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# The gating of human red cell $Ca^{2+}$-activated $K^+$-channels is strongly affected by the permeant cation species

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**Using inside-out patches, the effect of various permeant cations on the gating behaviour of the human red cell $Ca^{2+}$-activated $K^+$-channel was examined. For symmetric solutions the dwell time histograms indicated two shut and two open states. Mean open times as well as the open-state probability were affected by the permeant cation species: $Rb^+$ stabilised the channel in the open configuration, whereas $NH_4^+$ had a destabilising effect. Intermediate stability was obtained in $K^+$ solutions. Bi-ionic experiments indicated that the gating was influenced by the ion species occupying the channel, rather than by ions bound to external modifier sites.**

Ion channels constitute a group of membrane proteins that catalyse ion transport by offering an intermittent hydrophilic transport pathway through the hydrophobic lipid membrane. Contrary to carriers and pumps, the transport through ion channels occur without extensive changes in the protein conformation, allowing an exceedingly high transport rate [1]. Also distinct from other transport systems is the ability to switch between a non-conductive and a conductive form by the gating process. Conduction and gating usually are considered as independent aspects of the channel function: The gating is affected by physiological variables (membrane potential, chemical messengers, etc.) leading to activation or deactivation of the channel, whereas, once activated, the open channel specifies the single-channel conductance and ionic selectivity. The aim of the present study is to demonstrate that the gating kinetics and open state probability of the $Ca^{2+}$-activated $K^+$-channel from human red cells is affected by the nature of the permeant cation, showing that conduction and gating — in this channel at least — are not independent.

Patch clamp experiments [2] were performed at room temperature (20–22°C) on human erythrocytes suspended in the appropriate salt solutions (see figure legends). The electrodes were balanced to electrical zero with the open pipette in the bath. After giga seal formation the cell was destroyed to obtain an inside-out configuration of the excised patch. All experiments were performed with 22 $\mu$mol/l $Ca^{2+}$ in the bath, a concentration which is saturating with respect to activation of the channels. Only patches containing a single active channel were used in the kinetic analyses.

The signals from the patch clamp amplifier (EPC-7, List Electronic) were recorded with a total background noise (RMS) of 0.19–0.23 pA (3 kHz). Before digitalisation (44.1 ksamples/s) and storage on tape, the signal was low pass filtered (antialiasing, 10 kHz Butterworth). For analyses the digitised data were transferred to a computer. Calculations were performed on data that were further low pass filtered using a digital Gaussian filter. Current amplitude histograms were fitted to a sum of two normal distributions. The fit parameters (standard deviation, mean, and amplitude) were used to calculate the areas under the individual peaks, from which the open-state probability ($P_o$) was estimated. Mean life times ($\tau$) were obtained from fit of the open and closed dwell time distributions to a sum of exponentials.

Single-channel records from experiments with identical bath and pipette solutions (symmetrical conditions) of $K^+$, $NH_4^+$, or $Rb^+$, are shown in Fig. 1A. The conductance selectivity sequence ($g_K > g_{NH_4} > g_{Rb}$), corresponds to Eisenman's series IV or V [3], which has been shown to be the sequence for highly selective $K^+$-channels [4]. As seen from the figure, there are obvious differences in the gating kinetics in the three experiments: In symmetric $NH_4^+$ solutions short open

Abbreviations: EDTA, ethylenediaminetetraacetic acid; Mops, 3-(*N*-morpholino) propanesulfonic acid; NMGA, *N*-methyl-D-glucamine.

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periods were dominating, whereas much longer open periods were observed in $Rb^+$ solutions. In the $K^+$ experiment the open-state durations seemed to be intermediate between the ones observed in the $NH_4^+$ and $Rb^+$ experiments. In Fig. 1B typical current amplitude histograms are shown, illustrating the different conductances as well as different open-state probabilities. The short openings in the $NH_4^+$ experiment were largely unresolved at 0.5 kHz filtering resulting in an apparent presence of substates (compare with Fig. 1A), whereas at 5 kHz filtering the current histograms conformed to two distinct normal distributions. As shown in Table I, the value of $P_o$ depends strongly on the conductive ion: $0.943 \pm 0.056$ ($Rb^+$), $0.63 \pm 0.086$ ($K^+$), and $(0.190 \pm 0.071)$ $NH_4^+$, whereas varied negative potentials has no influence. The calculations were based on three different experiments with each cation, but identical results were obtained in a larger number ($n$) of experiments: $K^+$ ($n = 144$); $NH_4^+$ ($n = 11$); $Rb^+$ ($n = 17$).

