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Non-selective voltage-activated cation channel in the human red blood cell membrane

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Abstract

Using the patch-clamp technique, a non-selective voltage-activated Na^+ and K^+ channel in the human red blood cell membrane was found. The channel operates only at positive membrane potentials from about +30 mV (inside positive) onwards. For sodium and potassium ions, similar conductances of about 21 pS were determined. Together with the recently described $K^+(Na^+)/H^+$ exchanger, this channel is responsible for the increase of residual K^+ and Na^+ fluxes across the human red blood cell membrane when the cells are suspended in low ionic strength medium.

Keywords: Cation channel; Erythrocyte; Patch clamp; Residual transport; Low ionic strength

1. Introduction

It is now known that the ground-state permeability of the red blood cell membrane to K^+ and Na^+ is not caused by simple electrodiffusion [1–4]. In practice, this ground-state permeability (also referred to as residual transport) is defined as membrane transport under conditions where all known specific transport pathways (pumps, channels, carriers) are inhibited. It has been reported that the residual transport of K^+ and Na^+ across the human erythrocyte membrane does not depend on the membrane potential, within the range of −8 to +45 mV [1,2]. Recently, a previously unreported electroneutral $K^+(Na^+)/H^+$ antiporter has been described in the human erythrocyte membrane [2]. This $K^+(Na^+)/H^+$ antiporter can explain the increased K^+ efflux of erythrocytes suspended in low ionic strength (LIS) media up to an extracellular (NaCl+KCl) concentration of 7.5 mM, which corresponds to a membrane potential of approximately +45 mV. This LIS effect was originally observed by Davson [5] and has been investigated further by other groups [1,2,4,6–11]. Donlon and Rothstein [9] described a triphasic increase of the K^+ efflux when the extracellular NaCl concentration was reduced (osmolarity compensated by adding sucrose). When the membrane potential of human red blood cells was changed from 0 mV to about +40 mV, these authors also observed a significant increase of the K^+ efflux. However, changing the membrane potential to values higher than +40 mV produced a more pronounced increase of the K^+ flux, and at membrane potentials higher than +170 mV they observed a third phase with a dramatic increase of the K^+ flux [9]. Although the effect seen at very high voltage

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(+170 mV) can be explained by the beginning of the electrical breakdown of the membrane [9], there is no explanation for the enhancement of the increase of the K^+ efflux at potentials higher than +40 mV. On the basis of tracer kinetic experiments, Halperin et al. [12] proposed a voltage-dependent, non-specific cation channel, which opens at membrane potentials higher than +20 mV. The existence of such a channel, which does not discriminate between K^+ and Na^+, was demonstrated by Christophersen and Bennekou [13] using the patch-clamp technique. However, in contrast to the results reported by Halperin et al. [12], this channel was observed to begin opening at membrane potentials of −20 mV and showed an increased open probability at higher (positive) potentials.

The aim of the present paper, therefore, was to reconcile the discrepancy between the results of Halperin et al. [12] and those of Christophersen and Bennekou [13].

2. Materials and methods

2.1. Blood and solutions

Red blood cells from stored bank blood, 3–5 days old, from healthy donors (blood group O Rh(+)) were used. Erythrocytes were diluted 1:500 in the bath solution used for the patch-clamp experiments. An aliquot of 5–10 μl of this suspension was added to the measuring chamber containing 2 ml of the bath solution (final haematocrit $\sim 2\times10^{-4}$%).

The pipette solution contained 20 mM Na-tartrate. The compositions of the bath solutions were 70 mM Na-tartrate or 70 mM K-tartrate. In some cases during the experiment the K-tartrate solution was replaced by a solution containing Na-tartrate or a solution free of Na-tartrate and K-tartrate (sucrose replacement). In addition, all solutions contained (mM): $BaCl_2$, 2.5; bumetanide, 0.1; ouabain, 0.1; glucose, 10; morpholinopropane sulfonic acid (MOPS), 10. The pH was adjusted to 7.4 with NaOH (Na^+-containing solutions), KOH (K^+-containing solutions), or Tris(hydroxymethyl)aminomethane (Tris) (Na^+- and K^+-free solution), and the osmolarity adjusted to 300 mosmol (by the addition of sucrose, measured using a vapour pressure osmometer). All solutions were filtered using 0.2 μm filters (Nalgene, USA).

