Biochimica et Biophysica Acta 902 (1987) 159–168
Elsevier

BBA 73632

Temperature dependence of Ca^{2+}-activated K^+ currents in the membrane of human erythrocytes

Ryszard Grygorczyk

Max-Planck-Institut für Biophysik, Heinrich-Hoffmann-Strasse 7, D-6000 Frankfurt / Main 71 (F.R.G.)

(Received 19 March 1987)

Key words: Erythrocyte membrane; Potassium channel; Temperature dependence; Single-channel current; (Human)

The currents through single Ca^{2+}-activated K^+ channels were studied in excised inside-out membrane patches of human erythrocytes. The effects of temperature on single-channel conductance, on channel gating and on activation by Ca^{2+} were investigated in the temperature range from 0 up to 47 °C. The single-channel conductance shows a continuous increase with increasing temperature; an Arrhenius plot of the conductance gives the activation energy of 29.6 ± 0.4 kJ / mol. Reducing the temperature alters channel-gating kinetics which results in a significant increase of the probability of the channel being open (P_o). The calcium dependence of P_o is affected by temperature in different ways; the threshold concentration for activation by Ca^{2+} is not changed, the Ca^{2+} concentration of half-maximal channel activation is reduced from 2.1 μmol / l at 20 °C to 0.3 μmol / l at 0 °C, the saturation level of the dependence is reduced for temperatures higher then about 30 °C. The relevance of the obtained data for the interpretation of the results known from flux experiments on cells in suspensions is discussed.

Introduction

Cation movements across the red cell membrane can be separated pharmacologically into three major components: the ouabain-sensitive Na^+ and K^+ flux mediated by the sodium pump, $Cl^-/Na^+/K^+$ cotransport systems sensitive to 'loop' diuretics (e.g., bumetanide, furosemide), and the residual, ouabain- and loop diuretics-insensitive K^+ flux (for references see, for example Refs. 1 and 2). For full thermodynamical description of the transport processes changes of temperature and pressure are used as a complementary physical tool [1]. Especially the effects of temperature on Na^+ and K^+ transport in the erythrocyte membrane attract much attention [2–4]. Two of the flux components, the ouabain-sensitive and the loop diuretics-sensitive, show a continuous decrease with decresing temperature from 37 to 0 °C while the residual K^+ flux, in human red cells, shows a paradoxical increase when temperature is reduced below 12 °C [5,1]. Similar effects have been observed in red cells from rat, dog and guinea pig (when calcium was added to the medium) but not in other species (e.g., rodents [2,6]).

Since Ca^{2+}-activated K^+ channels seem to contribute to the elevated K^+ flux at reduced temperatures in red cells of at least some species [2] I wanted to investigate in more detail the effects of temperature on the Ca^{2+}-activated K^+ channels in the membrane of human erythrocytes.

The Ca^{2+}-activated K^+ channels in red cells

Correspondence (present address): R. Grygorczyk, Department of Pharmacology, Medical Science Building, University of Toronto, Toronto, Canada M5S 1A8.

(reviewed in Ref. [7]) have been extensively studied in this laboratory by the patch-clamp technique [8,9,10]. The direct measurements of single-channel conductance, channel gating and their modulation by different factors allowed a better interpretation and understanding of a series of results obtained in flux experiments on cells in suspensions. In particular it was possible to establish that Ca^{2+}-activated K^+ channels mediate so called Gàrdos effect consisting of an increased membrane permeability to K^+ at elevated intracellular calcium concentrations [11].

In this paper I shall present data from patch-clamp experiments in the temperature range from 0 to 47 °C; this may serve to a further characterization of the Ca^{2+}-activated K^+ channel.

Materials and Methods

Human red cells were obtained from Rh^+0 blood of healthy donors from Red Cross Blood Bank in Frankfurt. The cells were washed two to three times in isotonic KCl solution buffered to pH 7.4 by 4-morpholinepropane sulfonic acid (Mops).

Solutions

The standard solution contained 150 mmol/l KCl, 1 mmol/l $MgCl_2$ and was adjusted to pH 7.4 by 10 mmol/l Mops buffer. Concentrations of free Ca^{2+} below 5 μmol/l were established by buffering with 1 mmol/l ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA) (see Ref. 12). Solutions in the pipette were usually prepared without added $CaCl_2$. The stability constant of the Ca-EGTA complex, *K*, used for calculations of the free Ca^{2+} concentrations was not corrected for changes of the temperature. According to Ref. 13 *K* does not change significantly while changing the temperature between 0 and 35 °C (see also Discussion).

Patch-clamp recordings

The patch-clamp technique [14] was applied to record single-channel currents in cell-free, inside-out membrane patches of human red cells.

The bath temperature was controlled with a Peltier cooling device and monitored with a thermistor placed near the tip of the patch pipette.