To visualise the effect of the permeant cations on the gating kinetics, normalised open- and closed-state histograms are shown (Fig. 2). The closed dwell time distributions were described by a sum of two exponentials with mean closed times that were not significantly af-

TABLE I

*Open state probabilities at various membrane potentials in symmetric solutions of $NH_4^+$, $Rb^+$, or $K^+$ (130 mmol/l; solutions compositions as in Fig. 1*

The actual calculations are based on records lasting from 20 to 90 s, and the mean ± S.D. were obtained from three different experiments with each cation.

| $V_m$ (mV) | $P_o \pm$ S.D. | | |
|---|---|---|---|
| Cations: | $NH_4^+$ | $K^+$ | $Rb^+$ |
| −30 | | 0.574 ± 0.013 | 0.986 ± 0.021 |
| −40 | 0.259 ± 0.197 | 0.642 ± 0.050 | 0.912 ± 0.083 |
| −50 | | 0.661 ± 0.056 | 0.885 ± 0.150 |
| −60 | 0.229 ± 0.147 | 0.676 ± 0.105 | 0.953 ± 0.033 |
| −70 | | 0.669 ± 0.145 | 0.972 ± 0.023 |
| −80 | 0.121 ± 0.072 | 0.607 ± 0.060 | 0.970 ± 0.015 |
| −90 | | 0.564 ± 0.066 | 0.964 ± 0.001 |
| −100 | 0.072 ± 0.083 | 0.659 ± 0.066 | 0.944 ± 0.018 |

