Species-Dependent Differences in the Effect of Ionic Strength on Potassium Transport of Erythrocytes: The Role of Lipid Composition

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Abstract. The $Rb^+(K^+)$ efflux of erythrocytes from six mammalian species was investigated in solutions of physiological and low ionic strength. A species dependent increase of the $Rb^+(K^+)$ efflux in low ionic strength solution could be observed. The rate constant of $Rb^+(K^+)$ efflux of erythrocytes in physiological ionic strength solution correlates with the content of arachidonic acid of the membrane phospholipids. The same relation was observed in solution of low ionic strength with the exception of human erythrocytes. In addition, an age-dependent correlation of the rate constant of $Rb^+(K^+)$ efflux from calf erythrocytes in low ionic strength solution with the content of arachidonic acid of the membrane phospholipids was found.

The $Rb^+(K^+)$ efflux of human erythrocytes, which is enhanced in low ionic strength solution, decreases with the decreasing temperature. The temperature-dependent ESR order parameter of a fatty acid spin label for human and cow erythrocytes in solution of physiological and low ionic strength media suggested that the effect of low ionic strength on $Rb^+(K^+)$ efflux is not solely based on a change of membrane fluidity. The results are interpreted as being due to a specific influence of membrane phospholipids on the $Rb^+(K^+)$ efflux.

Key words: $Rb^+(K^+)$ efflux — Erythrocytes — Mammalian species — Membrane phospholipids — Membrane fluidity

Introduction

For a variety of integral membrane proteins it could be shown that their function is influenced by the surrounding lipids. This influence can be specific, reflecting a requirement for a particular lipid head group and/or fatty acid of a phospholipid; or more generally, it can be an effect of the physical state of the surrounding lipids (Farias et al. 1975; Sandermann 1978; Mead 1984). As shown in the last

few years, both mechanisms seem to be involved in the regulation of membrane protein function (Roelofsen 1977; Abeywardena and Charnock 1983; Castuma and Brenner 1983). Studying factors which can influence residual ion transport (i.e. ion transport which is neither mediated by an ATP consuming ion pump nor by a specific stoichiometrically coupled ion transport mechanism) through biological membranes these possibilities have to be taken into account. Increased residual cation transport has been interpreted either in terms of defects at the lipid/protein interface or in pore formation in proteins or protein aggregates (Anner 1981; van der Steen et al. 1982; Last et al. 1983; van Hoogevest et al. 1984; Bernhardt et al. 1988, 1991).

Measuring the $Rb^+(K^+)$ efflux of erythrocytes no participation of the ion transport via the Na^+/K^+-pump has to be taken into consideration. The K^+ transport via the Ca^{2+}-induced (Gardos) channel can be neglected if the intracellular Ca^{2+} concentration is not significantly increased. Therefore, the $Rb^+(K^+)$ efflux represents the residual as well as the possible stoichiometrically coupled ion transport involving K^+ (see e.g. Bernhardt et al. 1988).

As shown in an earlier paper using erythrocytes from different mammalian species the membrane fluidity could be one factor responsible for the increase of $Rb^+(K^+)$ efflux in low ionic strength solution (Erdmann et al. 1990). However, these data suggested that fluidity is not the only factor. In the present paper temperature changes were used to modify the erythrocyte membrane fluidity. In addition, differences in phospholipid composition of the membrane have to be considered. Kirk (1977) reported that the total K^+ influx rises with the increasing phosphatidylcholine content and the decreasing sphingomyelin content of the erythrocyte membrane. Also, phosphate uptake and chloride/bicarbonate exchange were found to correlate with the content of these membrane phospholipids (Gruber and Deuticke 1973; Lu and Chow 1982). In addition, phosphate uptake and chloride/bicarbonate exchange correlate also with the arachidonic acid content of the membrane phospholipids (Gruber and Deuticke 1973; Lu and Chow 1982). The aim of the present work was to investigate the possible influence of membrane lipid composition on the $Rb^+(K^+)$ efflux of erythrocytes.