The data were stored on magnetic tape, and were later transferred to a brush recorder (Gould) for evaluation by hand, or records were digitized at an appropriate sampling rate, transferred to floppy diskettes and analysed by means of an LSI 11/23 computer (for details see Refs. 10 and 15). The probability of the channel being open P_o was usually determined from amplitude histograms. At low temperatures (< 10 °C) records were dominated by long-lasting openings and show reduced frequency of brief closures: under these conditions estimates of P_o were often made by hand from data plotted by the brush recorder.

Results

The effects of temperature on single-channel conductance, on channel gating and on activation by Ca^{2+} of the K^+ channels were investigated in cell-free, inside-out membrane patches of human red cells. For convenience the studies were performed primarily on inward currents, i.e. for the membrane configuration used currents flowing from pipette to the bath, since the Ca^{2+}-activated K^+ channels exhibit inward rectification with outward currents usually not exceeding 1 pA.

Fig. 1 shows records of single-channel K^+ currents in a membrane patch of a human erythrocyte at different temperatures and at a holding potential of $V_H = -100$ mV. The bath solution in contact with the internal membrane surface contained 10 μmol/l free Ca^{2+}. The records demonstrate that the amplitude of single-channel events decreases with decreasing temperature (note the presence of two channels in the membrane patch). In addition to single-channel conductance, the channel gating is also strongly affected by temperature. Reducing the temperature by only 5 °C from 35 to 30 °C results in a significant increase in channel activity that consists of high-frequency brief-channel openings and closings. This 'flickering' is gradually replaced by long-lasting dwell times when the temperature is further reduced; the temperature dependence of this channel gating is considered in more detail below.