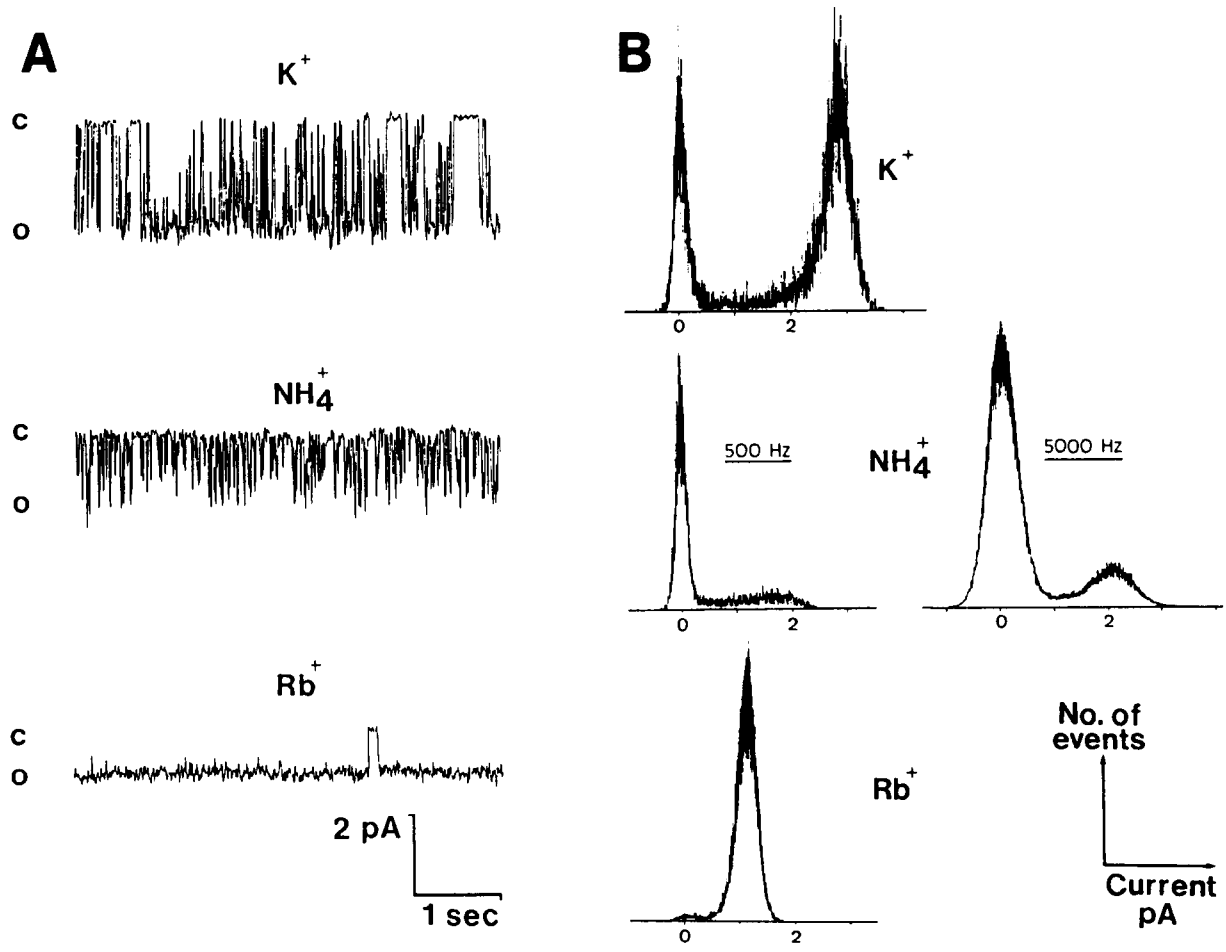


Fig. 1. (A) Single-channel fluctuations from patches exposed to symmetric solutions of $K^+$, $NH_4^+$, and $Rb^+$ ($X^+$), respectively. Solutions compositions: 130 mmol/l XCl, 5 mmol/l Mops, 3–4 mmol/l NMGA (pH = 7.4), 22 μmol/l $CaCl_2$. $V_m = -80$ mV. Filter frequency: 0.5 kHz. (B) Current amplitude histograms constructed from the experiments in A. Base-line peaks are adjusted to 0 pA. Binsize: 2.5 fA. Filter frequency: 0.5 kHz and 5 kHz.