2.2. Patch-clamp measurements

Pipettes were pulled from standard haematocrit tubes (Hirschmann Laborgeräte, Germany) using a vertical pipette-puller (Sutter Instruments, USA). The pipettes had a resistance of about 20 MΩ. The headstage, as well as the amplifier (Axopatch D1), was supplied by Axon (USA) and the ITC 16 (purchased from Instrutech Corp., USA) was used as an AD/DA converter. An IBM personal computer and the Wintida 3.0 software (obtained from HEKA Elektronik, Germany) was used to record and analyse the data. The cells were visualised using an inverted microscope (IMT-2, Olympus, Japan) and manipulated using a hydraulic micromanipulator (WR-89, Narishige, Japan). The measurements were carried out at room temperature (23°C). The measuring chamber was constructed in such a way that it was possible to exchange the bath solution (full exchange in 5 min). Since it is not possible to measure a whole-cell configuration using human red blood cells, only experiments using inside-out patches were performed. At inside-out configurations the holding potential and the membrane potential have opposite signs. After a gigaseal (5–10 GΩ) was formed, a variety of blocks and ramps in the range of ±120 mV were applied. The currents were low-pass filtered at 1 kHz (Bessel filter) and digitised at 3 to 5 kHz. For data analysis all-point amplitude histograms were used.

2.3. Reagents

Inorganic salts, sucrose and glucose were of analytical grade. Ouabain, bumetanide, Na-tartrate, and K-tartrate were obtained from Sigma Chemical Co. (St. Louis, USA). Tris was purchased from Fluka Chemie (Buchs, Switzerland) and MOPS was obtained from Serva (Heidelberg, Germany).

3. Results and discussion

Patch-clamp experiments were performed to investigate the so far unidentified ion channels which con-

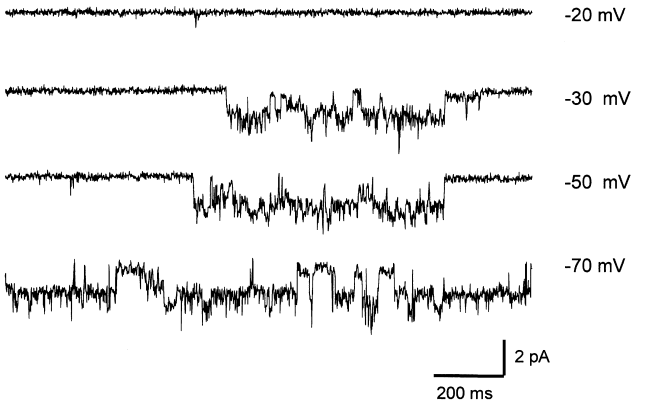

Fig. 1. Currents recorded from an inside-out patch of human red blood cells at different holding potentials. The pipette and the bath solution contained 20 mM Na-tartrate and 70 mM Na-tartrate, respectively (for additional components see Section 2).

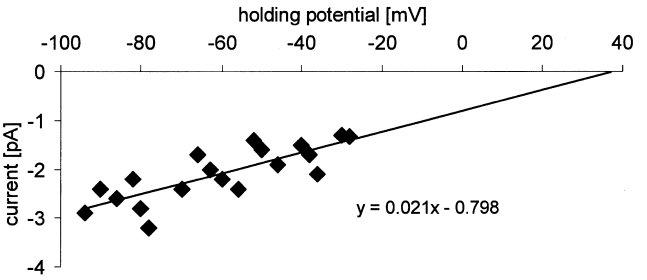

Fig. 2. Current–voltage relationship of channel activity taken from three inside-out patches of human red blood cells (data taken from recorded channel activity as shown in Fig. 1). The intersection of the voltage-axis (zero current) with the linear regression line represents the reversal potential for Na^+ of the channel activity (37 mV). The slope of the linear regression line represents the conductance of the channel of about 21 pS (see also Section 3).

tribute to the residual transport of K^+ and Na^+ across the human red blood cell membrane. One has to take into consideration that it has not yet proved possible to produce whole-cell patches of human red blood cells either in previous investigations in the literature or in our investigations.

