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TEMPERATURE DEPENDENCE OF MEMBRANE FUNCTION. DISPARITY BETWEEN ACTIVE POTASSIUM TRANSPORT AND $(Na^+ + K^+)$ATPase ACTIVITY

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Summary

Ouabain-sensitive K^+ influx in mammalian erythrocytes exhibits far less temperature sensitivity than the $(Na^+ + K^+)$ATPase prepared by hypotonic lysis from the same population of cells. The results are not in accord with lipid phase change as the critical mechanism of cold inhibition of intact pumps.

Much attention has been focused on the temperature dependence of membrane-bound $(Na^+ + K^+)$ATPase activity. Discontinuities in Arrhenius plots of enzyme activity have been interpreted in terms of a phase transition (e.g., refs. 1—7) or phase separation [8] in the membrane lipids, leading to a severe inhibition of enzyme function at low temperature. Additional support for this idea has been derived from fluorescence polarization spectroscopy [9]. Willis and Li [10] pointed out several years ago, however, that in mammalian kidney slices Na^+-dependent net uptake of K^+, and Na^+-sensitive respiration (supposedly a measure of in situ $(Na^+ + K^+)$ATPase [11,12]) were much less sensitive to cooling than was the $(Na^+ + K^+)$ATPase from a fraction of the same tissue. More recently, Zeidler and Willis [13] have observed that the ouabain-sensitive K^+ influx at 5°C into cultured kidney cells is about 10% of that at 37°C, a reduction which is an order of magnitude less than that seen in any $(Na^+ + K^+)$ATPase preparation from kidneys of the same species. These observations would seem to indicate a discrepancy in temperature sensitivity between the actual pump and the $(Na^+ + K^+)$ATPase, but at the same time they suffer from several difficulties of relating activity of a microsomal fraction prepared by cell rupture and detergent extraction to a function of intact cells. We undertook, therefore, to make a direct comparison between ion fluxes in erythrocytes and the $(Na^+ + K^+)$ATPase of broken cells freshly prepared by simple hypotonic lysis.

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Erythrocytes from guinea pigs, ground squirrels (*Citellus tridecemlineatus*) and groundhogs (*Marmota monax*) were used. The latter two are hibernating species and were chosen because the Na^+-pump of their red cells exhibits a low sensitivity to cooling, and a normal Na^+-K^+ coupling is maintained down to 5°C [14,15]. Blood samples (2—10 ml) were taken by cardiac puncture from lightly anesthetized animals into a heparinized glass syringe. The cells were washed three times by centrifugation (5 min, 3000 × *g*) in 10 ml of a medium containing 145 mM NaCl, 7.5 mM KCl, 2 mM $MgCl_2$, 10 mM glucose and 15 mM imidazole buffer. The pH was adjusted to 7.5 for each operational temperature. K^+ influx was measured by incubating cells in 1 ml of the medium modified to contain ^{42}KCl at a hematocrit of 0.03. Samples were incubated in triplicate, with ouabain added to half the tubes to a final concentration of 0.1 mM. After appropriate intervals (15—300 min) the cells were washed three times by centrifugation (10 s, 12 000 *g*) in a Beckman microfuge, lysed in 5% (w/v) trichloroacetic acid and their radioactivity measured by the Cerenkov effect in a liquid scintillation counter. The same samples were later analyzed for total Na^+ and K^+ by flame photometry. Three sampling times were used in each case to assure that computation of influx was based upon a linear uptake.

Ghosts were prepared by lysing washed packed cells in 14 vol. of an ice-cold solution of 10 mM imidazole and 1 mM EDTA, pH 7.1. After 10 min the membranes were centrifuged at 25 000 × *g* for 30 min at 5°C, washed twice in the imidazole EDTA medium, frozen and thawed quickly and washed three further times in a medium with only 10 mM imidazole, pH 7.2. The volume was then made up to that of the original volume of packed cells from which the ghosts were made.

In most instances the ghosts were used directly for estimation of (Na^+ + K^+)ATPase, but in some cases they were frozen and then stored for up to three days before use. No change in activity with storage was observed. ATPase activity was estimated by the release of inorganic phosphate as previously described [16] and the Na^+ + K^+ activation of the enzyme was determined by comparing the activity in tubes with and without 0.1 mM ouabain.

In Table I the effects of temperature on the (Na^+ + K^+)ATPase activity and on the ouabain-sensitive K^+ influx of erythrocytes of ground squirrels and guinea pigs are compared. At 37°C the molar ratio of K^+ influx to

TABLE I

A COMPARISON OF OUABAIN-SENSITIVE K^+ INFLUX AND ATPase ACTIVITY IN GUINEA PIG AND GROUND SQUIRREL RED CELLS AT VARIOUS TEMPERATURES

Data are expressed ± S.E.M. for four animals in each group. Each animal was measured at all temperatures.

