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# $Ca^{2+}$-Activated $K^+$ Channel from Human Erythrocyte Membranes: Single Channel Rectification and Selectivity

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**Summary.** The $Ca^{2+}$-activated $K^+$ channel of the human red cell membrane was characterized with respect to rectification and selectivity using the patch-clamp technique. In inside-out patches exposed to symmetric solutions of $K^+$, $Rb^+$, and $NH_4^+$, respectively, inward rectifying *i-V* curves were obtained. The zero current conductances were: $K^+$ (23.5 pS ± 3.2) > $NH_4^+$ (14.2 pS ± 1.2) > $Rb^+$ (11.4 pS ± 1.8). With low extracellular $K^+$ concentrations (substitution with $Na^+$) the current fluctuations reversed close to the Nernst potential for the K ion and the rectification as well as the *i-V* slopes decreased. With mixed intracellular solutions of $K^+$ and $Na^+$ enhanced rectification were observed due to a $Na^+$ block of outward currents. From bi-ionic reversal potentials the following permeability sequence ($P_K/P_X$) was calculated: $K^+$ (1.0) > $Rb^+$ (1.4 ± 0.1) > $NH_4^+$ (8.5 ± 1.3) > $Li^+$ (>50); $Na^+$ (>110); $Cs^+$ (≥5). $Li^+$, $Na^+$, and $Cs^+$ were not found to carry any current, and only minimum values of the permeability ratios were estimated. $Tl^+$ was permeant, but the permeability and conductance were difficult to quantify, since with this ion the single channel activity was extremely low and the channels seemed to inactivate. The inward rectification in symmetric solutions indicate an asymmetric open channel structure, and the different selectivity sequences based on conductances and permeabilities reflect interionic interactions in the permeation process.

**Key Words** erythrocyte · $K^+$ channel · selectivity · single channel · independence · rectification

## Introduction

In most cells an important regulatory response to intracellular ionized $Ca^{2+}$ is the activation of channels that are selective to $K^+$. In excitable cells a function of $Ca^{2+}$-activated $K^+$ channels is to repolarize bursting membranes, and thereby they participate in as diverse processes as regulation of pacemaker neuronal activity (Gorman, Herman & Thomas, 1982) and insulin secretion (Atwater et al., 1980). In nonexcitable cells they are important mediators of cell volume regulation (Hoffman & Simonsen, 1989) and epithelial transport (Hunter et al., 1986).

At present two main groups of $Ca^{2+}$-activated $K^+$ channels have been described: Maxi-channels with single channel conductances of 200–300 pS and mini-channels with smaller conductances, 5–40 pS. The mini-channels are more $Ca^{2+}$ sensitive than the maxi-channels, and most types are not opened by depolarizing voltages (for a review, *see* Blatz & Magleby, 1987). One type of the mini-channels found in human erythrocytes (Grygorczyk & Schwarz, 1983), HeLa cells (Sauvé et al., 1986) and aortic endothelial cells (Sauvé et al., 1988) is characterized by open channel inward rectification in symmetric $K^+$ solutions and an open state probability that increase slightly at hyperpolarizing voltages. In macroscopic flux experiments it was demonstrated that the erythrocyte channel shows a strong anomalous rectification at near physiological gradients of $K^+$ (Vestergaard-Bogind, Stampe & Christophersen, 1987). This group of mini-channels may be physiologically interesting because it can be activated by small $Ca^{2+}$ increments at highly negative resting membrane potentials.

Whereas the maxi-channels have been extensively characterized at the single-channel level (for a review, *see* Latorre, 1986), much has yet to be learned about both gating kinetics and open channel properties of the mini-channels. To gain information about chemical and physical structures in the open channel, detailed current-voltage functions and quantitative studies of channel selectivity must be obtained. Ideally, both equilibrium selectivity (binding parameters) as well as nonequilibrium selectivity (transport parameters) should be considered (Krasne, 1980).

In the present study the mini-channel from human red blood cells has been analyzed with the patch-clamp technique (Hamill et al., 1981). It was demonstrated that the channel showed inward rectification in symmetric solutions of both $K^+$, $NH_4^+$, and $Rb^+$, probably indicating a highly asymmetric channel structure. In mixed solutions of $K^+$ and $Na^+$ the rectification decreased at low extracellular $K^+$ concentrations, as if rectification depended on

the saturation of an outward facing channel site. $Na^+$ blocked the channel from the inside, resulting in an enhanced rectification. The selectivity to small inorganic cations were characterized, both from measurements of single-channel conductances in symmetric solutions and from bi-ionic reversal potentials. Sequences based on conductance ratios and permeability ratios were different, indicating that the independence principle is not obeyed for ion transport through this channel.

## Materials and Methods

### Cells

Blood from healthy, human donors was heparinized and centrifuged at 4500 × *g* for 3 min. Plasma and "buffy coat" were removed by aspiration. The cells were resuspended and washed in 30 vol ice-cold 156 mM KCl solution and stored on ice as a suspension (hematocrit 2%).

