found to be apparently linear functions of voltage over a wide range of V_m. The slope of these linear voltage functions [0.04, 0.11 and 0.17 μS/(cm^2 · mV)], were lower than the 0.25 μS/(cm^2 · mV) previously found at maximal activation of the conductance (Vestergaard-Bogind et al., 1987). Thus, the level as well as the slope of the basic conductance are strongly dependent on the degree of Ca^{2+} activation of the K^+ conductance. These apparently linear relationships between g_K(Ca) and V_m extend far into the range of positive V_m values.

That the concomitant decrease in extracellular pH and ionic strength are insignificant compared to the influence of V_m has been dealt with in the

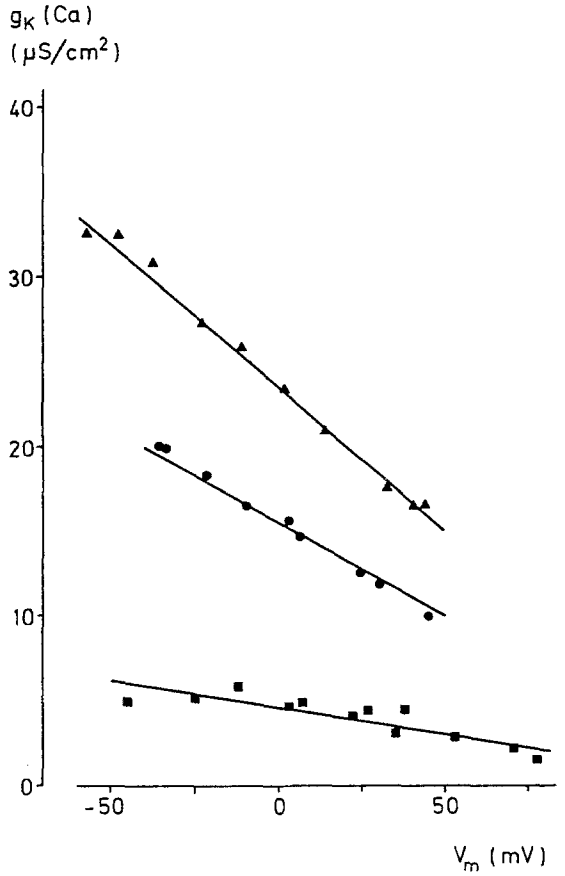

Fig. 6. Conductance (g_K(Ca) in μS/cm²) as a function of membrane potentials (V_m in mV) at three different levels of cellular ionized Ca [0.3 μM (■), 1.6 μM (●) and 3.0 μM (▲)]. Each point represents an experiment of the type shown in Fig. 1. In all experiments E_K was about −98 mV. Variation in V_m was obtained by substitution of sucrose for extracellular $NaNO_3$. The solid lines are calculated from Eq. (3) assuming V_0 = 0 mV and z' = 0.4 (*see* Discussion)