1. Temperature dependence of the single-channel current

Fig. 2 shows *I*–*V* (current–voltage) relations of

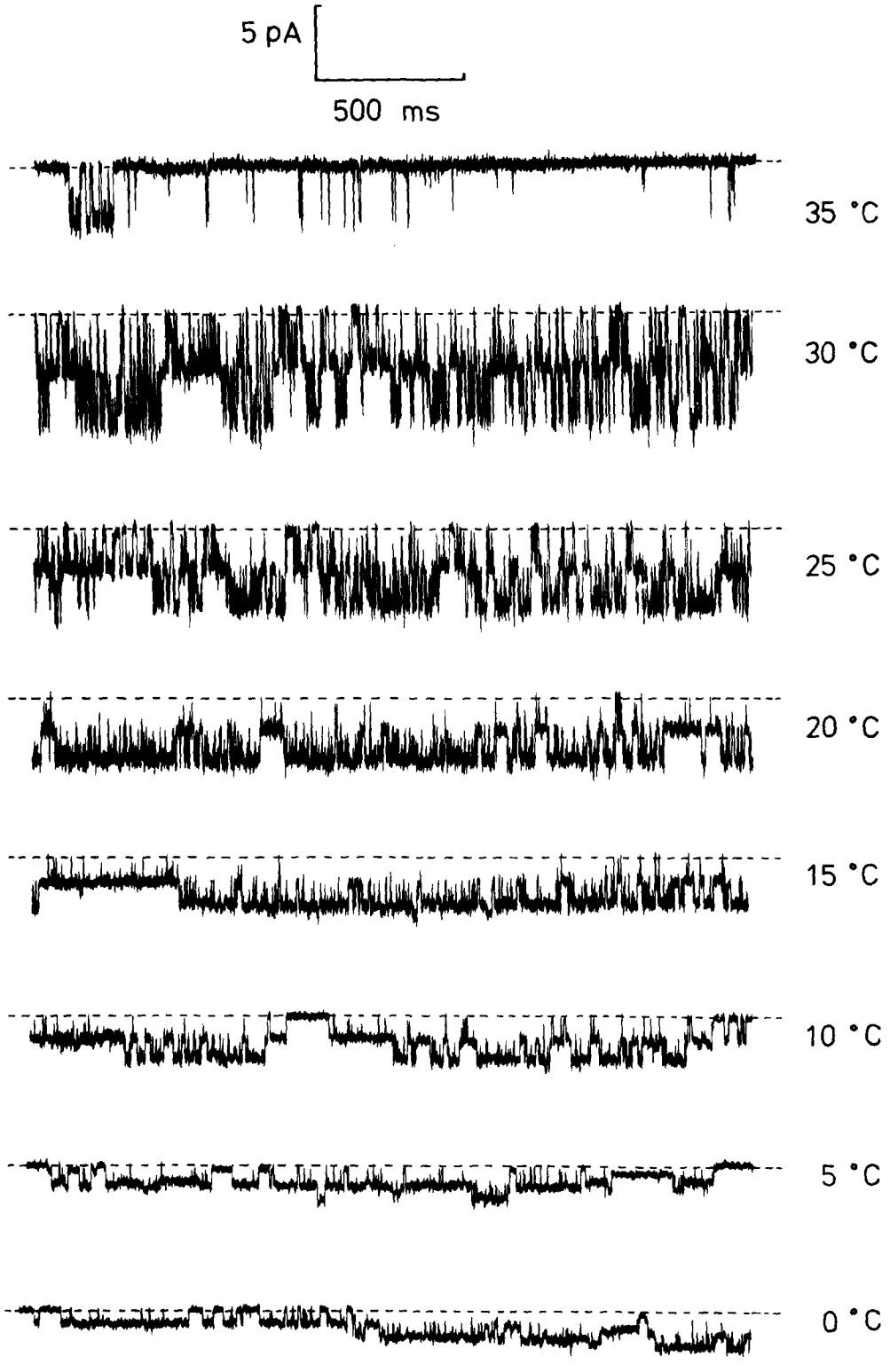

Fig. 1. Temperature dependence of single-channel K^+ currents in an excised membrane patch. Downward deflections represent inward current flowing from the pipette to the bath. Broken lines show the base line currnet level (closed channel). The temperatures are indicated on the right of each record. The pipette and bath contained the same standard solution (see Materials and Methods) with 10 μmol/l free Ca^{2+}. Holding potential $V_H = -100$ mV.

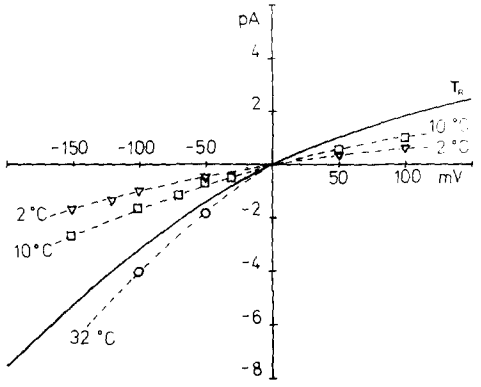

Fig. 2. Current–voltage (I–V) relations of the K^+ channel from one membrane patch measured at different temperatures. Pipette and bath contained the same solution as described in Fig. 1. The solid line represents I–V relation at room temperature (T_R) published previously (Grygorczyk et al. [9], Fig. 3); broken lines show the same but rescaled relation at 32 °C (○), 10 °C (□) and 2 °C (▽), respectively (see text for other details).

the channel for different temperatures. The solid line represents the I–V curve observed at room temperature (T_R) as published previously [9]. The broken lines represent the same curve multiplied for each temperature by a factor representing relative change of single-channel current observed at −100 mV with respect to the current at room temperature. Each curve fits well to the data points measured at the corresponding temperature indicating that decreasing temperature decreases the slope of the whole I–V curve by the same factor. Thus the temperature dependence of the channel conductance (represented by the slope of the I–V curve) can be described by the temperature dependence of single-channel current at a fixed membrane potential (e.g. −100 mV). Fig. 3A shows the dependence of the single-channel current recorded at −100 mV in the temperature range 0–47 °C. The Arrhenius plot of these data, Fig. 3B, is convincingly linear and has a slope which corresponds to an activation energy of 29.6 ± 0.4 kJ/mol.

2. Temperature dependence of channel gating

The records presented in Fig. 1 were obtained with 10 μmol/l free Ca^{2+} on the intracellular side of the membrane. This Ca^{2+} concentration is sufficient to give saturating channel activity at 20 °C, i.e. maximum probability of finding the channel in

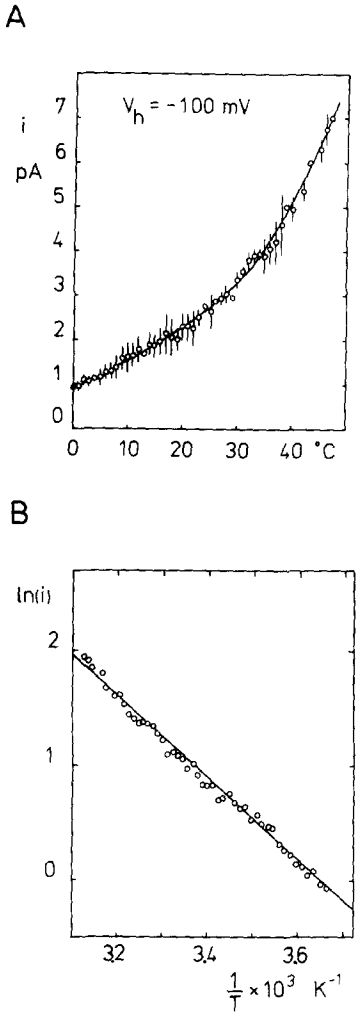

Fig. 3. (A) Temperature dependence of single-channel current recorded at −100 mV. Each point represents the mean value (±S.D.) from 2–9 experiments. Points without bars are from single experiment. (B) Arrhenius plot of the data shown in (A). The slope of the straight line, representing least-squares fit to the data, corresponds to an activation energy E_a of 29.6 ± 0.4 kJ/mol.

the open state, P_o (see Ref. 9). Fig. 4 shows records obtained with low (0.5 μmol/l) Ca^{2+} in the bath at higher (35 °C) and lower temperature (0 °C). One can easily recognize that at 35 °C only brief channel openings are present, while at 0 °C the record is dominated by openings lasting up to several seconds which is similar to the observations at 10 μmol/l Ca^{2+} (Fig. 1). This temperature dependence of channel gating corresponds to a

