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Evidence for a voltage-gated, non-selective cation channel in the human red cell membrane

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Using the patch clamp technique we have identified a voltage-dependent, non-selective cation channel in the human red cell membrane. Basic properties of this channel are reported, and it is proposed that it may be involved in the increased transport of cations which is seen when intact human red cells are suspended in a depolarising media.

The K^+ and Na^+ conductances of the human red cell are under physiological conditions orders of magnitudes lower than the corresponding conductances of most other cell types. However, when the intracellular Ca^{2+} activity is raised the K^+ conductance increases dramatically, resulting in a net efflux of K^+ and a concomitant hyperpolarisation. It is now well documented that this is due to activation of a voltage insensitive and highly K^+ selective 'mini' channel (20 pS) with inward rectifying properties [1,2].

Using the patch clamp technique [3] we demonstrate here that - besides the Ca^{2+}-activated K^+ channel - the human red cell membrane contains a Ca^{2+}-insensitive, non-selective cation channel. This channel, which is most frequently observed at high ion concentrations, exhibits activation by positive voltages with slow relaxations between steady-state holding potentials. The current-voltage characteristic is a symmetric, superlinear curve with a zero current conductance of 35 pS, when the patch is bathed in symmetric 500 mM salt solutions.

Red cells from freshly tapped human blood were washed in approx. 30 vol. ice-cold isotonic KCl and stored on ice. Small aliquots of the cell suspension were transferred to the experimental chamber and diluted to a final cytocrit of approx. 0.005%. After giga seal formation (5–50 GΩ) inside-out patches either formed spontaneously or were obtained by moving the pipette tip through the air-water interface. All experiments were performed at room temperature (20–22°C). The electrodes were zeroed with the open pipette in the bath and patch potentials were controlled by polarisation of the pipette. The membrane potential is defined as $V_{bath} - V_{pipette}$ and electric current from bath to pipette is taken to be positive. The signals from the patch clamp amplifier (EPC-7, List Electronic, Darmstadt, F.R.G.) were filtered (3 kHz), digitised (44100 samples/s, 16 bit datasize) and stored on tape. For analyses the digitised data were transferred to a computer.

A characteristic of the present channel was the infrequency of observations at physiological salt concentrations, whereas it was observed at least as frequently as the Ca^{2+}-activated K^+ channel at 500 mM salt. Thus the experiments presented here were carried out at this high salt concentration (solutions compositions in figure texts).

In Fig. 1 single channel records from experiments where the patch is exposed to identical, symmetric KCl solutions are shown. The patch contained a channel with conduction and kinetic properties being very distinct from the Ca^{2+}-activated K^+ channel. Steady-state current fluctuations were not observed at negative membrane potentials (inward currents), whereas current fluctuations of larger unit amplitude and longer mean duration, than observed for the Ca^{2+}-activated K^+ channel, were discernible at positive potentials. In experiments where the Ca^{2+} activity was above approx. 1 μM, and both channels were present, the two channel types were always distinguishable due to these differences. At positive membrane potentials, the current fluctuated between zero and a well-defined main state

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Mops, 3-(*N*-morpholino)propanesulphonic acid; NMGA, *N*-methyl-D-glucamine.

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(that is the most frequently observed current level). However, as demonstrated in fig. 1B, the channel shifted occasionally to states with different conductances. The examples presented show differences in several respects. In the upper trace the substate is characterised by a lower conductance than the main state, whereas the substate in the lower trace has a higher conductance. In both cases clear gating events are evident within the substate (see inset). The substate with the higher conductance is particularly interesting, since the transition from the main state involves a shift in both gating kinetics, open state conductance, and, most surprisingly, the apparent closed state conductance. This may reflect some relation between conduction and gating properties as has also been inferred for the Ca^{2+}-activated K^+ channel [4]. These substate phenomena are interesting but their interpretation is not straightforward and they will not be considered further in this paper.