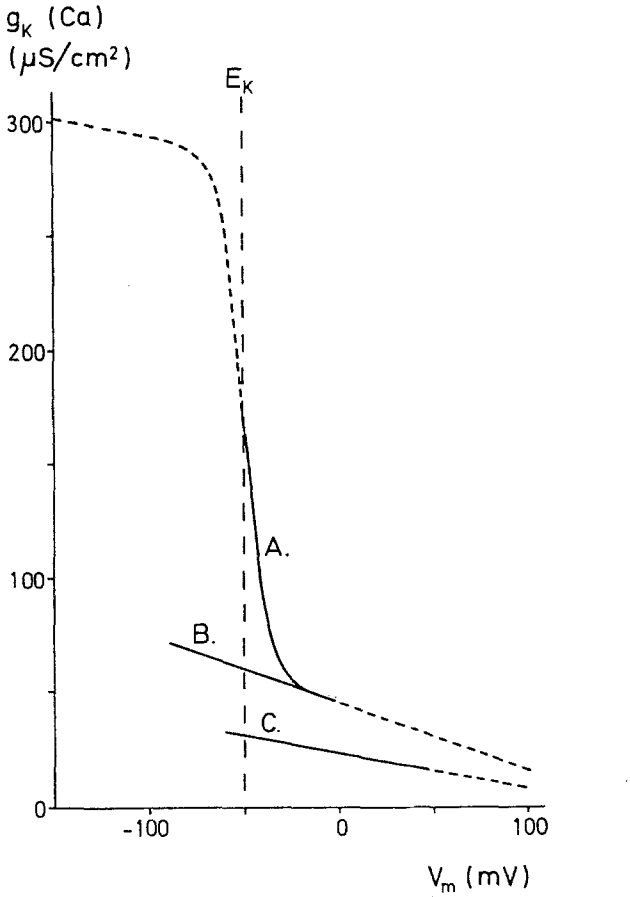

Fig. 7. g_K(Ca) in μS/cm² as functions of V_m (in mV) at about half (curve *C*) and maximal (curve *A* and *B*) Ca^{2+} activation of the conductance (concentration of cellular ionized calcium 3, respectively, 22 μM). Curves *A* and *B* are based on previously published data (Vestergaard-Bogind et al., 1987) and curve *C* is based on the same data as the upper curve in Fig. 6. In a simple two-state model the curves *B* and *C* were calculated from Eq. (3), inserting an equivalent valence z' of 0.4 and assuming chemical equilibrium at V_m = 0 mV. Curve *A* was calculated as the sum of two Boltzmann-type equations: the basic conductance (z' = 0.4, chemical equilibrium at V_m = 0) and an independent, inward-rectifying gating process (z' = 5, chemical equilibrium at the E_K value, in this case −50 mV). The solid lines represent experimental results were $[K^+]_{ex}$ was ~3.6 (curve *B* and *C*), respectively, 20 mM (curve *A*). The broken lines represent extrapolations into V_m ranges experimentally unachievable with the present technique

Results section. That the decrease in g_K(Ca) towards zero at higher V_m values reflects a voltage dependence of g_K(Ca) and not the decrease in extracellular ionic strength is also seen from Fig. 7, where the g_K(Ca) value of 45 μS/cm² at V_m = −2 mV (cellular Ca^{2+} concentration ~ 30 μM) was obtained in an experiment with only 10 mM salt in the extracellular phase. Accordingly, the only important variable is the membrane potential.

While the level of basic conductance was expected to be dependent on cellular Ca^{2+} concentration the maintained linearity of the voltage function over such a wide range was not obvious. A comparison of the results represented in Figs. 6 and 7 with those of the patch-clamp studies of Grygorczyk and Schwarz (1983) presents several problems. In experiments with symmetrical concentrations of ~140 mM KCl they find that the single-channel conductance γ varies from ~10 to ~40 pS as V_m is varied from +100 to −200 mV, the *I-V* function being significantly supralinear. At a concentration of ionized Ca of 5 μM the open state probability p was a slightly superlinear function of membrane potential varying from ~0.35 to ~0.70 as V_m was varied from +75 to −100 mV. Using chord conductance values evaluated from their Figures we have calculated the product $\gamma \cdot p$ at various voltage values. As expected this product, which should be proportional to the

membrane conductance, was found to be a superlinear function of V_m within the range of +50 to −50 mV.

Unfortunately, outward-current γ values at lower, physiological $[K^+]_{ex}$ values have not been determined in these patch-clamp studies and it is also uncertain whether the reported voltage dependence of the open state probability shifts along the voltage axis with changes in cellular ionized Ca or $[K^+]_{ex}$. All the same, compared to the single-channel data reported by Grygorczyk and Schwarz (1983) the apparently linear relationships between $g_K(Ca)$ and V_m shown in Fig. 6 are somewhat surprising.