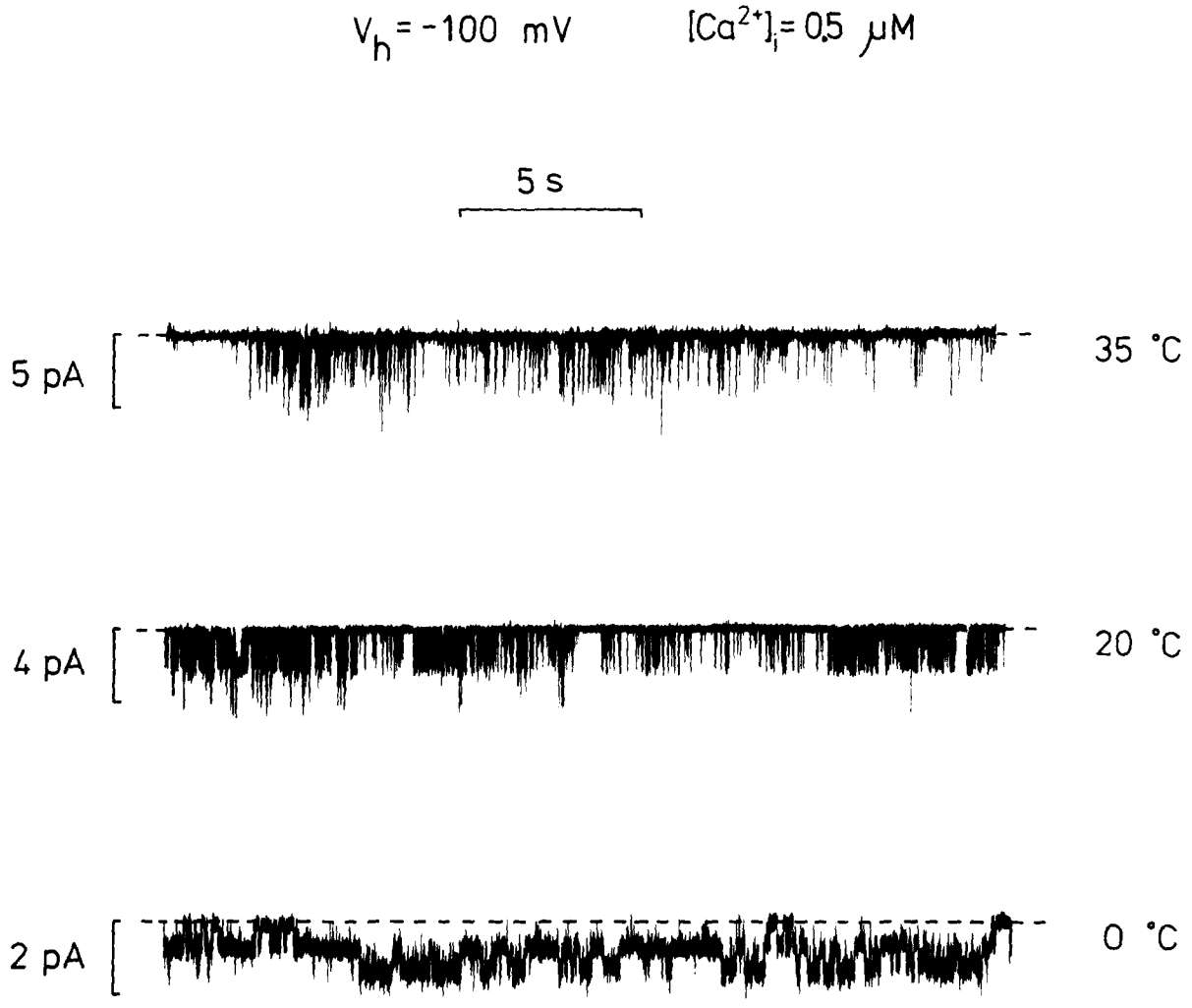

Fig. 4. Temperature dependence of channel activity recorded with 0.5 μmol/l Ca^{2+} in the solution in contact with the intracellular membrane surface. The membrane potential was set to −100 mV; temperatures are indicated on the right of each record. Broken lines represent baseline current levels. Note the presence of two channels in the membrane patch.

significant increase of P_o if the temperature is lowered. The effect is reversible and can be repeated during cycles of heating and cooling within the temperature range from 0 to 47 °C. Fig. 5 shows open- and closed-time distributions of the K^+ channels at 30 and 0 °C with 0.5 μmol/l Ca^{2+} in the bath solution. For 30 °C the open-time distribution could be satisfactorily described by a single exponential with a time constant corresponding to a mean open time of about 1.6 ms. In contrast the closed-time distribution has to be described by two components: a fast one with a mean closed time τ_{c1} of about 6.8 ms and a slow one with τ_{c2} = 40.0 ms. At 0 °C the situation is so to speak reversed; i.e. the open-time distribution now shows two components with mean open times of about τ_{o1} = 3.7 and τ_{o2} = 29.4 ms, and the closed-time distribution is now dominated by a fast component with a mean closed time of about 2.5 ms (the contribution of the slow component amounts to less then 2%).

