Biochimica et Biophysica Acta, 552 (1979) 540—545

BBA Report

BBA 71380

TEMPERATURE DEPENDENCE OF ACTIVE K^+ TRANSPORT IN CATION DIMORPHIC SHEEP ERYTHROCYTES

C.H. JOINER and P.K. LAUF

Department of Physiology, Duke University Medical Center Durham, NC 27710 (U.S.A.)

(Received December 5th, 1978)

Key words: Temperature dependence; K^+ transport; Cation polymorphism; Anti-L; (Sheep erythrocyte)

Summary

Arrhenius diagrams of K^+ pump fluxes measured between 15°C and 41°C were discontinuous in high K^+ but not in low K^+ sheep red cells. Exposure of low K^+ cells to anti-L caused a bimodal temperature response of K^+ pump flux with a transition temperature, *Tc*, similar to that found in high K^+ cells but with comparatively higher activation energies above *Tc*.

The temperature dependence of Na^+ and K^+ transport in intact cells has received only little attention. Active and passive cation movements were measured in red cells and cultured renal cortex cells of hibernators and nonhibernators to explain the 'cold resistance' of hibernating animals to loss of cellular K^+ at low body temperatures [1,2]. A disparity was reported between the temperature dependence of K^+ pumping in intact red cells and the (Na^+ + K^+) -ATPase of their hemoglobin free membranes in guinea pig and ground hog [3]. Numerous reports have associated discontinuities in Arrhenius plots of (Na^+ + K^+)-ATPase activity of microsomal preparations with lipid phase changes [4,5] or phase separation [6]. However, the applicability of such studies to transporting systems in intact cells has been questioned [3].

The Na^+ and K^+ transport system of high potassium (HK) and low potassium (LK) sheep red blood cells offers several advantages for the study of its temperature dependence. The two types of cells differ both kinetically in their internal cation affinities [7], as well as their maximum pump turnover rates [8]. Furthermore, the isoantibody to LK cells (anti-L) has been shown to affect both of these parameters when stimulating active transport in LK cells [9,10,11]. This system thus provided a unique opportunity to relate the temperature dependence of a transport system to its physiologic operation.

The red cells used in this study were obtained from sheep homozygous with respect to their cationic trait (HK or LK) and antigenic status (MM or LL). The genetic aspects of this system as well as details of the L antibody preparation have been reported elsewhere [12]. Media were prepared from reagent grade chemicals and ultrafiltered deionized water. Experiments were performed in sodium (Na^+) or tetramethylammonium chloride media. Previous work from this laboratory has shown that the presence of trimethylammonium in the medium did not impair maximum K^+ pump activity measured under conditions of cellular Na^+ loading [8,11]. This was to be expected since, unlike Na^+, trimethylammonium does not interact with the K^+ loading site of the Na^+ and K^+ pump. Sodium medium consisted of (mM): 140 NaCl, 5 KCl, 10 glucose, 10 imidazole-HCl. The composition of the trimethylammonium medium was: trimethylammonium 230 mosM, NaCl 20 mM, KCl 5 mM, glucose 10 mM, imidazole-HCl 10 mM. Two buffer systems were employed. In some experiments, media with pH 7.4 at each individual temperature were used. Alternatively, one solution with pH 7.4 at 37°C was used for all temperatures so that the actual pH of a given solution varied according to the temperature coefficient of the imidazole buffer (from pH 7.29 at 40°C to 8.11 at 5°C). Because experimental comparison of K^+ influx under these two conditions of pH showed only small differences not affecting the temperature dependence of either active or passive fluxes, most of the experiments presented here involved the more convenient technique of using a single solution of pH 7.4 at 37°C.

Measurement of cellular cations, and estimation of unidirectional K^+ influx by ^{42}K uptake have been reported previously [9,13,14]. In brief, red cells, suspended at a hematocrit of 10% in the buffers described above, in presence and absence of anti-L and with or without ouabain were pre-equilibrated for 20 min. The concentrations of anti-L (5 mg anti-L immunoglobulin/ml) and of ouabain (10^{-4} M) were more than sufficient to saturate all L antigenic sites and pumps sites, respectively, during this period of incubation and the temperatures selected. Duplicate samples were taken after one hour incubation of cell suspensions in presence of isotope in a series of waterbaths of various temperatures selected in the range of 15—41°C. K^+ pump influx ($^iM_K^P$) is defined as the ouabain-inhibitable component of total K^+ influx.