Materials and Methods

Erythrocyte preparation

Except for human blood, where 2-day-old whole blood (citrate preserved) of the 0 Rh^+ group was used, the blood of the animals studied was drawn by venipuncture into heparin (1.5 mg per 10 ml blood) as anticoagulant. Plasma and buffy coat were removed by centrifugation (2000 × *g*, 8 min, 20 °C) followed by aspiration of the upper layers. Subsequently, the erythrocytes were washed twice (2000 × *g*, 8 min. 20 °C) in physiological ionic strength solution containing (mmol/l): NaCl 141.3; KCl 5.7; glucose 5.0; and Na_2HPO_4/NaH_2PO_4 5.8, pH 7.4.

Determination of the rate constant of Rb-86 efflux

The method of continuous efflux measurements was described in detail elsewhere (Bernhardt et al. 1984). Briefly, 0.08 ml of $^{86}RbCl$ (Rb^+ as K^+ indicator) were added to 1 ml erythrocyte suspension (hematocrit 35%), the radiochemical concentration reaching a maximum of 13.7 MBq. This was followed by an incubation for 2 hours at 37 °C. The cell suspension was poured into a diffusion chamber covered with a millipore filter to allow ions to diffuse but to restrain the cells. The loss of radioactivity was detected continuously by means of scintillation counting. The flushing solution was either the physiological ionic strength solution or a solution of low ionic strength containing (mmol/l): KCl 5.7; glucose 5.0; sucrose 262.2; Na_2HPO_4/NaH_2PO_4, pH 7.4. The rate constant was calculated as the negative slope of the linear regression line obtained by semilogarithmic plot of counts against time, and is expressed in min^{-1}.

ESR measurements

The fatty acid spin label used in all experiments was 2-(3-carboxypropyl)-2-decyl-4,4-dimethyl-3-oxazolidinyloxyl (I(10,3)). A thin film of the label was prepared on the wall of a glass vessel by dissolving it (0.08 mg) in tetrachlormethane and evaporating the solvent in a stream of nitrogen. Two ml erythrocytes in physiological ionic strength solution (hematocrit 45%) were added and carefully shaken at room temperature for 20 minutes. The procedure is used to avoid interactions between the spin label molecules (Herrmann et al. 1982). The erythrocytes were then separated from the solution by centrifugation ($1000 \times g$, 5 min) and washed twice with 2 ml of either the physiological ionic strength solution or the solution of low ionic strength ($1000 \times g$, 8 min). ESR spectra were recorded on an ESR spectrometer (ZWG, Academy of Science, GDR), using a flat quartz cell for aqueous solution. All measurements were carried out under the following conditions: microwave power 20 mW, modulation amplitude 0.4 mT, scan time 6.6 min, time constant 0.03 s. The temperature was measured with an accuracy of ± 0.1 °C by a small thermistor inserted in the sample cell. The order parameter was calculated according to Griffith and Jost (1976) using the following T-tensors: $T_{xx} = 0.695$ mT, $T_{yy} = 0.535$ mT, $T_{zz} = 3.300$ mT.

Determination of fatty acid content of membrane phospholipids

Fatty acid composition of calf erythrocyte membrane phospholipids was determined by gas chromatography (2100 VARIAN) as described by Preiss et al. (1982).

Statistical treatment of result

The results are presented as mean ± S.D. of at least 3 independent experiments. When not shown, error bars have been smaller than the symbols. Student's t-test was used ($p = 0.05$) to test the significance of differences. In the Arrhenius plots (Figs. 4–7) the significance of differences in activation energies and frequency factors was estimated using regression line analysis based on F-test ($p = 0.05$).

Results

The rate constants of the $Rb^+(K^+)$ efflux of erythrocytes from six mammalian species (man, rat, horse, pig, rabbit, cow) were measured in physiological and low ionic strength solution. The main differences between the erythrocytes of the various mammalian species studied concern the relative contents of membrane phospholipids as well as the structure of the acyl chains of their fatty acids (Deuticke 1977). We attempted correlating the rate constants of $Rb^+(K^+)$ efflux obtained in physiological and low ionic strength solutions with the composition of membrane phospholipids reported for the different mammalian species (Nelson 1967; Wessels and Veerkamp 1973). For both solutions there was no significant correlation of the rate constants with the relative contents of the four major phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin; data not shown).