Most probably the linear relationships between $g_K(Ca)$ and V_m are apparent and reflect fractional decreases in $g_K(Ca)$ with increasing V_m. This assumption is supported by the decrease of the slope with decreasing Ca^{2+} activation of the channels (*see* Fig. 6). In a simple two-state theory (Hodgkin & Huxley,, 1952*b*) the slopes of the basic conductances at the three different degrees of Ca activation shown in Fig. 6 correspond to a gating particle with an equivalent valence z' of ~0.4, if in a Boltzmann-type equation

$$g_K(Ca) = A \frac{1}{\exp\left[\frac{z'F(V_m - V_o)}{RT}\right] + 1} \quad (3)$$

chemical equilibrium is assumed at 0 mV, that is if $V_o = 0$ mV and therefore $A = 2\ g_K(Ca)$ at $V_m = 0$ mV. R, T, and F have their usual meaning. The solid lines in Fig. 6 and curves B and C in Fig. 7 (C equal to upper curve of Fig. 6) have been calculated from Eq. (3). Curve B (solid line) in Fig. 7 represents the basic conductance as a function of V_m at maximal Ca activation (cellular concentration of ionized Ca was 27 μM) previously reported (Vestergaard-Bogind et al., 1987). The fit of the experimental values to the calculated line ($z' = 0.4$) was here just as good as seen from Fig. 6.

Thus the z' value calculated on the basis of four very different slopes, is calculated with a very low uncertainty. In contrast, the corresponding chemical equilibrium, assumed to be at $V_m = 0$ mV, could be anywhere within the interval of −10 to +10 mV, since the sigmoid part of the curves, because of the low z' value, are not within the actual V_m range.

The curves in Fig. 7 summarize our present knowledge of $g_K(Ca)$ as a function of V_m at different degrees of Ca activation including the previously reported (Vestergaard-Bogind et al., 1987) voltage dependence of the inward-rectifying process. As mentioned, the curves B and C have been calculated from Eq. (3) with a z' value of 0.4.

The steep inward-rectifying voltage dependence around E_K represents a different and independent gating process superimposed upon the basic conductance. Calculated from the sum of two Boltzmann-type equations the equivalent valence of the gating particle is here ~5 with chemical equilibrium at the E_k value in question. The equivalent valence of the gating particle of the inward rectification is thus very close to the one found for the delayed rectifier in the squid axon (Hodgkin & Huxley, 1952*a*).

In Fig. 7 the solid lines represent V_m values covered by experimental data while experimental verification of dash-line sections has been impossible at present. Thus, at maximal Ca activation the magnitude of $g_K(Ca)$ is so high that V_m values higher than about 0 mV cannot be obtained even with all cellular chloride replaced by nitrate ions and maximal replacement of extracellular $NaNO_3$ by sucrose (*see* Eq. 2). On the other hand g_{Cl} is so high that even with DIDS it is not possible, at an extracellular K^+ concentration of ~3 mM, to achieve V_m values which are close enough to the E_K value to demonstrate the steep inward-rectifying voltage dependence. This voltage dependence is therefore represented by a series of experiments performed at $[K^+]_{ex} = 20$ mM (curve A and the corresponding E_K line in Fig. 7). At an $[K^+]_{ex}$ value of ~3 mM curve A would have the same shape but be displaced to the left to a voltage range around −100 mV. At lower degrees of Ca activation of the K^+ conductance the steep voltage dependence of the rectification process would only be demonstrable at a correspondingly higher $[K^+]_{ex}$. (For a further discussion *see* Vestergaard-Bogind et al., 1987.)

With respect to the voltage dependence of the basic conductance an alternative to a gating particle with an equivalent valence of 0.4 would be a voltage-dependent block of $g_K(Ca)$ by an intracellular ion. Thus, higher intracellular concentrations of Na ions have been found to diminish the efflux of K ions from resealed ghosts, a depression released by increased $[K^+]_{ex}$ (*cf.* Yingst & Hoffman, 1984). Experiments, depicting a characterization of $g_K(Ca)$ as a function of the intracellular concentrations of K- and Na-ions, are in progress in our laboratory, and in these experiments a possible voltage dependence of the Na inhibition of $g_K(Ca)$ should be demonstrable.

The authors wish to thank Annelise Honig and Kirsten Abel for excellent technical assistance, and expert assistance in preparation of the manuscript by Susan Hargreaves and Villy Rasmussen. We are grateful to Dr. J. Sandblom (Gothenburg University) for reading the manuscript. This work was supported by The Carlsberg Foundation (1985/86 no. 341/IV) and the NOVO Foundation (1984-12-10).

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Received 17 July 1987; revised 9 November 1987