To ascertain that high or low temperature incubation or the media chosen did not induce artifacts in K^+ pump determinations due to alterations of cellular K^+, $[K^+]_c$, or ATP concentrations, these two parameters were checked. The $[K^+]_c$ in control LK cells was 11.2 mmol/l cells after 1 h incubation at 19°C, as compared to 11.7 at 40°C. LK cells treated with anti-L had $[K^+]_c$ of 11.0 and 12.8 at 19°C and 40°C, respectively. These small differences in $[K^+]_c$ would not be expected to alter K^+ pumping significantly in LK cells [7], especially in the presence of anti-L [9]. ATP contents, measured on perchloric acid extracts of red cell suspensions [15] were reduced slightly by high temperature incubation. In anti-L treated LK cells, ATP content was 0.63 mmol/l cells after 1 h at 40°C, as compared to 0.78 at 21°C. Control LK cells had ATP contents of 0.69 and 0.89 at 40°C and 21°C, respectively, while HK cells had 0.98 and 1.09 mmol/ATP/l cells at these temperatures. As with $[K^+]_c$ levels, these variations of ATP contents have previously not

been found to noticably affect the operation of the Na^+ and K^+ pump [8], unless a major shift in the affinities of the pump system for these ligands occurred with changing temperature.

Active K^+ influx ($\vec{M}_K^P$) was found to be highly temperature dependent in both HK and LK sheep erythrocytes, with K^+ pumping dropping in LK cells to immeasurable levels at temperatures lower than 15°C. Arrhenius plots of ln $\vec{M}_K^P$ versus reciprocal absolute temperature are shown for three typical experiments in Fig. 1. All such plots for HK sheep cells demonstrated a clear discontinuity as illustrated in panel A, and the data were well fitted by two straight lines. Such discontinuities were not apparent in Arrhenius plots of K^+ pump rates of control LK red cells (panels B and C, open circles), although the scatter of the data shown was clearly greater in these cells. However, close inspection of the data from six experiments led to the conclusion that straight lines best described the plots. The addition of anti-L to these cells caused a change in the temperature dependence of K^+ pumping and also reduced the scatter of the data (Fig. 1, panels B and C, filled circles). At temperatures above 30°C, anti-L-stimulated K^+ pumping fell at a lower rate with temperature than did control LK fluxes. The temperature of about 30°C constituted an inflection point below which antibody-stimulated fluxes were more rapidly inhibited.

Table I presents data collected from several experiments. The transition, or critical, temperatures (T_c) for K^+ pumping ranged from 28—32°C in HK cells, and in anti-L-treated LK cells (LK anti-L) was 30.9°C. Activation energies above T_c (Ea_I^{HK}) for HK cells were 9—11 kcal/mol and below T_c (Ea_{II}^{HK}) 18—27 kcal/mol. The activation energy (Ea^{LK}) for K^+ pumping in LK cells over the entire range of temperature varied between 19 and 30 kcal/mol. The alteration in temperature dependence produced by anti-L con-

TABLE I

ACTIVATION ENERGIES AND CRITICAL TEMPERATURES FOR K^+ PUMP FLUXES IN SHEEP RED CELLS, AND THE EFFECT OF ANTI-L

Values were determined from Arrhenius diagrams as described in Fig. 1. 'Averages' for anti-L treated LK cells are given as mean ± S.E.M. for the data listed.

Sheep	Media	Critical Temp (T_c)	Activation Energy (kcal/mol)	
			Above T_c	Below T_c
HK 63	Na	27.7	10.1	27.1
HK 86	Na	29.6	8.7	29.6
HK 86	Na	29.2	9.1	26.7
HK 86	TMA	32.4	11.0	18.4
LK 58	Na	none	21.5	
LK 58	Na	none	30.1	
LK 58	Na	none	18.5	
LK 51	TMA	none	27.3	
LK 52	TMA	none	23.1	
LK 71	TMA	none	20.0	
Anti-L treated				
LK 51	TMA	33.8	17.7	25.6
LK 52	TMA	29.3	15.8	32.4
LK 71	TMA	29.6	16.3	28.4
LK + Anti-L Averages		30.9 ± 1.5	16.6 ± 0.6	28.8 ± 2.0