Fig. 1*a* shows that in solution of physiological ionic strength, a significant correlation could be found between the rate constants of $Rb^+(K^+)$ efflux for erythrocytes of various mammalian species and the relative content of arachidonic acid in the membrane phospholipids. The same could be observed in low ionic strength solution with the exception of human erythrocytes (Fig. 1*b*). Although it is clear from Fig. 1*b* that a significant correlation can be established upon excluding other animals (e.g. rat), there is a reasonable explanation for neglecting data for human erythrocytes only (see Discussion). Furthermore, there was no correlation between the rate constants of $Rb^+(K^+)$ efflux in both solutions and the relative contents of any other phospholipid fatty acid measured.

In previous papers we could show the rate constant of $Rb^+(K^+)$ efflux of human erythrocytes to significantly increase in low ionic strength solution (Bernhardt et al. 1984; Erdmann et al. 1990). This phenomenon is absent in e.g. cow erythrocytes (cf. Figs. 1*a* and 1*b*; see also Erdmann et al. 1990). However, the erythrocytes of newborn calves show the same increase of the rate constant in solution of low ionic strength as do human erythrocytes (Fig. 2). The increase depends on the age of the calves. Investigations of the lipid composition of calf erythrocytes did not reveal any age-dependent change of the head group composition of the membrane phospholipids (data not shown). However, changes in the relative contents of arachidonic acid (20:4) and linoleic acid (18:2) could be detected (Fig. 3). Thus, the rate constant of $Rb^+(K^+)$ efflux from calf erythrocytes in low ionic solution can be correlated with the content of arachidonic acid depending on the age of the calves (correlation coefficient $r = 0.951$, $p = 0.05$). But in physiological ionic strength solution there is no significant correlation of the rate constant of $Rb^+(K^+)$ efflux and the arachidonic acid content of the membrane phospholipids. Since it has been demonstrated that the rate constants of the $Rb^+(K^+)$ efflux of erythrocytes from various mammalian species correlate with the membrane fluidity (measured

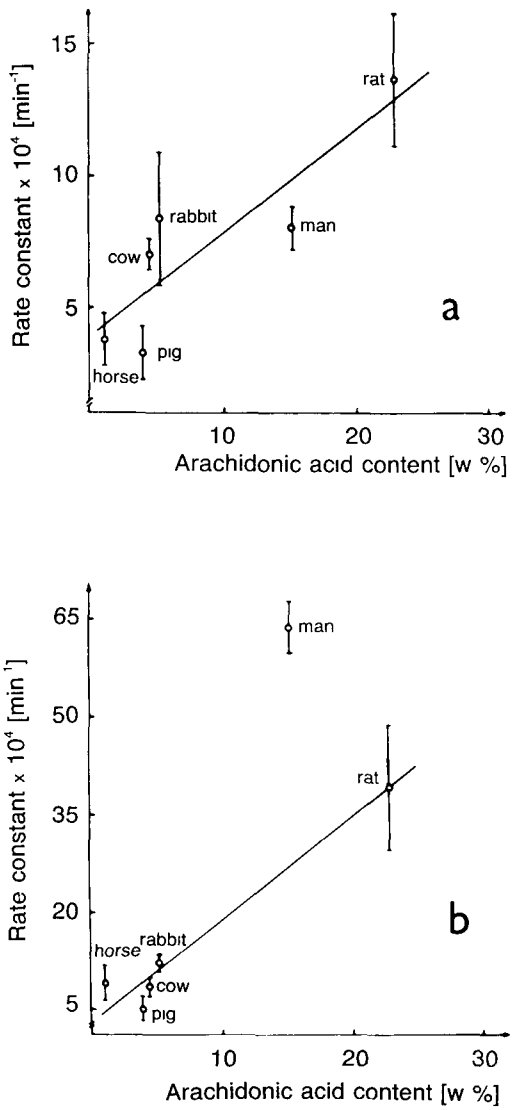

Figure 1. Rate constant of $Rb^+(K^+)$ efflux as a function of the arachidonic acid content of the erythrocyte membrane phospholipids (arachidonic acid values taken from Wessels and Veerkamp 1973). *a*) rate constants in solution of physiological ionic strength, $r = 0.877$, $p = 0.05$ (with human erythrocytes excluded: $r = 0.911$, $p = 0.05$); b) rate constants in solution of low ionic strength, $r = 0.797$, not significant (with human erythrocytes excluded: $r = 0.974$, $p = 0.01$). T=37 °C, pH 7.4, osmolality = 290 mOsmol/l