Fig. 1 shows a representative example of channel opening events at different holding potentials. Channel activity, which was measured in the presence of Na^+ in the pipette solution and Na^+ in the bath solution (at different concentrations), could be seen in at least seven patches. The same channel characteristic was observed when Na^+ in the bath solution was replaced by an identical amount of K^+ (data not shown). When the holding potential was changed from +120 mV to −20 mV, no channel current was observed. Only when the holding potential was −30 mV (or lower) could channel opening events be seen (Fig. 1). The current traces (Fig. 1) were analysed in terms of all-point amplitude histograms. Amplitudes corresponding to the main state (maximum of the amplitude histogram) were taken for a current–voltage relationship (Fig. 2). From the slope of the regression line in Fig. 2, the main conductance of the channel was estimated to be 21 pS.

An analysis of the amplitude histograms showed that the current traces consist of at least four discrete levels. Although the possibility that there is more than one channel per patch cannot be completely ruled out, it seems more realistic to assume a single channel with three substates. This assumption is based on the fact that all amplitude histograms result in approximately the same value of current of the main state. If, however, the current traces reflect the presence of more than one channel, these channels should be of one type since they show the same characteristics (see below, e.g., Fig. 4).

The dependence of measured channel activity on the holding potential can be analysed in terms of the open state probability of the channel. Since the inside-out configuration of the membrane (inside-out patch) was used in these experiments, a negative holding potential represents a positive membrane potential. The result, presented in Fig. 3, shows that the channel starts to open at a membrane potential of about +30 mV and shows an open-state probability of 1 at about +70 mV. Since the fine structure of the channel traces is not the subject of the present paper, no discrimination of substates was taken into consideration in Fig. 3. Therefore, the channel is regarded as open when it is not closed.

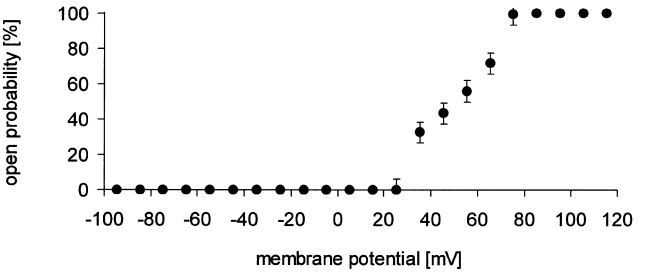

Fig. 3. The open probability of the channel as a function of the membrane potential (data taken from three sets of voltage ramps).

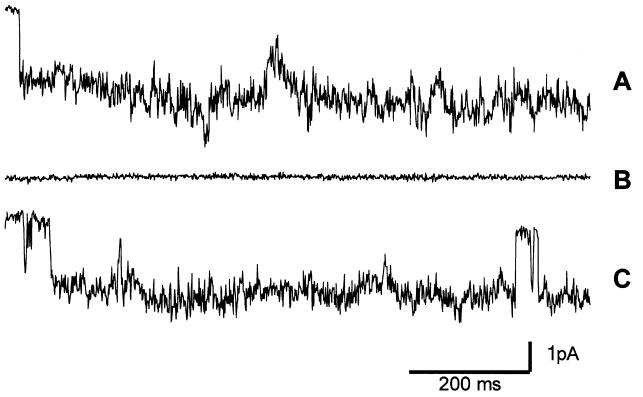

Fig. 4. Channel activity of an inside-out patch of human red blood cells in different bath solutions (replacement of the solution during the channel recording) recorded at a holding potential of about −60 mV. The pipette solution contained 20 mM Na-tartrate. The bath solution contained 70 mM K-tartrate (A) and was replaced by a solution containing no K^+ and no Na^+ (B). Finally, it was replaced again by a solution containing 70 mM K-tartrate (C). The additional components of the pipette and bath solutions are given in the legend of Fig. 1.