3. Temperature dependence of activation by Ca^{2+}

Fig. 6 shows more explicitely the temperature dependence of P_o at different Ca^{2+} concentrations in the bath solution (in Fig. 6A at 0.2, 0.5 and 1.0

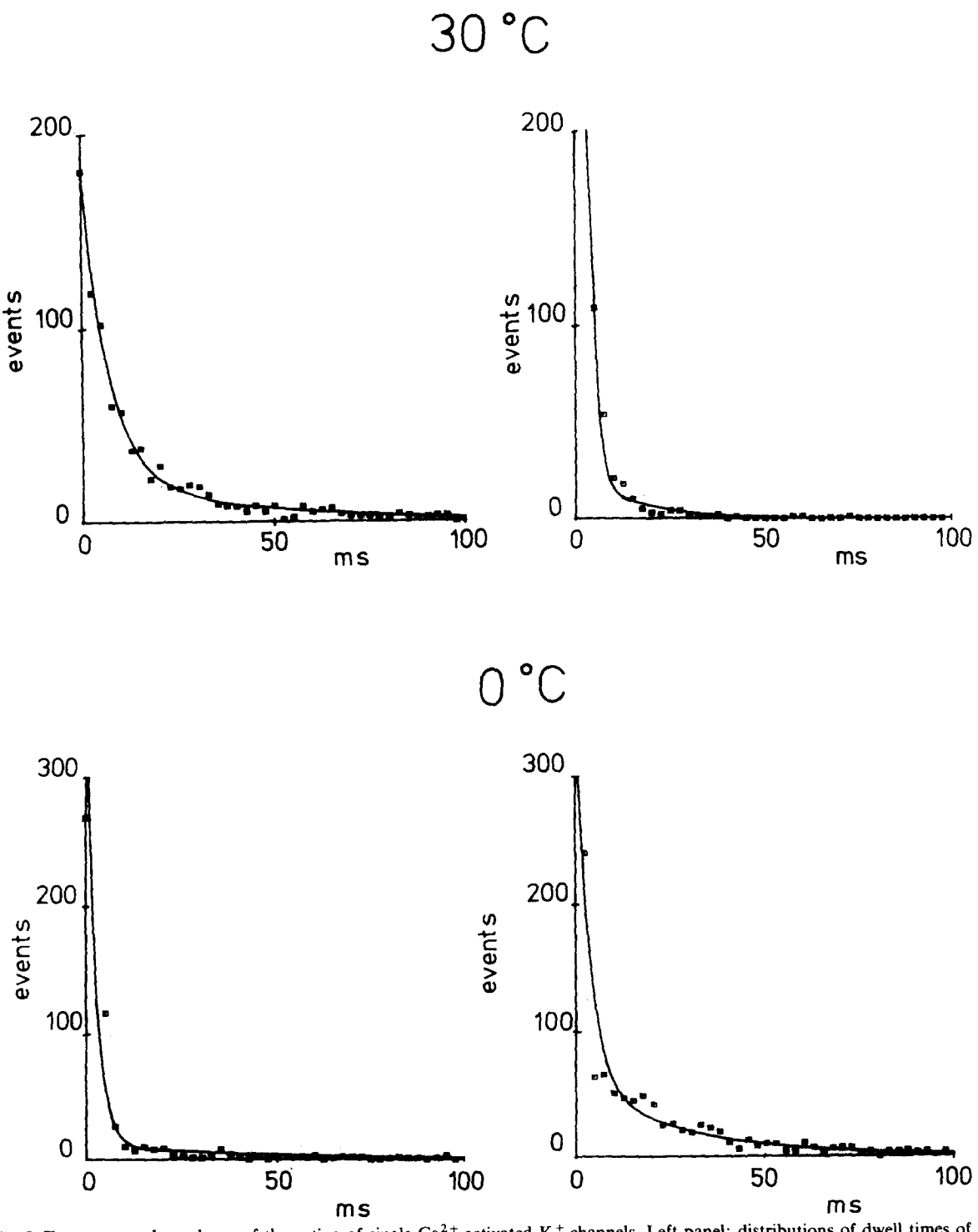

Fig. 5. Temperature dependence of the gating of single Ca^{2+}-activated K^+ channels. Left panel: distributions of dwell times of the closed state at 30 °C and 0 °C; right panel: distributions of dwell times of the open state at 30 °C and 0 °C. Records of single-channel currents were obtained at −100 mV and digitized at sampling rate of 400 Hz. The curves represent least-squares fits of $N = P_1 \exp(-t/\tau_1) + P_2 \exp(-t/\tau_2)$ to the data. Fit parameters for closed times distributions at 30 °C are: $\tau_{c1} = 6.8$ ms, $\tau_{c2} = 40.0$ ms, $P_{c1} = 88\%$, $P_{c2} = 12\%$ and at 0 °C $\tau_{c1} = 2.5$ ms, $\tau_{c2} = 76.9$ ms, $P_{c1} = 98\%$, $P_{c2} = 2\%$. For open-time distribution fit parameters at 30 °C are: $\tau_{o1} = 1.6$ ms, $\tau_{o2} = 14.3$ ms, $P_{o1} = 99\%$, $P_{o2} = 1\%$ and at 0 °C $\tau_{o1} = 3.7$ ms, $\tau_{o2} = 29.4$ ms, $P_{o1} = 84\%$, $P_{o2} = 16\%$.