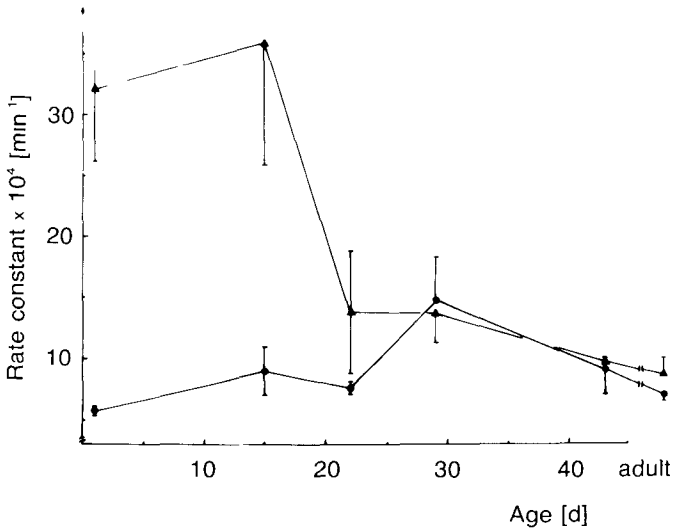

Figure 2. Rate constant of $Rb^+(K^+)$ efflux of calf erythrocytes in dependence on the age of the animals Filled circles, physiological ionic strength solution, filled triangles, low ionic strength solution T=37 °C, pH 7 4, osmolality = 290 mOsmol/l

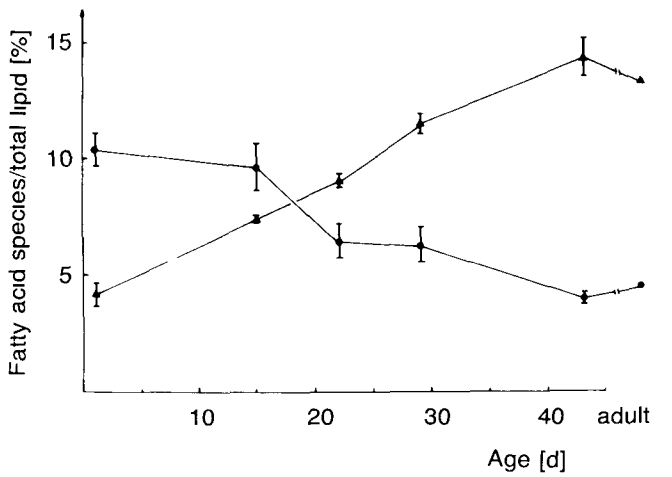

Figure 3. Arachidonic acid and linoleic acid contents of membrane phospolipids calf erythrocytes in dependence on the age of the animals Filled circles, arachidonic acid filled triangles linoleic acid (Data for adults from Haest, personal communication)

as the order parameter of the fatty acid spin label I(10,3) or taken as the mean number of double bonds of the phospholipids, Erdmann et al 1990) we measured the ESR order parameter of this spin label in calf erythrocytes in dependence on the age of the animals No significant changes could be observed (data not shown)

Furthermore, to decide whether the increase of the rate constant of the $R^+(K^+)$ efflux in low ionic strength solution is based on fluidity of the erythrocyte membrane phospholipids we measured the rate constants of the $Rb^+(K^+)$ efflux as well as the order parameters of the fatty acid spin label I(10,3) for human and cow erythrocytes in solutions of physiological and low ionic strength in dependence on temperature The results are presented in Figs 4–7 From Fig 4 it can be seen that the increase of the rate constant of the $Rb^+(K^+)$ efflux of human erythrocytes in low ionic strength solution is temperature-dependent The apparent activation energies of the $Rb^+(K^+)$ transport were 16 kJ/mol in physiological ionic strength solution and 52 kJ/mol in low ionic strength solution (the difference is significant) The frequency factors were estimated to be 0 46 min^{-1} in physiological ionic strength solution and 4 11 10^6 min^{-1} in low ionic strength solution (the dif ference is significant) The values for cow erythrocytes were found to be 11 kJ/mol and 13 kJ/mol (insignificant difference) and 0 07 min^{-1} and 0 20 min^{-1} (insignif icant difference), respectively (Fig 5) From Figs 6 and 7 it can be seen that