In the case of outward going currents at positive membrane potentials, the *i-V* curve for the open channel was easily obtained from steady-state experiments as those in Fig. 1. In an attempt to obtain inward going currents at negative membrane potentials, the experimental protocol illustrated in Fig. 2 was chosen. The patch was initially clamped at a positive holding potential to achieve the characteristic steady-state current fluctuations. When an open state burst was detected the membrane potential was switched to a new voltage. Inward currents were detected for a few seconds after the voltage step to a negative membrane potential. Thus, the kinetic relaxation for voltage inactivation of the channel is slow compared to most other voltage-gated channels. A quantitative analysis of steady-state and relaxation kinetics are in progress.

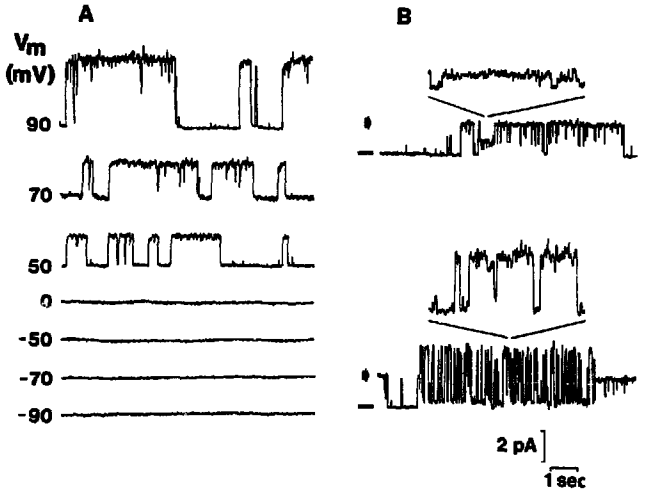

Fig. 1. (A) Single channel recordings showing the effect of the membrane potential on the normal gating and conductance mode of the channel. (B) Single channel records exhibiting shifts in gating and conductance mode. Bars and arrows indicate the normal closed and open current levels, respectively. $V_m = 50$ mV. Solutions: 500 mM KCl, 5 mM Mops, 3–4 mM NMGA, 22 μM $CaCl_2$, 1 mM EGTA, (pH = 7.4). Cut-off frequency: 500 Hz (insert 1000 Hz). Duration of insets are 100 ms.

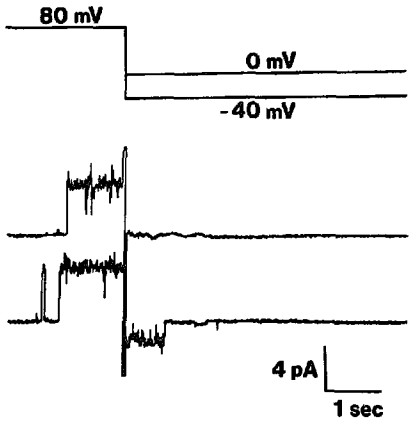

Fig. 2. The experimental protocol for obtaining single-channel currents at negative membrane potentials. The patch was clamped at a holding potential of 80 mV. During an opening event the potential was alternately stepped to (A) 0 mV (the equilibrium potential for K^+) and (B) −40 mV (the equilibrium potential for Cl^-). Single channel fluctuations were obtained in 11 of 30 steps to E_{Cl}, whereas single channel events were never obtained after a step to E_K. Solutions: pipette: 500 mM KCl, 5 mM Mops, 3–4 mM NMGA (pH = 7.4). Bath: 100 mM KCl, 400 mM potassium glyconate, 5 mM Mops, 3–4 mM NMGA, 22 μM Ca^{2+}, 1 mM EGTA (pH = 7.4). The traces have been corrected for seal currents and capacitative transients. Cut-off frequency: 400 Hz.