### Salt Solutions

All salt solutions were made from high grade salts and double distilled water and filtered through a syringe filter with a pore size of 0.22 μm. The salts were chloride salts except for thallium, which was the nitrate salt. The experiments were performed in hypotonic media to increase the size of the cells. The solutions contained (in mM): 130 salt, 5 MOPS,[1] pH 7.4, adjusted by addition of 3–5 mM of NMGA or the appropriate hydroxide. To the bath solutions (the intracellular phases) were added 20 μM $CaCl_2$ (total concentration 22 μM), while 1 mM of EDTA was included in the pipette solutions (the extra cellular phase). In the experiments with $TlNO_3$ in the pipettes 1 mM of HCl was added to the solutions to stabilize the electrode potentials.

### Pipettes and Electrodes

Patch-clamp pipettes were pulled from hematocrit capillaries (Cee Bee) by a two-state process and coated with Sylgard. After fire polishing the resistances were 15–20 MΩ. The reference pipette and the patch-clamp pipette always contained the same chloride concentration. Usually the bath solution and the solution in the reference pipette were identical.

### Electronics

The patch-clamp amplifier was an EPC-7 (List Electronic). Experimental data were low-pass filtered (0.5 or 3 kHz) and stored on tape. Single-channel currents were measured either directly by hand on a transcript from a digital storage oscilloscope or data were transferred to a computer and converted to current amplitude histograms.

### Experimental Procedure

Prior to an experiment a sample of the cell suspension was centrifuged and the cells were washed once in the bath solution before being transferred to the experimental chamber. After giga-seal formation inside-out patches were obtained by moving the cell through the air/water interface. All experiments were carried out at room temperature (20–22°C).

### Errors

The electrode offset was zeroed with the open pipette in the bath solution. The possible error due to a liquid junction potential between different pipette and bath solutions was negligible in the bi-ionic experiments with $NH_4^+$, $Rb^+$, and $Cs^+$, while potentials up to 3 and 6 mV could be detected with $Na^+$ and $Li^+$, respectively. After an experiment the patch was destroyed and the offset was measured again. An electrode drift within ±3 mV was accepted (usually the drift was within ±1 mV). No corrections were performed.

### Conventions

The standard electrophysiological conventions were followed. The clamp potential (from now on called the membrane potential, $V_m$) is defined as the inside (bath) potential minus the outside (pipette) potential. Outward-going single channel currents ($i$), (from bath to pipette) are defined as positive.

### Identification of the Channels

In solutions of foreign cations the $Ca^{2+}$-activated $K^+$ channel was recognized from its dependence on internal $Ca^{2+}$.

### Calculations

The selectivity ratios between $K^+$ and the test ion ($X^+$) were either expressed as a permeability ratio or as a single channel conductance ratio.

Single channel chord conductances, $g_c$ (Hodgkin & Huxley, 1952), were calculated from $i$-$V$ curves obtained in experiments with identical solutions of the test ion on the two sides of the membrane (symmetric condition).

Permeability ratios were obtained from zero current potentials (Eisenman & Horn, 1983). In the particularly simple case where the inside solution contains $K^+$ only and the outside solution contains a test ion ($X^+$) only, and $C_K(i) = C_X(o)$ (bi-ionic condition), the permeability ratio is defined as

$$\frac{P_K}{P_X} = \exp\left[-\frac{V_r \cdot F}{R \cdot T}\right]. \quad (1)$$

$F$, $R$ and $T$ have their usual meanings, $V_r$ is the reversal potential and $P$ is the permeability coefficient. Generally the $i$-$V$ curves were nonlinear functions and the reversal potentials were esti-

[1] *Abbreviations:* EDTA, ethylene diamine tetraacetic acid; MOPS, 3-[N-morpholino] propanesulfonic acid; NMGA, N-methyl D-glucamin.

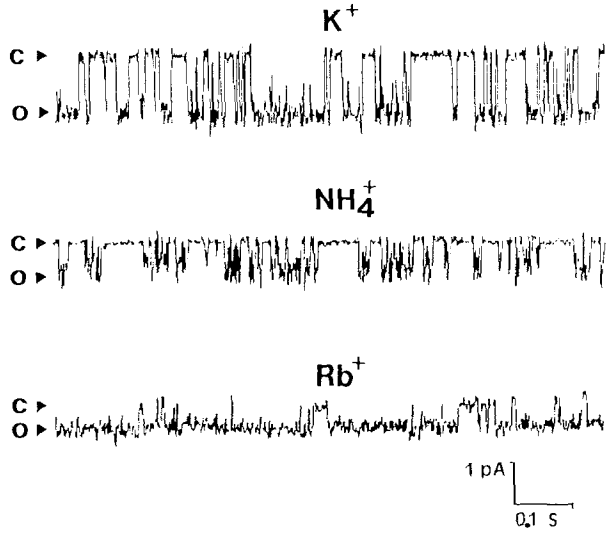


**Fig. 1.** Patch-clamp recordings from inside-out patches exposed to symmetric solutions of $K^+$, $Rb^+$ and $NH_4^+$ (130 mM), respectively. $V_m$: −60 mV. $Ca^{2+}$ concentration: 22 μM. *C:* Closed state, *O:* Open state. Filter frequency: 500 Hz

mated by a polynomial interpolating routine (Asystant, Macmillian Software).