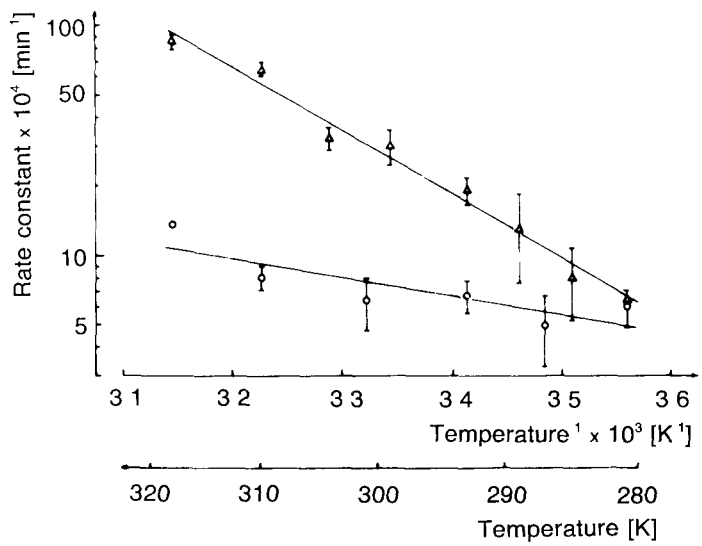

Figure 4. $Rb^+(K^+)$ efflux rate constant for human erythrocytes as a function of temperature (Arrhenius plot) Empty circles, physiological ionic strength solution, empty triangles, low ionic strength solution pH 7 4, osmolality = 290 mOsmol/l

the I(10,3) spin label order parameters for both human and cow erythrocytes are decreased (i.e. membrane fluidity is increased) upon reducing the ionic strength of the medium. The apparent activation energies of the order parameters for human erythrocytes were −4.8 kJ/mol in physiological ionic strength solution and −4.9 kJ/mol in low ionic strength solution (insignificant difference). The values for the frequency factor were 0.10 both in the physiological and the low ionic strength solution. The corresponding values for cow erythrocytes were −5.7 kJ/mol and −4.4 kJ/mol (insignificant difference), and 0.08 and 0.12 (insignificant difference), respectively.

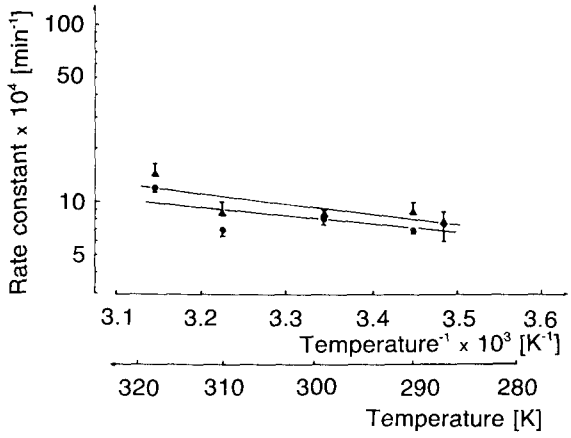

Figure 5. $Rb^+(K^+)$ efflux rate constant for cow erythrocytes as a function of temperature (Arrhenius plot). Filled circles, physiological ionic strength solution; filled triangles, low ionic strength solution. pH 7.4, osmolality = 290 mOsmol/l.

Interestingly, in human erythrocytes a comparable change in membrane fluidity produced by changing either the temperature or the ionic strength of the solution does not result in an equivalent change of the rate constant of the $Rb^+(K^+)$ efflux. In addition, a comparison of the rate constants of human and cow erythrocytes in low ionic strength solution at different temperatures (but at the same order parameter) shows that the increase of the rate constant of the $Rb^+(K^+)$ efflux is not equivalent.