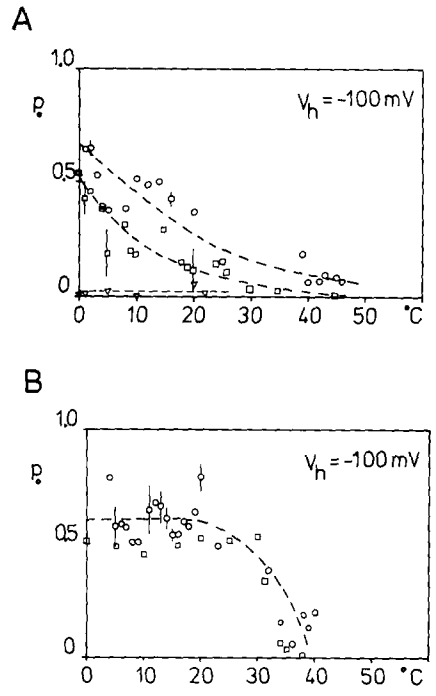

Fig. 6. Dependence of the probability of the channel being open (P_o) on temperature at different internal Ca^{2+} activity: (A): 0.2 (▽), 0.5 (□) and 1.0 μmol/l (○); B: 5 (○) and 10 μmol/l (□). The estimates of P_o were made from records of single-channel currents measured at membanre potential of −100 mV. Symbols represent mean values (±S.D.) from two to four experiments. Symbols without bars are from single experiments.

μmol/l Ca^{2+}, and in Fig. 6B at 5 and 10 μmol/l Ca^{2+}). The values of P_o represent a 'total' probability of the channel being open, i.e., they were calculated without distinguishing between different open states of the K^+ channel whose existence was demonstrated in the open and closed-time distributions (Fig. 5). The phenomenon of increasing P_o with decresing temperature is clearly seen for 0.5 and 1.0 μmol/l Ca^{2+} while for lower calcium concentrations (0.2 μmol/l and less) P_o remains low also at reduced temperatures. For higher calcium concentrations (5 and 10 μmol/l) a drop of P_o could be only seen above 30 °C while at lower temperatures P_o remains at the saturating level. Fig. 7 shows the dependence of probability of a channel being open, P_o, on calcium concentration at three different temperatures: 35, 20 and 0 °C. It demonstrates that at 0 °C the steepness of the dependence is much higher than at 20 and 35 °C while the threshold concentration for activation by calcium (approx. 0.2 μmol/l) seems not to be changed. At 35 °C, in addition, the saturating level of P_o is significantly reduced. The estimated calcium concentrations corresponding to half-maximum channel activation, $K_{1/2}$, are 0.3, 2.1 and 1.2 μmol/l at 0, 20 and 35 °C, respectively. While the dependence of P_o on Ca^{2+} concentration is not very steep at 35 °C, the $K_{1/2}$ is lower than at 20 °C due to the reduced saturating level of P_o.

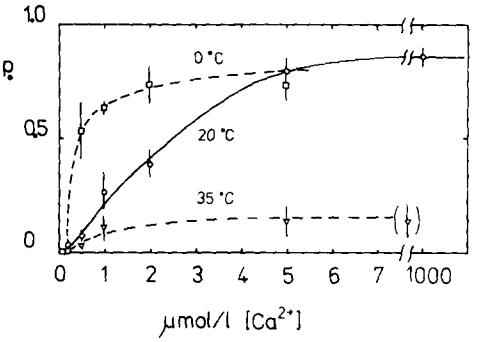

Fig. 7. Dependence of the probability of the channel being open, P_o, on internal Ca^{2+} activity at 0 °C (□), 20 °C (○) and 35 °C (▽). Symbols represent mean values (±S.D.) The curve measured at 20 °C includes in part data published previously (Grygorczyk et al. [10], (Fig. 4). The point in the brackets (▽) (see curve measured at 35 °C) was obtained with 10 μmol/l free Ca^{2+}.