## Results

### *i-V* Curves in Symmetric Cation Solutions

To obtain basic information about the open channel energy profile and the selectivity to various ions, experiments with the extra- and intracellular solutions containing identical concentrations of test ions were performed. Patch-clamp records demonstrating single channel fluctuations in solutions of both $K^+$, $Rb^+$, and $NH_4^+$ are shown in Fig. 1. The current voltage relations are plotted in Fig. 2. Nonlinear, inward rectifying *i-V* curves were found and the rectification seemed to be most pronounced in $K^+$ solutions, while it was barely detectable in the $Rb^+$ experiments. The most conductive ion was $K^+$ with a single channel chord conductance varying from 50 pS at −120 mV to 13 pS at 120 mV. In symmetric $NH_4^+$ or $Rb^+$, the conductances varied from 25 pS (−120 mV) to 8 pS (−100 mV) for $NH_4^+$ and from 18 pS (−120 mV) to 7 pS (80 mV) for $Rb^+$. In symmetric solutions of $Na^+$, $Cs^+$, and $Li^+$ no current fluctuations were detected. Therefore, the single channel conductances with these ions are concluded to be less than ≈2 pS. (The presence of active channels were controlled by perfusion with a $K^+$ solution).

### *i-V* Curves in Asymmetric $K^+$ Solutions

Current-voltage experiments were performed at different concentrations and gradients of $K^+$, with either the extracellular or the intracellular concentration of $K^+$ kept constant. In all experiments $K^+$ was substituted with $Na^+$. Typical results are plotted in the *i-V* diagram of Fig. 3. While lowering the concentration of $K^+$ in the outside solution (filled symbols), characteristic changes of the *i-V* curves were observed: (i) The reversal potential shifted along the voltage axis approximately following the Nernst equation for the K ion. (ii) At any fixed value of the membrane potential the slopes of the *i-V* curves decreased. (iii) In the range of membrane potentials investigated the current-voltage relations became increasingly linear. Accordingly, the shape of the current-voltage relationships are dependent on voltage and the extracellular concentration of $K^+$. (iv) For large positive membrane potentials the outward-going currents approached a saturating value of about 1.5 pA, regardless of the concentration of the extracellular $K^+$ concentration, indicating a very low (or zero) limiting slope conductance for large positive driving forces.

In the experiments with different intracellular $K^+$ concentrations (open symbols), it was impossible to determine the reversal potentials, since outward single channel currents could not be detected at low intracellular $K^+$. At negative membrane potentials the inward currents were only slightly affected by the intracellular $K^+$ concentration. This indicates that for large negative values of the driving forces, the currents reach the same limiting slope conductance. Obviously in these experiments the rectification is more pronounced than in symmetric $K^+$ solutions. Since in macroscopic experiments it has been shown that intracellular $Na^+$ is a voltage-dependent blocker (Stampe & Vestergaard-Bogind, 1989) the enhanced rectification was suspected to be due, at least partly, to $Na^+$ block of the outward-going $K^+$ currents. In Fig. 4 an experiment is shown that clearly demonstrates that $Na^+$ blocks the channel. The individual blocking events are unresolved at 200 Hz filtering, indicating a fast or flickery type of blockage (Yellen, 1984).

From *i-V* curves obtained at various extracellular $K^+$ concentrations single channel conductances were calculated. In Fig. 5 the conductances at zero membrane potential are plotted *vs.* the extracellular concentration of $K^+$. This curve clearly demonstrates that the channel conductance saturates at an extracellular $K^+$ concentration of about 30 mM. The apparent maximum conductance is 25 pS, and the apparent half saturation concentration is 4 mM. The corresponding curve ($V_m$ = 0 mV) for variation

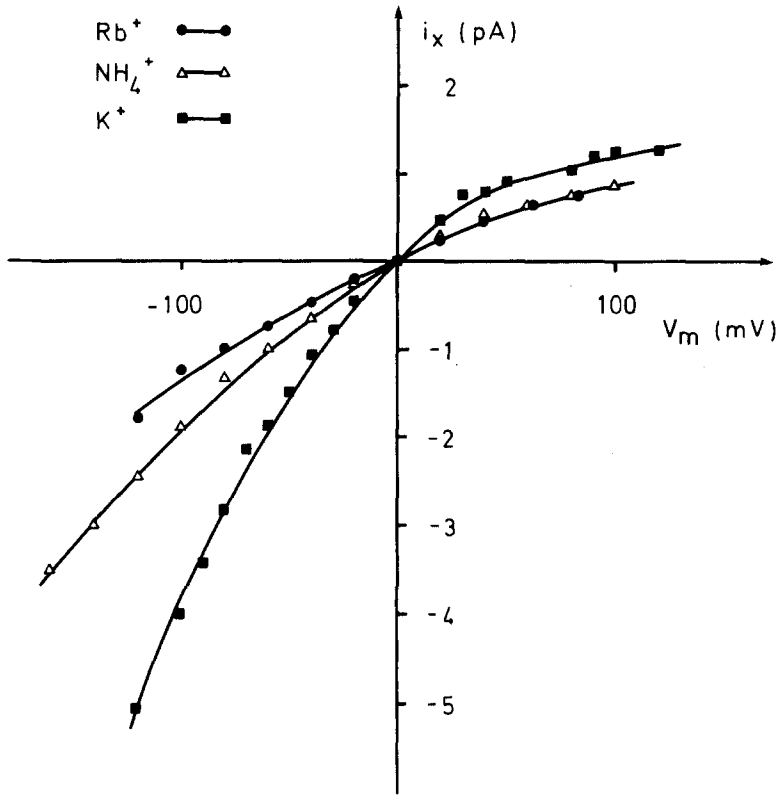


**Fig. 2.** *i-V* curves obtained in symmetric solutions of the test cations. Inside (bath) solution: 130 mM *X*Cl, 5 mM MOPS, ≈4 mM NMGA (pH = 7.4), 22 $\mu$M $CaCl_2$. Outside (pipette) solution: 130 mM *X*Cl, 5 mM MOPS ≈4 mM NMGA, 1 mM EDTA (pH = 7.4). (■) $X = K^+$ ($n = 8$); (△) $X = NH_4^+$ ($n = 3$); (●) $X = Rb^+$ ($n = 4$). Lines were drawn by eye