Discussion

The results presented in this paper continue the investigations of Erdmann et al. (1990) who stressed membrane fluidity as a possible factor influencing the $Rb^+(K^+)$

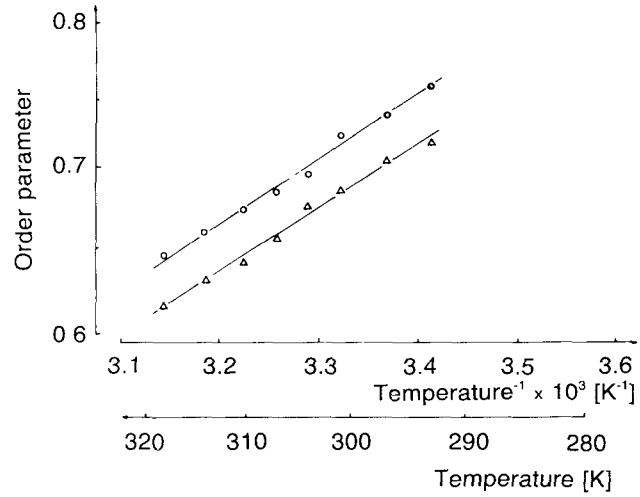

Figure 6. Order parameter of the fatty acid spin label I(10.3), incorporated in the membrane of human erythrocytes, as a function of temperature (Arrhenius plot). Empty circles, physiological ionic strength solution; empty triangles, low ionic strength solution. pH 7.4, osmolality = 290 mOsmol/l.

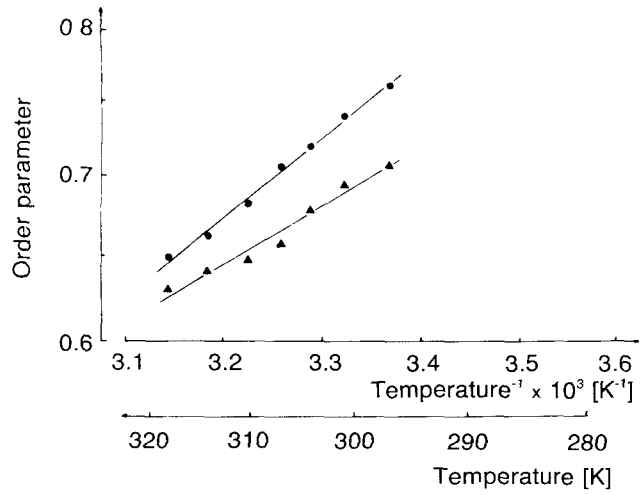

Figure 7. Order parameter of the fatty acid spin label I(10.3), incorporated in the membrane of cow erythrocytes, as a function of temperature (Arrhenius plot). Filled circles, physiological ionic strength solution; filled triangles, low ionic strength solution. pH 7.4, osmolality = 290 mOsmol/l.

efflux of erythrocytes The rate constants of the $Rb^+(K^+)$ efflux from erythrocytes of seven mammalian species in solution of physiological as well as low ionic strength could be shown to correlate with the ESR order parameter of the fatty acid spin label I(10,3), with the values for human erythrocytes being excluded The appearance of significant Na^+, K^+, Cl^- cotransport in human eryhrocytes was proposed as the reason for this different behaviour of human red blood cells (Erdmann et al 1990)

Since it is known that the erythrocyte membranes from different mammalian species differ in their lipid composition the phenomenon reported by Erdmann et al (1990) could reflect also a more specific effect of the membrane composition, possibly influencing also the membrane fluidity There is some evidence that the transport of nonelectrolytes (glycerol, erythritol) and anions (phosphate, chloride) is related to the phospholipid pattern of the erythrocyte membrane (Gruber and Deuticke 1973, Deuticke et al 1980, Lu and Chow 1982) Now we add another evidence to show that the membrane phospholipid composition may be of importance for ion transport regulation The term membrane fluidity is used though there are different interpretations for the physical meaning depending on the method of the fluidity determination The order parameter calculated from the ESR spectrum using the fatty acid spin label I(10,3) refers to the static rather than to the dynamic components of fluidity (Stubbs and Smith 1984) As shown in the present paper the arachidonic acid content of the phospholipids seems to be of importance in case of $Rb^+(K^+)$ efflux, in particular for results obtained in low ionic strength solution This is supported by results of Kuypers et al (1984) who could show that human erythrocytes will be leaky for K^+ if the native phosphatidylcholine is partly replaced by phosphatidylcholine containing arachidonic acid A change of the content of a highly unsaturated fatty acid of the membrane phospholipids is known to change membrane fluidity (Boggs 1980)