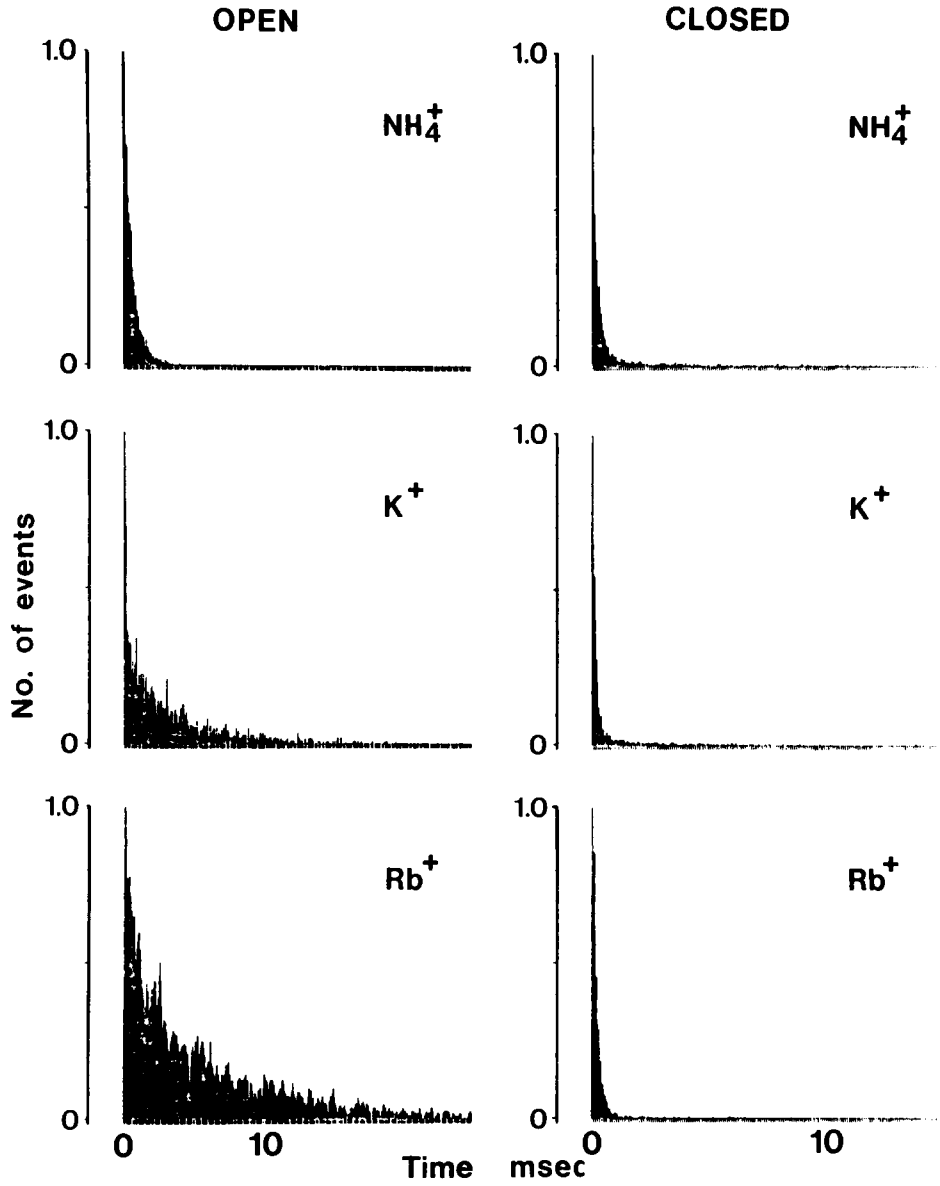


Fig. 2. Closed and open state histograms obtained from experiments with symmetrically distributed cations. All histograms were normalised with respect to the (fitted) Y-axis intercept. $V_m = -80$ mV. Binsize: 45 μs. Filter frequency: $K^+$ and $NH_4^+$ experiments: 5 kHz. $Rb^+$ experiment: 2 kHz.

fected by the nature of the permeant cations: 0.35 ms and 5.52 ms. The open state distributions could likewise be described by two exponentials; however, with mean open times that were strongly affected by the cation species: 0.3 ms and 0.6 ms ($NH_4^+$), 0.1 ms and 2.8 ms ($K^+$), and 0.8 ms and 5.9 ms ($Rb^+$), respectively. The difference in the open-state probability with various cations thus reflects a change in the open-state dwell times. Put in another way the permeant ions do not affect the frequency of openings, rather they determine the mean time that the channel stays in the open state after an opening event.

Assuming Markov behaviour [5] the minimum kinetic scheme must include at least two closed (C) and two open (O) states:

$$C_I \rightleftarrows C_A \rightleftarrows O_1 \rightleftarrows O_2$$

This is a sequential model where only the activated closed state, $C_A$, is able to shift to the open state, and the transition from the inactivated closed state, $C_I$, to $C_A$ represents the binding of $Ca^{2+}$. From $O_1$ the channel may return to the closed state, or it may switch to another open state, $O_2$, with identical unit conductance.

$O_2$ constitutes the stabilised open channel and it is suggested that the equilibrium is displaced towards $O_1$ in $NH_4^+$ solutions, towards $O_2$ in $Rb^+$ solutions, and is more evenly distributed in $K^+$ solutions. The variation of the mean life time for the second open state in these experiments may then indicate that the transition of $O_2$ to $O_1$ is influenced by the different cations.

The ion effects may be interpreted as a result of different binding affinities to superficially placed modifier site(s), the existence of which has been suggested before [6]. Alternatively, a direct effect of binding to internal sites in the channel could be imagined. Without a detailed knowledge of the concentration dependence of gating kinetics and occupancy for each cation, a sharp distinction between these alternatives are not possible. However, the results of the following experiments seem to favour the occupancy hypothesis rather than the modifier site hypothesis.

In Fig. 3A the $i - V$ curve from a bi-ionic experiment with $Rb^+$ (inside) and $NH_4^+$ (outside) is shown. At zero membrane potential an outward going single-channel current of 0.4 pA were observed. The outward current carried by $Rb^+$ reversed to an inward going current of $NH_4^+$ at a potential of $-50$ mV, indicating that $Rb^+$ is more permeant than $NH_4^+$. The value of the reversal potential, $V_r$, corresponds to a constant field permeability ratio of 7.2. In the present context it is important that the gating for outward current ($Rb^+$) is dominated by long open periods, whereas the gating for inward current ($NH_4^+$) is dominated by short open periods (Fig. 3B). Similar bi-ionic experiments have been performed with $K^+/Rb^+$ and with $K^+/NH_4^+$, which also demonstrated gating characteristics that changed with the direction of the current.

Using red cell suspensions ($K^+$, $Na^+$) it has been demonstrated that the present channel is of the multi-ion type that shows single-file diffusion [7]. If the single-file condition applies also in bi-ionic experiments with $NH_4^+$ and $Rb^+$, then at $(V_m - V_r) > 20$ mV the net flux through the channel is nearly identical to the *cis* → *trans* single flux. Accordingly, occupancy of the channel sites by ions from the *cis* side are expected to increase sharply as $(V_m - V_r)$ increases. Since a superficial modifier site is sensitive to the cations in the bulk solution on the same side of the membrane, the change in the gating characteristics in the bi-ionic experiment is most straightforwardly interpreted as a shift from a $Rb^+$ filled pore (stabilisation) to a $NH_4^+$ filled pore (destabilisation.