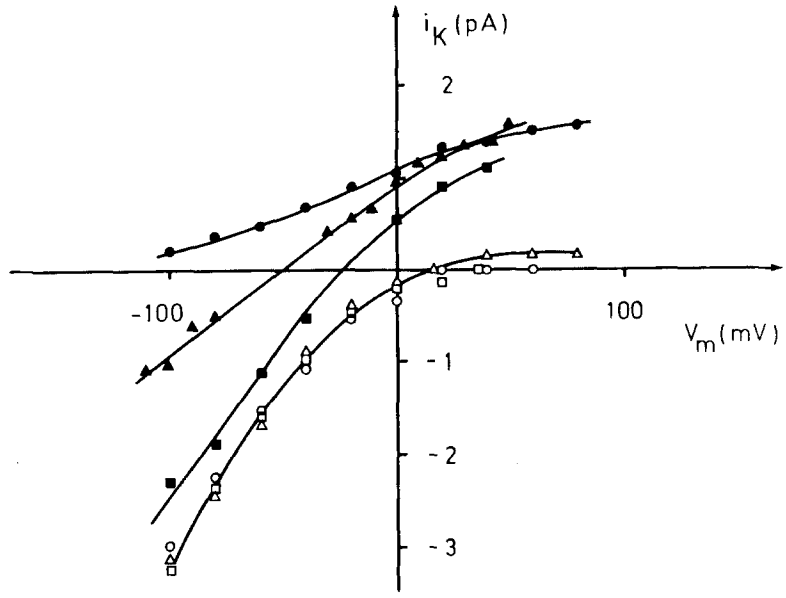


**Fig. 3.** *i-V* curves obtained in asymmetric $K^+$ solutions. Inside (bath) solutions: 130 mM (KCl + NaCl), 5 mM MOPS (pH = 7.4), 22 $\mu$M $CaCl_2$. Outside (pipette) solutions. 130 mM (KCl + NaCl), 5 mM MOPS (pH = 7.4), 1 mM EGTA. First series (filled symbols): inside solution: 130 mM KCl. Outside solutions: 50 mM KCl (■); 15 mM KCl (▲); 0 mM KCl (●). Second series (open symbols): inside solution: 5 mM KCl (○); 30 mM KCl (□); 50 mM KCl (△). Outside solution: 130 mM KCl. Lines were drawn by eye

of the intracellular concentration of $K^+$ was not a saturating function, due to various degrees of $Na^+$ blocking (*not shown*).

## Bi-Ionic *i-V* Curves and Permeability Ratios

The above experiments demonstrated different single channel conductances in symmetric solutions of the various cations. Alternatively, selectivity may also be expressed as permeability sequences based on measurements of reversal potentials. Since the two approaches elucidate different aspects of the selectivity mechanism, permeability sequences were also calculated in the present study. This also allows direct comparison with permeability studies of the classical $K^+$ channels from excitable tissues.

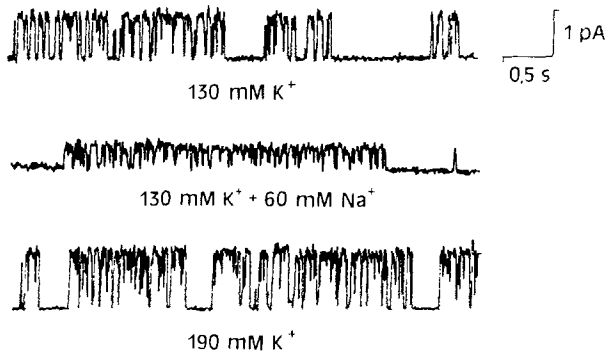


**Fig. 4.** An experiment that demonstrates $Na^+$ block from the inside of the membrane. Inside (bath) solution: 190 mosm/liter (KCl + NaCl + sucrose), 5 mM MOPS (pH = 7.4), 22 $\mu$M $CaCl_2$. *Upper trace:* 130 mM KCl + 100 mM surcrose. *Middle trace:* 130 mM KCl + 60 mM NaCl. *Lower trace:* 190 mM KCl. Outside (pipette) solution: 130 mM NaCl, 100 mM sucrose, 5 mM MOPS (pH = 7.4), 1 mM EDTA. $V_m$ = 0 mV. Filter frequency: 200 Hz