There are two possibilities to explain the measured channel activity. Taking into consideration that our measurements were performed in the inside-out membrane configuration, cations could move from the bath solution into the pipette, or anions could flow out of the pipette. There is evidence that the channel activities shown in Fig. 1 represent a cation channel. The reasons to assume a cation channel is present are as follows: (i) the intersection of the linear regression line (see Fig. 2) with the voltage axis (zero current), which represents the reversal potential, gives a value of 37 mV (this value is in good agreement with the calculated Nernst potential for Na^+ (32 mV) under the applied experimental conditions); (ii) the replacement of Na^+ in the bath solution by K^+ during one experiment (one patch) resulted in small changes of the conductance, and it is known from tracer flux experiments that the flux of tartrate ions across a red blood cell membrane in general is negligible; (iii) the replacement of the Na^+- or K^+-containing bath solution by a Na^+- and K^+-free solution (replacement by sucrose) during channel recording resulted in the disappearance of the channel activity (for K^+ replacement see Fig. 4).

As yet, there have been no reports of non-selective, voltage-activated channels in the membranes of non-excitable cells. For excitable cell membranes, however, this kind of channel has been reported for example in smooth muscle and cardiac tissues [14–16]. These channels are probably based on more than one channel type and vary in conductance from as low as 1 pS up to 40 pS [17,18].

The number of cation channels per red blood cell membrane can be easily estimated assuming that the residual flux of monovalent cations (see Section 1) is mainly due to ion movement through the channel under investigation. This happens under conditions where the membrane potential is high and the channel is open (see also below). Taking into account the conductance and the open state probability of the channel (this paper) as well as the residual cation flux across the red blood cell membrane [2], and assuming a random distribution in the cell population, a value of 0.25 channels per red blood cell can be calculated (see Appendix A). Based on this result and assuming a patch to cell surface area ratio of about 1:30, one would expect one channel record per 120 patches. However, we were able to detect channel activity in one out of 10 patches. One possible explanation for this discrepancy, and consistent with the calculated low number of channels per cell, is the fact that the bath solution in the patch-clamp experiments is different from the intracellular solution of intact red blood cells (the possibility that the composition of the intracellular solution can influence the activation of the channel must be taken into account). Therefore, it may be more realistic to compare the patch-clamp data (channel conductance) with the residual cation flux measured in resealed ghosts. Given that the residual cation flux is 10 times higher than that measured in intact red blood cells [19], a value of 2.5 channels per red blood cell is calculated. Such a value is consistent with the observed frequency (10%) of detection of channel activity in the patch-clamp experiments described above. In addition, only a certain fraction of the cell population may express the channel, albeit at a higher frequency. If, for example, 8% of the population were to have three channels each, the population would appear to have 0.25 channels per cell, in accord with our conductance measurements. If a frequency of one channel per 120 patches is to be expected, however, implicit in this assumption must be that our techniques bias towards selection of cells in which channels are expressed (which may in part

explain why only one in 20 attempts to form a giga-seal met with success).

One type of cation channel present in the human erythrocyte membrane is the Ca^{2+}-activated K^+ (Gardos) channel [20]. Patch-clamp investigations of the red blood cell membrane have been carried out to characterise this channel in more detail [21–27], e.g., showing a zero-voltage conductance of 18–22 pS [21,23]. To block the Gardos channel in our measurements, Ca^{2+}-free solutions that contained 2.5 mM Ba^{2+} were used in all experiments. It is easy to see that the channel described in the present paper is not consistent with the Gardos channel although the conductance is very similar. The main difference is that the channel described in the present paper was recorded in both K^+-containing and in Na^+-containing (K^+-free) solutions. Furthermore, the Gardos channel opens at positive as well as negative membrane potentials [21–23].

In addition to the Gardos channel and possibly other cation channels (see below), an anion-selective channel with a conductance of 6 pS has been observed in the human red blood cell membrane [27,28]. To reduce anion channel activity, tartrate-containing solutions were used. The concentration of chloride ions was kept to a minimum, just sufficient (5 mM) to keep the silver/silver chloride electrodes functioning correctly. However, in a few experiments an additional channel activity was observed (data not shown). This channel had a zero-voltage conductance of about 10 pS, close to the conductance of the anion channel previously described by Passow et al. [27] and Schwarz et al. [28], and this channel was therefore not investigated further.