The tendency to stabilise the channel in the conductive configuration is expressed by the following sequence:

$$Rb^+ > K^+ > NH_4^+$$

It is noteworthy that this 'selectivity sequence' is different from both the conductance sequence ($K^+ > NH_4^+ > Rb^+$) and from the permeability sequence ($K^+ > Rb^+ > NH_4^+$) found in this channel (Christophersen, in press).

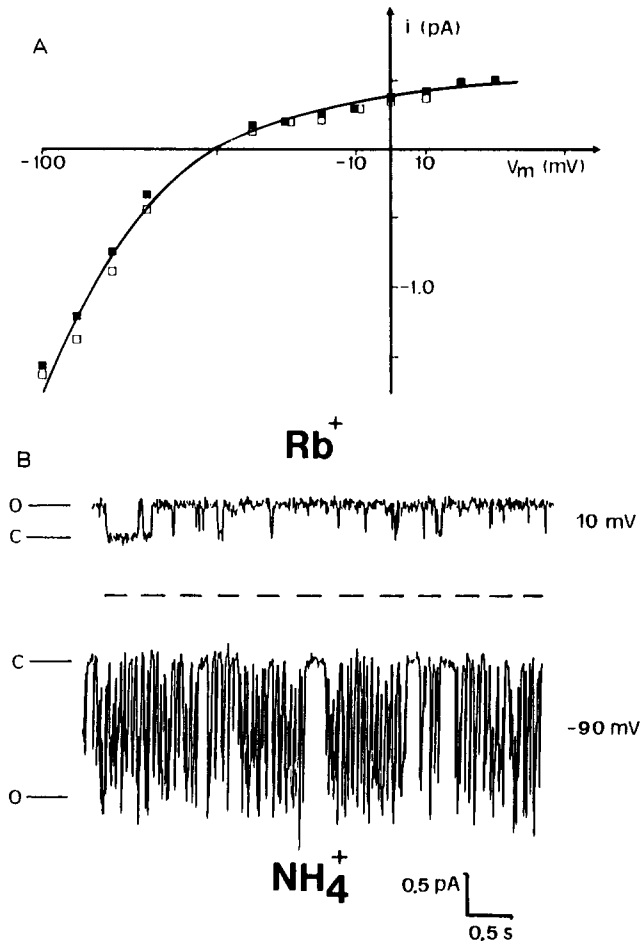


Fig. 3. (A) The single-channel $i - V$ curve from a bi-ionic experiment with $Rb^+$ (inside) and $NH_4^+$ (outside). Solutions: 130 mmol/l XCl, 5 mmol/l Mops, 3–4 mmol/l NMGA (pH = 7.4), 22 μmol/l $CaCl_2$. Data from two different experiments. (B) Single-channel fluctuations from the bi-ionic experiment in A. Filter frequency: 0.5 kHz.

Gating modulating effects of permeant ions are quantitatively well described for the gramicidin A channel, where ion occupancy has been showed to slow dissociation of the dimeric complex [8]. For membrane channels, where gating probably is a more complicated process than the gramicidin gating, reports on the role of the permeant ions are few. Investigating the voltage dependent decay of the current through a population of delayed rectifier $K^+$ channels, Swenson and Armstrong [9] showed that the channel closing rate were slowed, relative to a pure $Na^+$ medium, by the presence (100 mmol/l) of external $K^+$ (factor 1.7) or $Rb^+$ (factor 2.9). They suggested that $Rb^+$ slowed the closing more effectively than $K^+$ due to the higher $Rb^+$ dwell time in the channel. Cukierman et al. [10] reported that at high concentrations of $Cs^+$ the cation channel from sarcoplasmic reticulum remains open all the time. The effects were interpreted as a tight binding in the channel pore,

and it was suggested that the SR-channel closes only when it is empty. This, however, cannot be a general property of $K^+$-channels, since both the delayed rectifier [11] and the $Ca^{2+}$-activated maxi $K^+$-channel [12] are able to close with a blocking ion in the channel pore.

The open-channel stabilisation/destabilisation demonstrated in the present study is of interest from a biophysical point of view, emphazising the relationship between conduction and gating. However, it would be interesting to know if the equilibrium between the conductive states could also be affected by physiological parameters, which would then represent an independent regulatory mechanism of the open-state probability at high $Ca^{2+}$ concentrations.

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