The *i-V* curves for the main conductive state presented in Fig. 3 consist partly of data taken at constant holding voltages and partly of data obtained immediately after a voltage step. For positive voltages the two methods gave identical results. Under both mono- and bi-ionic conditions with equal salt concentrations on the two sides of the membrane, conductances, reversal potentials and *i-V* shapes showed a striking independence of the nature of small inorganic cations (K^+, Rb^+, NH_4^+, Na^+). The current reversed at zero mV and the *i-V* curve was nearly linear at low potentials (±40 mV) with a conductance of 35 pS, whereas it was superlinear at larger potentials with a single channel chord conductance increasing to 70 pS at a ±90 mV driving force. Substituting 80% of the Cl^- in the bath solution with glyconate changed neither the reversal potential nor the single channel conductance (see Fig. 2). These results demonstrate that the channel is permeant to small inorganic cations, but impermeant to Cl^-. The lack of selectivity holds whether permeabilities (bi-ionic condition) or single channel conductances (symmetric condition) are considered. The symmetric *i-V* curves, from experiments with identical cation concentrations on the two sides of the membrane, indicate a symmetric potential energy profile for the permeating ions. However, the non-linearity is incompatible with a simple diffusion regime.

As shown in Fig. 2, a voltage-dependent gating, rather than an extreme outward rectification, is the

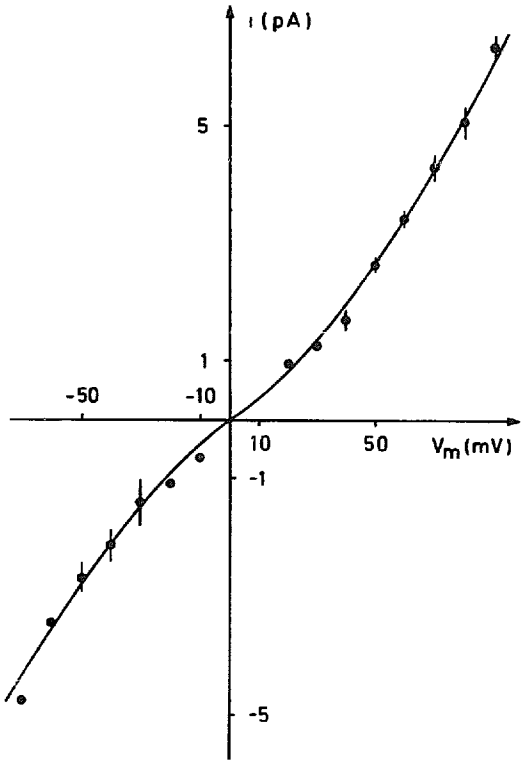

Fig. 3. The current voltage characteristic (voltage range: −70 mV; 90 mV) for the main open state of the channel bathed with salt concentrations of 500 mM in bath as well as pipette solutions. Identical results were obtained with all cations tested (K^+, Rb^+, NH_4^+ and Na^+). Points represent single determinations or mean values ±1 S.D. from 3–11 independent experiments. The line was drawn by eye.

basis for the lack of observations of inward currents at negative holding potentials. It is therefore possible to calculate a steady-state open state probability (P_o) as a function of the membrane potential also at negative potentials, see Fig. 4. P_o increases steeply from 0 at negative potentials to about 0.85 at positive voltages.

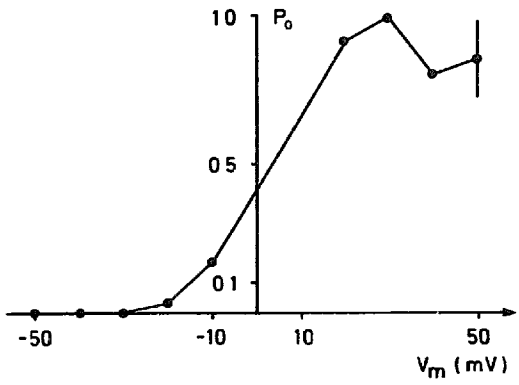

Fig. 4. The steady-state open probability (P_o) as a function of V_m. P_o was calculated by fitting current amplitude histograms (obtained from 1 min of continuous recording at each potential) to a sum of two Gaussians. To avoid artefacts due to possible non-stationary gating, P_o at $V_m = 50$ mV was calculated between each shift to a new potential. The experimental result at this potential, therefore, is the mean value ±1 S.D. from nine runs. Solutions: 500 mM KCl, 5 mM Mops, 3–4 mM NMGA (pH = 7.4).