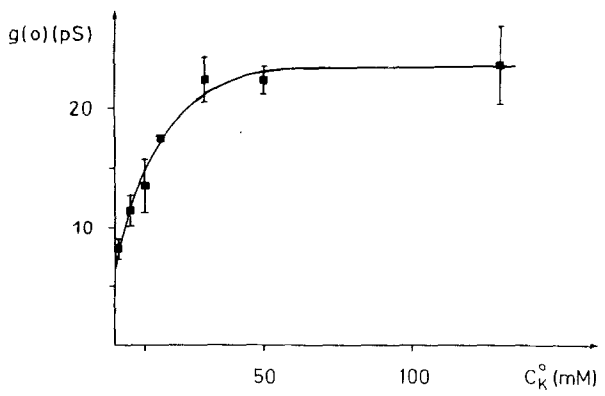


**Fig. 5.** The single channel conductance as a function of the extracellular concentration of $K^+$. Inside (bath) solution: 130 mM KCl, 5 mM MOPS (pH = 7.4), 22 $\mu$M $CaCl_2$. Outside (pipette) solution: 130 mM (NaCl + KCl), 5 mM MOPS, 1 mM EDTA (pH = 7.4). The bars represents 1 × SD of from 3–8 independent experiments. $V_m$ = 0 mV. The line was drawn by eye

In Fig. 6 *i-V* records from experiments with different bi-ionic distributions are shown: The inside solution is $K^+$, while the cation in the outside solution is either $Li^+$, $NH_4^+$, or $Rb^+$. At a membrane potential of 0 mV, outwardly directed current fluctuations of ≈1 pA were observed in the experiment with $Li^+$ and $NH_4^+$, whereas with $Rb^+$ only very small currents (≈0.2 pA) were detected. In the experiments with $Rb^+$ and $NH_4^+$, respectively, the $K^+$ current decreased and reversed to inward going currents at negative membrane potentials, while no inward current could be detected in the $Li^+$ experiment.

Results from the experiments with $Li^+$, $Cs^+$, and $Na^+$ in the pipette solutions are plotted in the *i-V* diagram of Fig. 7*A*. The outward $K^+$ currents saturated at positive membrane potentials and approached zero at negative potentials. It was impossible to demonstrate inward-going currents carried by any of the pipette cations. However, with $Rb^+$ or $NH_4^+$ in the pipette (Fig. 7*B*) reversal potentials could be determined. For $Rb^+$ the result was −9.0 mV ± 1.4 ($n$ = 4) and for $NH_4^+$ the result was −54.0 mV ± 4.1 ($n$ = 5). In the Table (column 2) the reversal potentials have been converted to permeability ratios. In case of the "impermeant" cations, $Li^+$, $Na^+$, and $Cs^+$, maximum values for the reversal potentials were estimated by extrapolation of the *i-V* curves. This approach allows calculation only of minimum values for the permeability ratios.

In a different approach ($n$ = 3), with $K^+$ in the bath and $Na^+$ in the pipette, the bath solution was exchanged with a $Tl^+$ solution (*see* Fig. 8). At least four active channels were initially observed. After perfusion, infrequent outward-going single channel currents (carried by $Tl^+$) were detected. Hence, $Tl^+$ is permeant. However, single channel events were never observed in symmetric $Tl^+$ solutions nor in bi-ionic $K^+/Tl^+$ experiments comparable with the experiments described above. This may be due to inhibitory effects of $Tl^+$ (Latorra, Vergara & Moczydlowski, 1983), since after flush with the original $K^+$ solution, only the activity from one channel remained. In the Table (column 3) the zero potential current of the K ion relative to various test ions are calculated. Since the driving forces in these experiments are in principle identical (nominally infinite), it is tentatively suggested that $Tl^+$ is slightly more conductive than both $NH_4^+$ and $Rb^+$, but less conductive than $K^+$.

## Discussion

### Open Channel Conduction Properties

Important results that must be accounted for are the inward rectifying *i-V* curves in symmetric solutions, the decreasing rectification at low extracellular $K^+$ concentrations, the saturation of the conductance at high extracellular $K^+$ concentrations, and the saturating outward currents at large positive potentials. These observations show that permeation is more complicated than simple diffusion.

The rectification is not due to a fast voltage-dependent block of the outward currents by some intracellular blocker ion. In the present experiments the only candidate for such blocking is $Ca^{2+}$, or in some cases, NMGA. However, a strong block by these cations is ruled out by the observations that