Temp. (°C)	Guinea pig ouabain-sensitive ($mmol \cdot l\ cells^{-1} \cdot h^{-1}$)		Ratio	Ground squirrel ouabain-sensitive ($mmol \cdot l\ cells^{-1} \cdot h^{-1}$)		Ratio
	K^+ influx	ATPase		K^+ influx	ATPase	
10	0.067 ± 0.007	0.0048 ± 0.0005	14	0.19 ± 0.02	0.0070 ± 0.0008	27
15	0.25 ± 0.02	0.016 ± 0.001	15.6	0.40 ± 0.03	0.043 ± 0.009	9.3
20	0.60 ± 0.06	0.052 ± 0.002	11.5	0.72 ± 0.12	0.14 ± 0.01	5.1
25	1.22 ± 0.09	0.21 ± 0.01	5.8	1.33 ± 0.12	0.32 ± 0.05	4.2
30	1.84 ± 0.06	0.64 ± 0.06	2.9	2.28 ± 0.29	1.19 ± 0.09	1.9
37	2.75 ± 0.18	1.25 ± 0.08	2.2	2.95 ± 0.22	1.25 ± 0.15	2.4

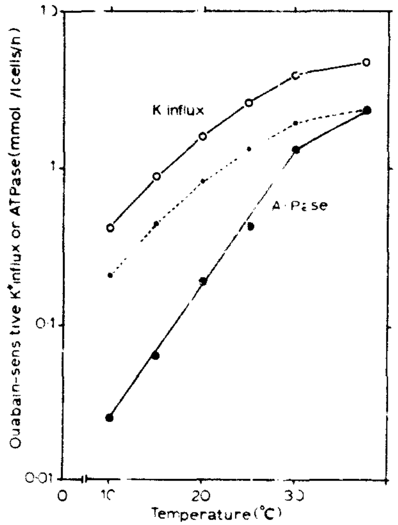

Fig. 1. Temperature dependence of ouabain-sensitive K^+ influx and ATPase activity in red cells from a hibernating groundhog. The washed red cells were stored at 5°C for 72 h in a medium containing 150 mM NaCl, 20 mM imidazole, 10 mM glucose, pH 7.3, and then preincubated at 37°C for 1 h to raise intracellular ATP levels. Fluxes were measured at a hematocrit of 0.03 for 3 times points in the period 0.5—5 h. ATPase activity in a broken ghost preparation was measured at a hematocrit of 0.05—0.3 over similar time intervals in a medium containing 150 mM NaCl, 10 mM KCl, 2 mM ATP, 15 mM imidazole pH 7.5, ± 0.1 mM ouabain. Intracellular Na^+ and K^+ concentrations measured at the start and end of the influx were Na^+ 38—43, K^+ 64—69 mmol · l cells^{-1}. The broken line represents an extrapolated ATPase activity assuming that the K^+/ATP ratio remained constant at 2 over the whole temperature range (as actually measured at 37°C).

$(Na^+ + K^+)$ATPase, based upon equivalent populations of cells, is between 2 and 3. At temperatures below 30°C, cooling inhibits the ATPase more strongly than it inhibits K^+ influx, so that at 10°C the K^+/ATP ratio is 27 in ground squirrels and 14 in guinea pigs.

This divergence is illustrated graphically in Fig. 1 with results on ATPase and flux in erythrocytes of a single groundhog, by comparing the actual decline in $(Na^+ + K^+)$ATPase activity with values computed on the assumption of a K^+/ATP ratio of 2 (as was measured at 37°C) at all temperatures. This experiment was useful also in that the cells had been stored for 72 h at 5°C, so that the Na^+ in the cells had risen to 40 mM. Hence, it is unlikely in this case that the K^+ influx was elevated at low temperature by a spurious increase of Na^+ content due to short-term incubation in the cold.

It is clear that even under conditions which may not yield maximal fluxes, the active transport of K^+ in intact cells is significantly greater at low temperature than would be expected from the measurement of enzyme activity. The fact that the two activities correlate well at 37°C indicates that the discrepancy at lower temperatures can not be attributed to loss of membrane or to simple inactivation of enzyme during its preparation.

It seems more likely that the difference is due to structural or compositional alteration resulting either from the preparation of the ghosts or from the lack of asymmetric conditions during their incubation. Thus, the difference in concentration of Na^+, K^+, Ca^{2+} or other components between the inside and outside of the cell may be important for the preservation of the integrity of the membrane or the local environment of the $(Na^+ + K^+)$ATPase. On the other hand, even though preparation of the ghosts requires no solubilization or disruption of the membrane, the essential operations of EDTA treatment and freezing and thawing may still render the membrane more vulnerable to higher order effects of changing temperature.

While these results seem to support the conclusions based on earlier studies with kidney tissue [10,13], it nevertheless may be that the discrepancy shown here reflects peculiar features of erythrocytes or of the procedures we used, rather than a general phenomenon. In this regard, two studies may be particularly relevant. The first is the proposal of Proverbio and Hoffman [17, 18] that in erythrocytes at low temperature a local membrane pool of ATP, to which the $(Na^+ + K^+)$ATPase has access, cannot exchange rapidly with bulk-phase ATP. (This hypothesis, based upon temperature-dependent changes in the ease of labelling $(Na^+ + K^+)$ATPase with exogenous [^{32}P]ATP, further states that the local pool is replenished by either cytoplasmic ATP or adjacent membrane-bound diphosphoglycerate kinase at 37°C, but only from the latter source at low temperature.)

The other pertinent observation is that Tanaka and Ohnishi [19] found a marked asymmetry in the distribution of specific spin-labelled phospholipids, which was reduced on preparing ghosts by hypotonic lysis. Such a rearrangement of lipids in the membranes of ghosts following hypotonic lysis could conceivably cause a change in the thermal stability of a membrane-bound enzyme.

Even if one of these two possibilities should account for our immediate observations, the fact that such gentle treatment alters the temperature-specific behavior of $(Na^+ + K^+)$ATPase places a severe constraint upon the applicability to intact systems of results and conclusions based upon temperature studies with far more molested enzymes.

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