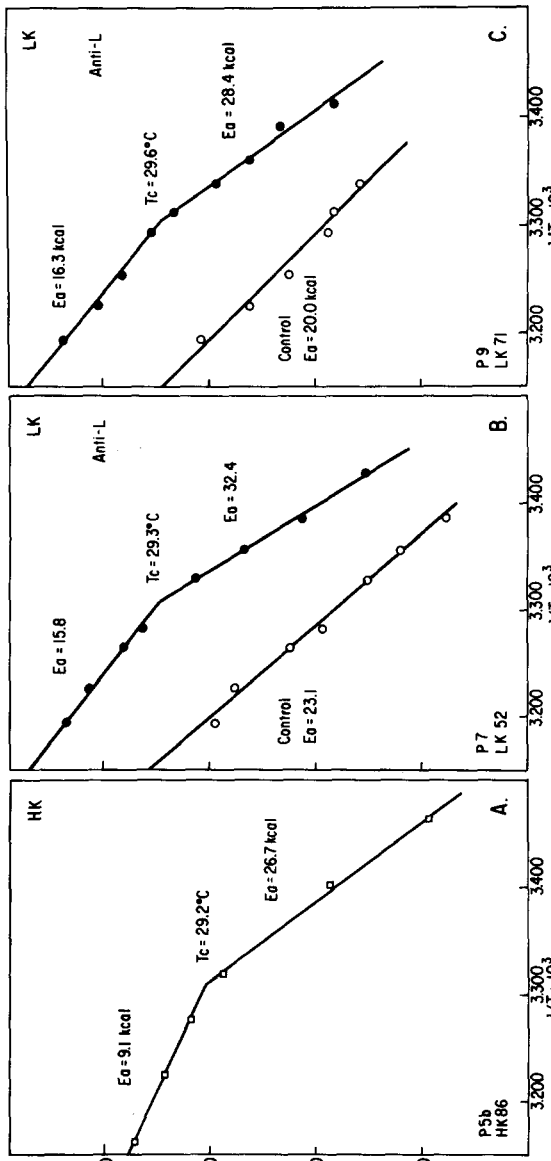

Fig. 1. Arrhenius plots of active K^+ influx in sheep red blood cells. Washed cells were preincubated 20 min at room temperature with and without 10^{-4} M ouabain to ensure complete inhibition of K^+ pumping in treated cells. If anti-L (S—44) was used, it was dialysed overnight at 4°C in appropriate medium and included in the preincubation at 1/6 dilution relative to serum. After the cells had equilibrated 20 min in the various temperature baths, ^{42}K was added. The lines depicted are derived from least squares regression analysis of the data. Critical temperatures were determined by the simultaneous solution of the two regression equations involved. Panel A: HK red cells; panels B and C illustrate two experiments with the cells from two LK (LL) sheep, without ("Control", ○) and with anti-L (●).

sisted of changing Ea^{LK} of K^+ pumping above 30° C to 16.6 kcal/mol (Ea_I^{AL}) along with the introduction of a transition temperature. This average Ea_I^{AL} value was clearly lower than the range of Ea_I^{LK} values and higher than that of Ea_I^{HK} values, whereas below T_c, Ea_{II}^{AL} for anti-L treated LK cells (28.8 kcal/mol) fell well into the range of the Ea^{LK} values in the absence of anti-L.

In contrast to our findings, the temperature dependence of (Na^+ + K^+)-ATP-ase obtained from numerous mammalian tissues generally has revealed inflections in Arrhenius plots between 16 and 22° C; activation energies ranged from 14—21 kcal/mol above T_c and from 32—45 kcal/mol below [5, 16—18]. Our experiments yielded activation energies for K^+ pumping which were lower, and the T_c values found in the range between 28 and 32° C for HK cells and between 30—34° C for LK cells were clearly higher than reported for (Na^+ + K^+)-ATPase preparations. This finding is consistent with reports that K^+ pumping in intact cells was less temperature sensitive than the (Na^+ + K^+)-ATPase activity of broken membrane preparations [2,3].

Although the interpretation of the temperature dependence of transport processes in biological membranes is compounded with difficulties, the possible origin of the change of the activation energy of K^+ transport occurring at critical temperatures in HK and anti-L treated LK cells warrants consideration in its relation to the monotonic Ea^{LK} found in untreated LK red cells. Generally, the activation energy is associated with the rate limiting step of a process; a change in this parameter (produced at T_c by anti-L or a difference in cell type) reflects some alteration of the rate controlling step. Studies on (Na^+ + K^+)-ATPase preparations have revealed that only the overall enzyme activity shows a nonlinear temperature response, while its Na^+ supported phosphorylation and K^+ dependent dephosphorylation steps exhibit linear Arrhenius plots [6,21], suggesting that the interconversion from a Na^+ to a K^+ sensitive conformation is being altered at T_c. This interpretarion is not easily applicable to the K^+ pump behaviour of our study and hence it is not possible to identify the rate limiting steps involved in the shift at T_c nor to decide whether only a quantitative change occurs in a particular reaction step.