A great variety of specific transport pathways for K^+ and Na^+ exist in the human red blood cell membrane (e.g., see [3,29]). In general, the transport of monovalent cations by different carrier mechanisms is electroneutral. Only the Na^+/K^+ pump is electrogenic since it exchanges 3 Na^+ for 2 K^+. However, in the absence of ATP there is no electrogenic ion transport via the Na^+/K^+ pump. Although not necessary, in the patch-clamp experiments carried out ouabain and bumetanide, inhibitors of ion transport via the Na^+/K^+ pump and the $Na^+/K^+/2Cl^-$ cotransporter, respectively, were added to the solutions. This was done to allow comparison of the patch-clamp data with that obtained from classical tracer kinetic experiments where ouabain and bumetanide are usually present in the flux medium to measure the residual K^+ transport.

Some properties of the channel described in the present paper correlate well with data describing a non-specific cation channel found by Christophersen and Bennekou [13,30]. In contrast to our findings, these authors were also able to generate channel openings at negative membrane potentials. In this respect, our experimental data are more consistent with results obtained by Halperin et al. [12] from tracer kinetic experiments. These authors described increased fluxes of monovalent cations at membrane potentials higher than +20 mV and assumed the involvement of a non-specific cation channel as the cause of this effect. Since Christophersen and Bennekou [13] described the channel only in 500 mM salt solutions, comparison of their results with the results presented in this paper and with those of Halperin et al. [12] are complicated.

Based on the presented data, the LIS effect described by many investigators (see Section 1) seems to be explained: two transport pathways for K^+ and Na^+ participate in the effect of the increase of K^+ and Na^+ fluxes across the human red blood cell membrane in solutions with reduced ionic strength. Firstly, an electroneutral $K^+(Na^+)/H^+$ exchanger operates independently of the membrane potential. The ion transport via this pathway is increased when the ionic strength of the extracellular solution is reduced, since the decrease of the outer surface potential (more negative) at low ionic strength leads to an increase in the cation concentration near the membrane surface and, therefore, to an enhancement of the carrier-mediated flux [2]. Secondly, reducing the ionic strength of the solution to levels corresponding to membrane potentials of +20 mV [12] or +30 to +40 mV ([9]; Schatzmann, personal communication; this paper), leads to the opening of a non-specific cation channel, which results in a further and more pronounced increase of the flux. The physiological relevance of this ion channel, however, remains unclear. Investigations of red blood cells with increased leak cation fluxes (e.g., sickle cells, malaria-infected red blood cells [31]) are necessary to characterise the involvement of the non-specific cation channel in these diseases.

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Appendix A. Calculation of the number of channels per cell

The part of the residual cation flux (J) that should be explained by the channel is assumed to be approximately 25 mmol/h for 1 l red blood cells at a membrane potential (U) of 45 mV (taken from [2] under LIS condition). This flux has to be divided by the number of the red blood cells present in one litre ($n = 8 \times 10^{12}$) to get the residual flux of a single cell (J_{sc}):

$$J_{sc} = \frac{J}{n} \tag{1}$$

On the other hand, the flux of a single cell (J_{sc}) can be derived from microscopic data and is equal to the product of the number of channels per cell (x), the open probability of the channels (p) at the applied membrane potential and the number of cations (N) that pass the channel in a time (t) divided by Avogadro's number (N_a):

$$J_{sc} = \frac{xpN}{N_a t} \tag{2}$$

Since

$$N = \frac{Q}{q_{el}} \tag{3}$$

where Q and q_{el} are the whole charge passing the channel and the elementary charge, respectively, and:

$$Q = It = gUT \tag{4}$$

where I, g, U and t are the current, conductance, applied membrane potential and time, respectively, it follows that

$$J_{sc} = \frac{xpgU}{q_{el}N_a} \tag{5}$$

From Eq. A1 and Eq. A5 it follows for the number of channels per cell (x) that:

$$x = \frac{N_a q_{el}}{ngUp} J \tag{6}$$

One has to take into account that the fluxes presented in Eqs. A1 and A5 are given in mmol/h and mmol/s, respectively. Assuming an open probability of 0.35 at a membrane potential of 45 mV (this paper, see Fig. 3) and a channel conductance of 21 pS (this paper), the average number of channels per red blood cell can be calculated as approximately 0.25.