The reason for a significant correlation of the rate constant of $Rb^+(K^+)$ efflux in low ionic strength solution with the content of arachidonic acid of the membrane phospholipids (only with the value for human erythrocytes being excluded) is the existence of Na^+,K^+,Cl^- cotransport (same interpretation as for the order parameter, see above) This cotransport pathway is of significant extent in human erythrocytes only (Ellory et al 1982) Furosemide, an inhibitor of Na^+,K^+,Cl^- cotransport, reduced the rate constant of the $Rb^+(K^+)$ efflux of human erythrocytes by about 50% both in solution of physiological and low ionic strength (Bernhardt et al 1987) The rate constants of $Rb^+(K^+)$ efflux for human erythrocytes at 37 °C in the presence of 10^{-4} mol/l furosemide was $4\,2 \pm 0\,7\ 10^{-4}$ min^{-1} in physiological ionic strength solution and $32\,6 \pm 3\,0\ 10^{-4}$ min^{-1} in low ionic strength solution No significant changes were recorded with cow and horse erythrocytes in the presence of furosemide (data not shown) Using these values for human erythrocytes, a close correlation ($r = 0\,969$, $p = 0\,01$) was obtained (Fig 1b) It should be noticed that

in physiological ionic strength solution (Fig 1*a*) no significant correlation could be found between the rate constant of $Rb^+(K^+)$ efflux and the arachidonic acid content of the membrane phospholipids, using the rate constant for $Rb^+(K^+)$ efflux of human erythrocytes in the presence of furosemide ($r = 0\ 688$) However, using this value for human erythrocytes a significant correlation could be found between the rate constant and the ESR order parameter of the fatty acid spin label I(10,3) measured by Erdmann et al (1990) ($r = -0\ 859$, $p = 0\ 05$)

Upon inhibiting Na^+ K^+,Cl^+ cotransport by furosemide (see above) equal rate constants for human and cow erythrocytes cannot be obtained at the same order parameter (different temperatures) even when accounting for the decrease of the $Rb^+(K^+)$ efflux rate constant of human erythrocytes in low ionic strength solution Therefore the conclusion has to be drawn that the increase of $Rb^+(K^+)$ efflux in low ionic strength solution is not solely based on a change of membrane fluidity

As could be shown in previous papers the enhancement of the residual transport of monovalent cations through the erythrocyte membrane after reducing the ionic strength of the extracellular solution cannot be explained on the basis of electrodiffusion (Bernhardt et al 1988, 1991, Erdmann et al 1990, 1991) Obviously there must be an additional and essential factor influencing the residual cation transport through the erythrocyte membrane Although the mechanism of the residual cation transport is not known, from experiments with SH-reacting substances it is reasonable to assume that membrane proteins are involved (Bernhardt et al 1991) Therefore, it is possible that the surrounding lipids can influence the proteins participating in this process by changing their conformation and/or by modifying the state of the lipid protein interface

The results presented herein may be interpreted in the following way Different actions which change the membrane fluidity (temperature, ionic strength) might influence the interaction between lipids and proteins in a different way, and therefore differently affect the residual cation transport In some case (e g in physiological ionic strength solution), the physical properties of the membrane reflected by the ESR order parameter seem to be decisive, under different conditions (e g in low ionic strength solution) the role of special fatty acids of the membrane phospholipids is of more importance for residual cation transport In this respect a specific effect of arachidonic acid was observed, although it cannot be ruled out that this is a specific effect including other highly unsaturated fatty acids present in the membrane phospholipids in minor amounts only In addition, a species-dependent occurrence of a membrane protein (or proteins) participating in the residual transport of monovalent cations (possibly also varying with the ageing of the animals) should be considered This protein could also be differently influenced by the above mentioned conditions

The results presented underline the idea that the fatty acid composition of the membrane phospholipids is of importance for the residual transport of monovalent

cations through the erythrocyte membrane although it will also influence membrane fluidity This should be considered when investigating cation transport phenomena in relation to membrane phospholipids

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Final version accepted February 26, 1992