The value at negative potentials was inevitably close to zero, while the degree of activation at positive potentials varied from one experiment to another. We have not yet been able to account for this variation. However, the basic features of the voltage-dependence is unequivocal, since it was demonstrated in all experiments.

An intriguing observation from the present study was the more frequent occurrence of the cation channel at high salt concentrations. The channel was not entirely inactivated at lower concentrations, but regarding the low number of observations a quantitative study was difficult to carry out. The activation mechanism is unknown. It may be indirect via the osmotic stress imposed on the erythrocytes during the patch preparation or direct via a stabilising effect of the transported ions on the ion channel itself.

Non-selective cation channels have been described from a variety of cells and tissues, including nerve, heart muscle, and epithelial cells (for a review, see Ref. 5). This class of channels is heterogeneous both with respect to conductance, selectivity and activation mechanisms. In salt solutions of near physiological osmolarity of alkali metal cations, linear *i-V* curves with single channel conductances in the range of 20–35 pS are usually found. Most types are activated by internal Ca^{2+} concentrations in the physiological range [6,7] but activation by membrane stretch [8] and depolarised membrane potentials in combination with Ca^{2+} are also reported [9].

It is well known that the passive fluxes of K^+ and Na^+ from intact human erythrocytes are increased when the cells are suspended in solutions of low ionic strength [10,11] (for a review see Ref. 12). The fluxes were suggested to be conductive and the activation was attributed to an effect of the positive membrane potential, which results from the reversed Cl^- gradient, rather than to an effect of the low ionic strength "per se". Inhibition of the fluxes by 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) were interpreted as involvement of the band III protein [13]. From flux measurements combined with an independent determination of the membrane potential by the carbonylcyanide-*m*-chlorophenylhydrazone (CCCP)-method, it has recently been shown that K^+, Na^+ and Ca^{2+} conductances increase at positive potentials and that the activation can be inhibited by ruthenium red [12]. A voltage-activated cation pathway with identical properties were demonstrated in HK sheep red blood cells, but not in LK cells from sheep and dog [14]. It was proposed, that the fluxes were mediated by a transport system not previously characterised, but some relation to the Na^+, K^+-pump was considered.

Although further work at physiological salt concentrations is required, we suggest that the voltage-activated cation fluxes from intact red cells may - at least in the case of K^+ and Na^+ - be mediated by the

voltage-dependent cation channel described in this study. Presently, we are not tempted to suggest any relation to other red cell transport systems, since we feel that a genuine ion channel most probably represents an independent transport pathway. The channel is not identical to any of the above-mentioned cation channels from other cells, since it is independent of cellular Ca^{2+}, it is voltage-dependent and the *i-V* curve is symmetric non-linear.

Acknowledgements

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References

1 Grygorczyk, R. and Schwarz, W. (1983) Cell Calcium 4, 49–510.
2 Christophersen, P. (1991) J. Membr. Biol. 119, 75–83.
3 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
4 Bennekou, P. and Christophersen, P. (1990) Biochim. Biophys. Acta 1030, 183–187.
5 Partridge, L.D. and Swandulla, D. (1988). Trends Neurol. Sci. 11, 69–72.
6 Yellen, G. (1982) Nature 296, 357–359.
7 Von Tscharner, V., Prod'hom, B., Baggiolini, M. and Reuter, H. (1986) Nature 324, 369–372.
8 Christensen, O. (1987) Nature 330, 66–68.
9 Gray, M.A. and Argent, B.E. (1990) Biochim. Biophys. Acta 1029, 33–42.
10 Lacelle, P.A. and Rothstein, A. (1966) J. Gen. Physiol. 50, 171–188.
11 Donlon, J.A. and Rothstein, A. (1969) J. Membr. Biol. 1, 37–52.
12 Halperin, J.A., Brugnara, C., Tosteson, M.T., Ha, T.V. and Tosteson, D.C. (1989) Am. J. Physiol. 257, C986–C996.
13 Jones, G.S. and Knauf, P.A. (1985) J. Gen. Physiol. 86, 721–738.
14 Halperin, J.A., Brugnara, C., Ha, T.V. and Tosteson, D.C. (1990) Am. J. Physiol. 258, C1169–C1172.