Discussion

The data presented give further evidence that Ca^{2+}-activated K^+ channel in human red cells has several characteristics of K^+ channels in other cell membranes. The single-channel conductance depends on temperature as could be expected for ion diffusion in a water filled pore. Channel gating properties reveals several states of the channel, similarily to other Ca^{2+}-activated K^+ channels [16,17]. These Ca^{2+}-activated K^+ channels, which possibly form the major pathway of the residual (ouabain- and loop diuretics-insensitive) K^+ flux in the erythrocyte membrane, are also responsible for the anomalous increase in K^+ permeability observed in red cells of guinea pig at temperatures below 15 °C [2]. This transport system possibly

accounts for a similar effect in red cells of other non-hibernating species (rat, dog) observed when Ca^{2+} was present in the media. Human red cells seem to be an exception, since minimum K^+ permeability at 12°C was even observed in the absence of Ca^{2+} in the bathing medium [5]. The results from the patch-clamp experiments show that the temperature dependence of the K^+ flux mediated by the Ca^{2+}-activated K^+ channel is complex; it reflects the superimposed effects of temperature on single-channel conductance, on channel gating and on the activation by Ca^{2+}. With decreasing temperature a continuous decrease of channel conductance is observed, but simultaneously, altered gating kinetics results in an increase of channel opening probability at reduced temperatures. These effects could be relevant for the interpretation of data obtained in flux experiments.

Single-channel conductance decreases with decreasing temperature

Decrease of the channel conductance with decreasing temperature has already been described for several channels in different preparations, e.g. acetylcholine-activated channel in cultured skeletal muscle of chicken [18,19], glutamate-activated channel in locust muscle [20] and for Ca^{2+}-activated K^+ channel in cultured rat muscle [21]. In the later case the single-channel conductance increase from about 100 pS at 1°C to about 300 pS at 37°C i.e. by a factor of about 3 which is comparable to the value of about 4.3 as observed in my experiments on human red cells for the same temperature range. Decreasing single-channel conductance with decreasing temperature could be understood as a simple consequence of reduced thermal agitation of ions diffusing in a water filled pore [22]. The activation energies E_a for diffusion of ions in such pores usually lie in the range from 12 to 25 kJ/mol (for reference see Ref. 23) which is of the same order of magnitude as E_a for K^+ ions diffusion in a bulk solution (17 kJ/mol [24]). A similar value of E_a was found in the present study for activation energy of channel conductance (29.6 kJ/mol) in the temperature range 0–47°C. This is also similar to the value (28.7 kJ/mol) obtained by Hall et al. [1] from flux measurements of the passive ouabain- plus bumetanide-insensitive K^+ permeability in human red cells in the temperature range of 18 to 37°C, i.e. above the observed minimum of K^+ flux at 12°C (see also Ref. 6). On the other hand, Steward and Ellory [3] reported for E_a values of 50 and 36 kJ/mol in normal red cells and in red cells from patients with familial xerocitosis, respectively. The former value is similar to the activation energy of about 60 kJ/mol which could be estimated from the temperature dependence of Ca^{2+}-stimulated K^+ efflux from human red cell ghosts given by Simons (see Fig. 4 in Ref. 25). One possible explanation for the higher activation energies observed sometimes in K^+ flux experiments compared to my observations is that the number of active channels may alter with decreasing temperature. In some of my experiments with membrane patches containing several K^+ channels I got the impression that at lower temperatures the number of active channels is reduced but the effect was not studied in detail. In any case, one could suspect that the temperature dependence of single-channel conductance could account, at least in part, for the observed decrease of the K^+ flux observed with decreasing temperature in the temperature range above the minimum at about 12°C. Since channel conductance decreases continuously in the whole temperature range from 47 to 0°C this effect can not be responsible for the paradoxical increase of K^+ permeability below 12°C seen in flux measurements [5].

Channel gating is affected by temperature

The analysis of gating of the Ca^{2+}-activated K^+ channel in human red cells at different temperatures (Fig. 5) indicates that any plausible model for this channel has to be composed of at least two open and three closed states including a component with very short closures (see Ref. 10) not resolved at the sampling rate used to construct the histograms shown in Fig. 5. Thus the kinetics of the Ca^{2+}-activated K^+ channel in human red cells is as complex as for other Ca^{2+}-activated K^+ channels (see, for example, Refs. 16 and 17). Decreasing temperature slows down transition rates between channel states which leads to alterations of the dwell times of the corresponding channel states. The dwell times could increase or decrease depending on the relative temperature sensitivity

of transition rates between the different channel states. For the Ca^{2+}-activated K^+ channel studied here, an increase of channel dwell time in the open state with long-lasting openings is observed and simultaneously a decrease of channel dwell time in the closed state with long-lasting closures. This results in a significant overall increase of the probability of the channel being open, P_o, at reduced temperatures since $P_o = \tau_o/(\tau_0 + \tau_c)$ (see Figs. 6 and 7). The above effect reflects directly changes of the transition rates between channel states with decreasing temperature and cannot be accounted by e.g. increased free Ca^{2+} level due to the temperature dependent buffering of Ca^{2+} by EGTA. The temperature dependence of the stability constant of the Ca-EGTA complex is rather small and tends in opposite direction, i.e., to reduce free Ca^{2+} level with decreasing temperature.

An increase of mean channel open time with decreasing temperature has also been demonstrated for acetylcholine-activated channels in chick skeletal muscle by noise analysis (see for example, Ref. 20) as well as in patch-clamp experiments [19].