References

[1] I. Bernhardt, A.C. Hall, J.C. Ellory, J. Physiol. (Lond.). 434 (1991) 489–506.
[2] S. Richter, J. Hamann, D. Kummerow, I. Bernhardt, Biophys. J. 73 (1997) 733–745.
[3] I. Bernhardt, A.C. Hall, J.C. Ellory, Studia Biophys. 126 (1988) 5–21.
[4] K. Denner, R. Heinrich, I. Bernhardt, J. Membr. Biol. 132 (1993) 137–145.
[5] H. Davson, Biochem. J. 33 (1939) 389–401.
[6] W. Wilbrandt, Pflügers Arch. 242 (1940) 537–556.
[7] W. Wilbrandt, H.J. Schatzmann, Ciba Found. Study Group 5 (1960) 34–52.
[8] P.L. LaCelle, A. Rothstein, J. Gen. Physiol. 50 (1966) 171–188.
[9] J.A. Donlon, A. Rothstein, J. Membr. Biol. 1 (1969) 37–52.
[10] I. Bernhardt, E. Donath, R. Glaser, J. Membr. Biol. 78 (1984) 249–255.
[11] G.S. Jones, P.A. Knauf, J. Gen. Physiol. 86 (1985) 721–738.
[12] J.A. Halperin, C. Brugnara, M.T. Tosteson, T.V. Ha, D.C. Tosteson, Am. J. Physiol. 257 (1989) C987–C996.
[13] P. Christopherson, P. Bennekou, Biochim. Biophys. Acta 1065 (1991) 103–106.
[14] D. Noble, The Initiation of the Heartbeat, Clarendon Press, Oxford, 1979.
[15] D. DiFrancesco, C. Tromba, in: H.M. Piper, G. Isenberg (Eds.), Isolated Adult Cardiomyocytes, vol. II, CRC Press, Boca Raton, FL, 1989, pp. 97–115.
[16] N. Hagiwara, H. Irisawa, J. Physiol. 409 (1989) 121–141.
[17] G. Isenberg, in: D. Siemen, J. Hescheler (Eds.), Nonselective Cation Channels, Birkhäuser, Basel, 1993, pp. 247–260.
[18] T. Hisada, R.W. Ordway, M.T. Kirber, J.J. Singer, J.V. Walsh, Pflügers Arch. 417 (1991) 493–499.
[19] P. Müller, A. Herrmann, I. Bernhardt, R. Glaser, Biosci. Rep. 5 (1985) 425–432.
[20] G. Gardos, Biochim. Biophys. Acta 30 (1958) 653–654.
[21] O.P. Hamill, J. Physiol. 319 (1981) 97P–98P.
[22] R. Grygorczyk, W. Schwarz, Cell Calcium 4 (1983) 499–510.
[23] R. Grygorczyk, W. Schwarz, H. Passow, Biophys. J. 45 (1984) 693–698.

[24] M. Shields, R. Grygorczyk, G.F. Fuhrmann, W. Schwarz, H. Passow, Biochim. Biophys. Acta 815 (1985) 223–232.
[25] G.F. Fuhrmann, W. Schwarz, R. Kersten, H. Sdun, Biochim. Biophys. Acta 820 (1985) 223–234.
[26] R. Grygorczyk, Biochim. Biophys. Acta 902 (1987) 159–168.
[27] H. Passow, W. Schwarz, M. Glibowicka, N. Aranibar, M. Raida, in: F. Palmieri, E. Quagliariello (Eds.), Molecular Basis of Biomembrane Transport, Elsevier Science, Amsterdam, 1988, pp. 121–139.
[28] W. Schwarz, R. Grygorczyk, D. Hof, Methods Enzymol. 173 (1989) 112–121.
[29] B. Deuticke, R. Grebe, C.M.W. Haest, in: J.R. Harris (Ed.), Blood Cell Biochemistry, vol. 1, Erythroid Cells, Plenum, New York, 1990, pp. 475–529.
[30] P. Bennekou, Biochim. Biophys. Acta 1147 (1993) 165–167.
[31] H. Staines, Cation Transport in *Plasmodium falciparum*-Infected Human Erythrocytes, Ph.D. Thesis, Oxford University, 1998.