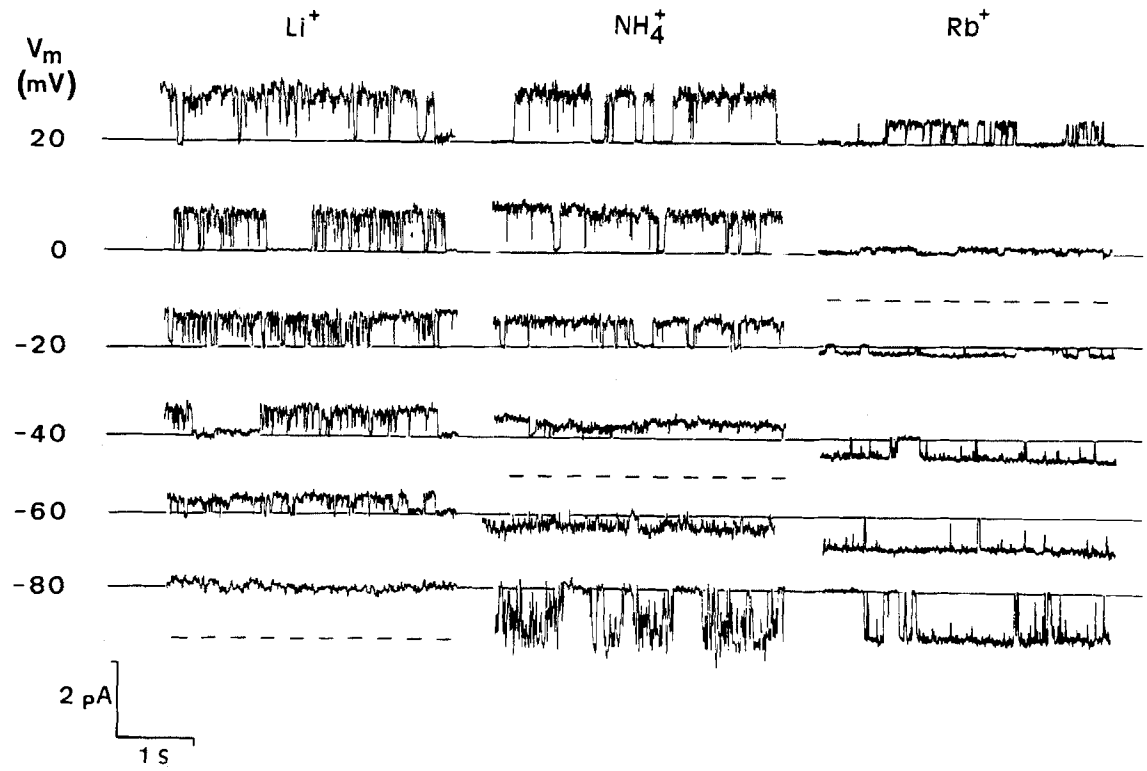


**Fig. 6.** *i-V* records illustrating the reversal potential method. All experiments were performed under bi-ionic conditions with $K^+$ in the intracellular solutions and different test cations in the extracellular solutions. Inside (bath) solution: 130 mM KCl, 5 mM MOPS, ≈4 mM NMGA (pH = 7.4), 22 μM $CaCl_2$. Outside (pipette) solution: 130 mM *X*Cl, 5 mM MOPS, ≈4 mM NMGA, 1 mM EDTA (pH = 7.4). *Left traces:* $X$ = Li; *middle traces:* $X$ = $NH_4$; *right traces:* $X$ = Rb. The dashed lines indicate the approximate reversal potentials. Filter frequency: 200 Hz

identical *i-V* curves were obtained with $Ca^{2+}$ concentrations from 10 to 100 μM and with and without NMGA (3–5 mM) added to the experimental solutions. Further, in a separate series of experiments only a weak blocking effect could be demonstrated when very high concentrations of NMGA (160 mM) were tested. Hence, it is concluded that the open channel rectification demonstrated in the present study is due to an asymmetry of the transport pathway. The lack of rectification at low extracellular $K^+$ concentrations (at near physiological levels) may be explained as desaturation of channel site(s) that lowers the single channel conductance (Fig. 5).

Sauvé et al. (1986) reported that similar inward rectifying *i-V* curves obtained with the mini-channel from HeLa cells and aortic endothelial cells (Sauvé et al., 1988) could be described by a very simple one-site-two-barrier model with the binding site placed in an electrical distance of 85% from the inside. In principle, this model does not apply to the erythrocyte channel for the following reasons: In macroscopic flux experiments the channel shows single-file diffusion with a flux ratio exponent of 2.7 (Vestergaard-Bogind, Stampe & Christophersen, 1985) and voltage-dependent $Na^+$ blockage characterized by an effective valence larger than 1 (Stampe & Vestergaard-Bogind, 1989). Hence, the channel is a multi-ion channel containing at least three binding sites in the permeation pathway (Hille & Schwarz, 1978). Therefore, even a minimal model based on the theory of absolute rates (Eyring, 1935) is complicated and contains a high number of free parameters (Läuger, 1980). A quantitative interpretation in terms of such a model will not be attempted.

## Selectivity

The $Ca^{2+}$-activated $K^+$ channel from human erythrocytes has been shown to be permeable to the $K^+$ analogs $Rb^+$, $NH_4^+$, and $Tl^+$, while the permeability to $Na^+$, $Cs^+$, and $Li^+$ are immeasurably low. This conclusion is based on two distinct approaches to ionic selectivity: (i) bi-ionic reversal potentials; and (ii) single channel conductance measurements.