Ca^{2+} sensitivity is increased at lower temperatures

The higher steepness of the dependence of P_o on Ca^{2+} concentration at 0 °C as compared to the dependence at 20 and at 35 °C (Fig. 7) indicates that transitions to the long-lasting open state (which dominate at reduced temperature) requires the binding of more Ca ions than transition to the short-lasting open state (dominating at higher temperature). Similar observations were made for Ca^{2+}-activated K^+ channel in cultured rat muscle [16,17]. Note however that the plot shown in Fig. 7 represents P_o of compound open states since transitions to different open states were lumped together. More detailed study would require analysis of channel activity separated into individual channel states like that done by Magleby and Pallota [16,17].

The K^+ flux mediated by Ca^{2+}-activated K^+ channel is proportional to the product of channel conductance γ, probability of the channel being open P_o, and number of K^+ channels. The opposing effects of decreasing temperature on P_o and γ could result in a minimum in K^+ flux if only intracellular Ca^{2+} concentration exceeds about 0.2 μmol/l. The effect could be then seen even with fixed intracellular Ca^{2+} concentration i.e. without participation of other low temperature effects on e.g. intracellular free Ca^{2+} control. The physiological, free Ca^{2+} level in human red cells is reported to be of the order of 10 to 30 nmol/l [26]. Some other estimates of the upper limit are in the range from 0.25 to 1.0 μmol/l; however, it is believed that the later are rather overestimates [27,28]. Thus one can not definitely exclude that, in addition, temperature-dependent control of intracellular free Ca^{2+} is involved in the observed increase of K^+ flux at temperatures below 12 °C as suggested in Ref. 29.

Acknowledgements

The author is grateful to Dr. W. Schwarz for helpful discussions and to Dr. H. Passow and Dr. P. Wood for reading the manuscript.

References

1 Hall, A.C., Ellory, J.C. and Klein, R.A. (1982) J. Membrane Biol. 68, 47–56
2 Hall, A.C. and Willis, J.S. (1984) J. Physiol. 348, 629–643
3 Stewart, G.W. and Ellory, J.C. (1985) Clin. Sci. 69, 309–319
4 Meenaghan, M., Follett, G.F. and Brophy, P.J. (1985) Biochim. Biophys. Acta 821, 72–78
5 Stewart, G.W., Ellory, J.C. and Klein, R.A. (1980) Nature 286, 403–404
6 Hall, A.C. and Willis, J.S. (1984) J. Physiol. (London) 354, 49 P
7 Schwarz, W. and Passow, H. (1983) Annu. Rev. Physiol. 45, 359–374
8 Grygorczyk, R. and Schwarz, W. (1983) Cell Calcium 4, 499–510
9 Grygorczyk, R., Schwarz, W. and Passow, H. (1984) Biophys. J. 45, 693–698
10 Grygorczyk, R. and Schwarz, W. (1985) Eur. Biophys. J. 12, 57–65
11 Gàrdos, G. (1958) Biochim. Biophys. Acta 30, 653–654
12 Methfessel, C. and Boheim, G. (1982) Biophys. Struct. Mech. 9, 3–60
13 Fabiato, A. and Fabiato, F. (1979) J. Physiol. (Paris) 75, 463–505
14 Hamill, O.P., Marty, A., Neher, E., Sackmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100
15 Schwarz, W., Grygorczyk, R. and Hof, D. (1987) in Methods in Enzymology; Biomembrane, Biological Transport, in the press
16 Magleby, K.L. and Pallota, B.S. (1983) J. Physiol. (London) 344, 585–604
17 Magleby, K.L. and Pallota, B.S. (1983) J. Physiol. (London) 344, 605–623

18 Sachs, F. and Lecar, H. (1977) Biophys. J. 17, 129–143
19 Nelson, D.J. and Sachs, F. (1979) Nature 282, 861–863
20 Anderson, C.R., Cul-Candy, S.G. and Miledi, R. (1977) Nature 268, 663–665
21 Barret, J.N., Magleby, K.L. and Pallota, B.S. (1982) J. Physiol. (London) 331, 211–230
22 Hille, B. (1984) in Ionic Channels of Excitable Membranes, Sinauer Associates Inc. pp. 153–162
23 Latorre, R. and Miller, C. (1983) J. Membrane Biol. 71, 11–30
24 Bruins, H.R. (1929) in International Critical Tables, Vol. 5, (Washburn, E.W., ed.), pp. 63–76, McGraw-Hill, New York
25 Simons, T.J.B. (1976) J. Physiol. (London) 256, 209–225
26 Lew, V.L., Tsien, R.Y., Miner, C. and Bookchin, R.M. (1982) Nature 298, 478–481
27 Schatzmann, H.J. (1973) J. Physiol. (London) 253, 551–569
28 Simons, T.J.B. (1982) J. Membrane Biol. 66, 235–247
29 Ellory, J.C. and Hall, A.C. (1983) J. Physiol. (London) 345, 148P