Discontinuities in Arrhenius plots of (Na^+ + K^+)-ATPase preparations as well as hexose transport systems [19] have been associated with lipid phase changes or transitions [4—6,20]. It has been suggested that the structural transition occurring around 20° C is due to a change in the lipid microenvironment of the (Na^+ + K^+)-ATPase rather than to liquid-crystal-gel phase transitions of the bulk lipid [22]. Lack of information on temperature dependence of active cation transport in intact cells precludes analogies to the work on isolated (Na^+ + K^+)-ATPase preparations as well as a useful correlation with our K^+ pump flux studies in sheep red cells. The lipid composition in sheep red cells is dissimilar to that of other cells because sheep cells contain sphingomyelin instead of phosphatidyl choline. Work on lipid mixtures suggests that the cholesterol has a higher affinity for sphingomyelin than for phosphatidyl choline [23]. Perhaps the presence of sphingomyelin or its tighter interaction with cholesterol changes the microenvironment of the Na^+ and K^+ pumps and hence contributes to the elevated T_c values found for K^+ pump flux in HK cells. Anti-L, therefore, may alter the reciprocal interaction between lipids and the pump proteins, a possibility not excluded by the fact that HK and LK red cell membranes have

identical lipid compositions [24,25] and that anti-L has not been shown yet to alter membrane lipid structure and composition. On the other hand, the origin of the critical temperature for active K^+ transport in HK and anti-L treated LK cells may simply reside in the protein moiety of the Na^+ and K^+ pump. In particular, the affinities for various ligands [26], the ionization state of the protein complex, and pump turnover could determine the temperature sensitivity of the system. In this regard it is of interest that the transition temperatures for Cl^- and Br^- transport were different (15° C versus 25° C) in human red cells but occurred at the same turnover rate of the system [27]. It would be of interest to analyze the temperature sensitivity of the K^+ pump in Na^+ loaded HK and LK cells under conditions of maximum transport rates.

This work was supported by USPHS Grant HL 2 PO1-12 157.

References

1 Kimzey, S.L. and Willis, J.S. (1971) J. Gen. Physiol. 58, 634—649
2 Zeidler, R.B. and Willis, J.S. (1976) Biochim. Biophys. Acta 436, 628—651
3 Ellory, J.C. and Willis, N.S. (1976) Biochim. Biophys. Acta 443, 301—305
4 Kimmelberg, H.K. and Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 282, 277—292
5 Charnock, J.S. and Simonson, L.P. (1977) Comp. Biochem. 58, 381—389
6 Barnett, R.E. and Palazzotto, J. (1974) Ann. N.Y. Acad. Sci. 242, 69—76
7 Hoffman, P.G. and Tosteson, D.C. (1971) J. Gen. Physiol. 58, 438—466
8 Joiner, C.H. and Lauf, P.K. (1978a) J. Gen. Physiol. (Lond.) 283, 155—175
9 Lauf, P.K., Rasmusen, B.A., Hoffman, P.G., Dunham, P.B., Cook, P., Parmelee, M.L. and Tosteson, D.C. (1970) J. Membrane Biol. 3, 1—13
10 Glynn, I.M. and Ellory J.C. (1972) in Roles of Membranes in Secretory Processes (Bolis, L., Keynes, R.D. and Wilbrandt, W., Eds.), pp. 224—237, Elsevier, New York
11 Joiner, C.H. and Lauf, P.K. (1978b) J. Physiol. (Lond.) 283, 177—196
12 Lauf, P.K. (1975) Biochim. Biophys. Acta 415, 173—229
13 Joiner, C.H. and Lauf, P.K. (1975) J. Membrane Biol. 21, 99—112
14 Lauf, P.K., Stiehl, B.J. and Joiner, C.H. (1977) J. Gen. Physiol. 70, 221—242
15 Lowry, O.H., Passoneau, J.V., Hasselberger, F.X. and Schultz, D.W. (1964) J. Biol. Gem. 239, 18—30
16 Charnock, J.S., Cook, C.A. and Casey, R. (1971) Arch. Biochem. Biophys. 147, 323—329
17 Grisham, C.M. and Barnett, R.E. (1974) Biochemistry 12, 2635—2637
18 Tanaka, R. and Teruya, A. (1973) Biochim. Biophys. Acta 323, 584—591
19 Wilson, G. and Fox, C.F. (1971) J. Mol. Biol. 55, 49—60
20 Charnock, J.S., Cook, D.A., Almeida, A.F. and To, R. (1973) Arch. Biochem. Biophys. 159, 393—399
21 Neufeld, A.H. and Levy, H.M. (1970) J. Biol. Chem. 245, 4962—4967
22 Barnett, R.E. (1977) in Membrane Proteins and Their Interactions With Lipids (Capaldi, R.A., ed.), Vol. 1, pp. 189—206, M. Dekker, New York
23 Demel, R.A., Jansen, J.W.C.M., Van Dijk, P.W.M. and van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 465, 1—10
24 Nelson, G.J. (1967) Lipids 2, 64—71
25 Nelson, G.J. (1969) Lipids 4, 350—355
26 Ellory, J.C. and Willis, J.S. (1978) J. Physiol. (Lond.) 274, 80p
27 Brahm, J. (1977) J. Gen. Physiol. 70, 283—306