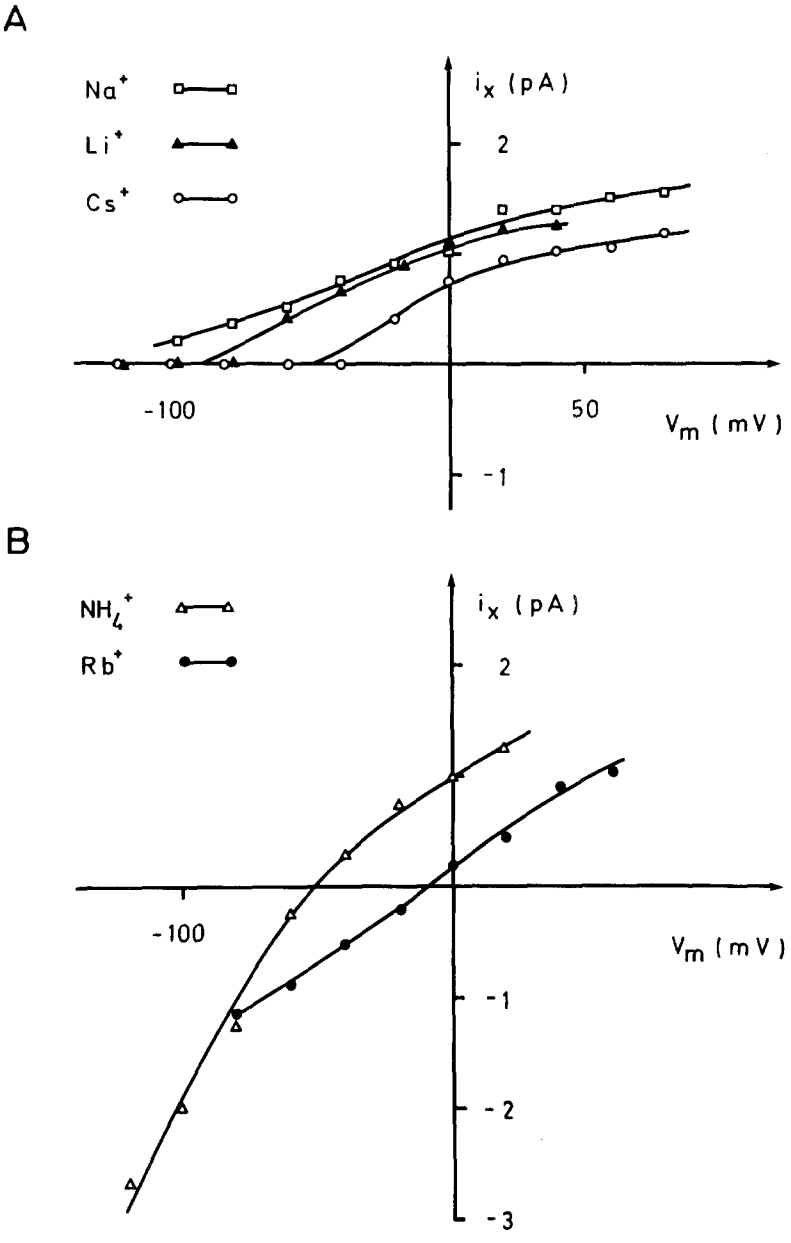


**Fig. 7.** *i-V* curves obtained under bi-ionic conditions with $K^+$ in the intracellular solutions and different test cations in the extracellular solutions. Inside (bath) solution: 130 mM KCl, 5 mM MOPS, ≈4 mM NMGA (pH = 7.4), 22 μM $CaCl_2$. Outside (pipette) solution: 130 mM *X*Cl, 5 mM MOPS, ≈4 mM NMGA, 1 mM EDTA (pH = 7.4). (*A*) *X* = Na ($n$ = 3) (□); *X* = Li ($n$ = 2) (▲); *X* = Cs ($n$ = 2) (○). (*B*) *X* = $NH_4$ ($n$ = 5) (△); *x* = Rb ($n$ = 4) (●). Lines were drawn by eye

As judged from i, the selectivity sequence ($P_K/P_X$) is:

$K^+$ (1.0) ≥ $Rb^+$ (1.4 ± 0.1) ≥ $NH_4^+$ (8.5 ± 1.3) ≫ $Na^+$ (>110); $Cs^+$ (≫5); Li (>50).

As judged from ii, the selectivity sequence ($g_K/g_X$) is:

$K^+$ (1.0 ± 0.1) ≥ $Tl^+$ ≥ $NH_4^+$ (1.7 ± 0.3) ≥ $Rb^+$ (2.1 ± 0.4) ≫ $Na^+$; $Cs^+$; $Li^+$ (>15).

Although the conductances and permeabilities are complex transport parameters, both sequences correspond to the relative affinities for equilibrium binding of the alkali metal ions to a site of intermediate field strength [Eisenman's series IV or V (Eisenman, 1962)]. However, it is interesting to note that the absolute values of the selectivity ratios as well as the relative position of $Rb^+$ and $NH_4^+$ are different in the two sequences. These results are not conflicting, rather they violate the independence principle (Hodgkin & Huxley, 1952) for ion transport through this channel.

**Table 1.** Summary of the selectivity parameters characterizing the human erythrocyte $Ca^{2+}$-activated $K^+$ channel[a]

| Ionic gradients | In \| Out $K^+ \mid X^+$ | In \| Out $X^+ \mid X^+$ | In \| Out $X^+ \mid Na^+$ |
|---|---|---|---|
| Test-ion ($X^+$) | $P_K/P_X$ | $g_K^o/g_X^o$ | $i_K^o/i_X^o$ |
| $K^+$ | 1.0 | 1.0 ± 0.1 | 1.0 |
| $Rb^+$ | 1.4 ± 0.1 | 2.1 ± 0.4 | 3.0 |
| $Na^+$ | >110 | >15 | — |
| $Cs^+$ | ≫ 5 | >15 | — |
| $Li^+$ | > 50 | >15 | — |
| $NH_4^+$ | 8.5 ± 1.3 | 1.7 ± 0.3 | 2.5 |
| $Tl^+$ | — | — | 2.0 |

[a] The permeability ratios ($P_K/P_X$) were calculated from bi-ionic reversal potentials (130 mM; $K^+$ in the bath solution and the test cation ($X^+$) in the pipette solution. The conductance ratios ($g_K^o/g_X^o$) were calculated from the zero current conductances obtained in independent experiments with symmetric solutions (130 mM) of the test cations. The current ratios ($i_K^o/i^o{}_X$) are based on the outward going currents obtained at $V_m = 0$ mV in bi-ionic experiments (130 mM) with the test on the intracellular side of the membrane and the effectively impermeant Na ion on the extracellular side.

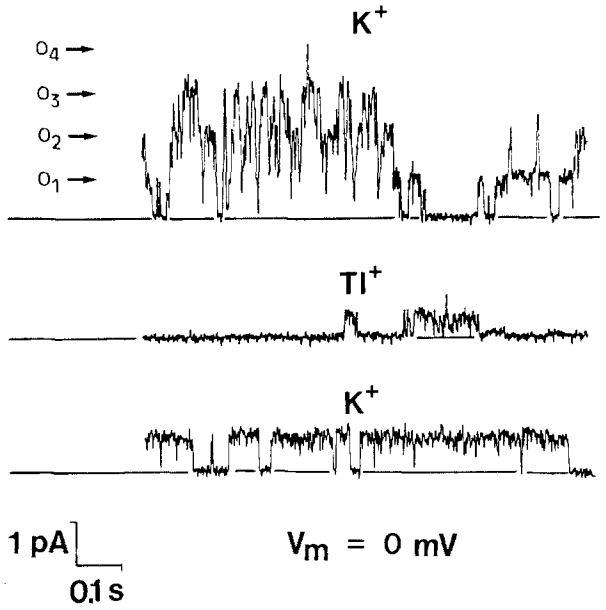


**Fig. 8.** *Upper trace:* Distinct single channel fluctuations between five current levels indicating that the patch contains at least four active channels. Inside (bath) solution: 130 mM $KNO_3$, 5 mM MOPS, ≈4 mM NMGA (pH = 7.4), 22 μM $Ca(NO_3)_2$. Outside (pipette) solution: 130 mM NaCl, 5 mM MOPS, ≈4 mM NMGA, 1 mM EDTA (pH = 7.4). $V_m = 0$ mV. *Middle trace:* Single channel fluctuations from the patch after extensive perfusion of the bath with a Tl-solution: 130 mM $TlNO_3$, 5 mM MOPS, ≈4 mM NMGA (pH = 7.4), 22 μM $Ca(NO_3)_2$. $V_m = 0$ mV. *Lower trace:* Single channel fluctuations from the patch after perfusion with the original K solution (from upper trace). $V_m = 0$ mV. Filter frequency: 200 Hz

Studying the human erythrocyte $K^+$ channel with the patch-clamp technique, Grygorczyk & Schwarz (1985) found from several potentials the following selectivity sequence: $K^+$ (1.0) > $Rb^+$ (1.3) > $Na^+$ (17) ≫ $Cs^+$ (immeasurably high). However, their permeability sequence is partly based on experiments with mixed ion solutions ($Na^+$) and partly on bi-ionic experiments ($Rb^+$). Hence the results are not directly comparable and give only a qualitative estimate of the permeability sequence. The result for $Na^+$ deviates strongly from the bi-ionic permeability ratio obtained in the present study as well as from results obtained in macroscopic flux experiments (Simons, 1976; Lew & Ferreira, 1978). Grygorczyk and Schwarz made no systematic *g*-sequence analysis, but they did observe that the inward-going currents carried by $Rb^+$ in the bi-ionic experiment were less than should be expected from a permeability ratio of only 1.3.

## Comparison with Other $K^+$ Channels

The relative selectivity sequence of the $Ca^{2+}$-activated $K^+$ channel from the human erythrocyte seems to parallel that of most delayed rectifiers as well as inward rectifiers (Hille, 1973; Hagiwara & Takahashi, 1974). The only permeant ion species are $Tl^+$, $K^+$, $Rb^+$, and $NH_4^+$, while the permeability to $Na^+$, $Cs^+$, and $Li^+$ are immeasurably low. The erythrocyte channel also resembles the $Ca^{2+}$-activated maxi-$K^+$ channel from T-tubules. The selectivity of this channel has been extensively characterized at the single channel level both from bi-ionic permeability ratios (Blatz & Magleby, 1984) and from measurements of single channel conductances (Eisenman, Latorre & Miller, 1986). Although the maxi-channel is also a multi-ion channel (Eisenman et al., 1986), at some points it is very different from the erythrocyte channel: The single channel conductance in symmetric $K^+$ is 250 pS, the current-voltage relations are symmetric and deviates only from linearity at large voltages, and the *i-V* shapes depend on the nature of the permeating ion. This is in contrast to the erythrocyte channel with a zero current conductance of 20 pS, inward rectifying *i-V* curves, and *i-V* shapes that depends little on the ionic species. Due to the high conductance of the maxi-channel, it is surprising that it is at least as selective as the small erythrocyte channel (Latorre & Miller, 1983). In general, it seems noteworthy that $K^+$ channels with highly different conduction properties are very similar when ionic selectivity is considered.

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