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INFLUENCES OF CELL VOLUME AND ADRENALECTOMY ON CATION FLUX IN DOG RED BLOOD CELLS

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SUMMARY

Rates of ^{24}Na and ^{42}K entry into dog red blood cells were found to be strongly influenced by cell volume. The kinetics of isotope movement were complex, and the cells were not in a steady state. By applying a simple, two-compartment equation to the early time points, values for flux were calculated and corrected for the changes in surface/volume ratio which occur when cells are shrunken or swollen. Curves were thus generated showing Na and K influx as functions of cell water content. A reinvestigation of the effects of adrenalectomy showed that all the observed changes in Na flux could be explained on the basis of alterations in red cell volume.

INTRODUCTION

A major determinant of cation permeability in dog red blood cells is their volume or water content [1–3]. The present work shows that some of the reported effects of adrenalectomy [4, 5] on Na influx in dog red blood cells are mediated by changes in cell volume.

METHODS

Femoral arterial blood was drawn from mongrel dogs into heparinized syringes 10–15 min before the start of each experiment. After discarding the plasma and buffy coat, the red cells were washed twice by centrifugation in a solution containing 148 mM NaCl, 5 mM KCl and 17 mM Tris or glycylglycine (pH 7.5 at 25 °C). The cells were washed twice more with the solutions in which influx was to be measured (for details see legends to Fig. 1 and Table I). After resuspension at a cell/medium volume ratio of 1 : 9 the isotope was added (40–100 μCi per 60 ml suspension), and an initial sample was taken. The suspension was then placed in a 37 °C water bath/shaker,

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and at intervals further aliquots were removed. Each sample was centrifuged at 20 000 $\times g$ (10 °C) in a special lucite tube with a narrow well at the bottom for collection of the cell pellet. The supernatant and top layer of cells were carefully removed. Appropriate dilutions of packed cells, supernatant, and hemolyzed whole suspension were prepared and assayed as follows: Radioactivity was determined with a well-type gamma crystal scintillation counter. Na and K concentrations were measured by flame photometry, using appropriate corrections for trapped medium [6]. Absorbance at 540 nm was used as an index of hemoglobin concentration in the packed cells and whole suspension, thus permitting a calculation of the packed cell volume (herein referred to as the hematocrit) of the suspension.

For the computation of influx a steady-state, two compartment model adapted from Sheppard's book [7] was used. The appropriateness of this treatment of the data will be discussed. The defining equation is:

$$\ln\left(1-\frac{a_c}{a_\infty}\right) = -M\left[\frac{1}{S_m(1-H)}+\frac{1}{S_c(H)}\right]t \tag{1}$$

where S_c and S_m are the concentrations of substance S in the cells and medium, respectively (mmol/liter); a_c and a_∞ are the specific activities of substance S in the cells and hemolyzed whole suspension, respectively (counts/mmol); H is the hematocrit. H and $1-H$, since they are, respectively, proportional to the volume of cells and medium in the suspension, are given in dimensions of volume (liters); t is the time in hours; and M is the flux in mmol per h. Flux (mmol/liter cells · h) is equal to M/H, and in eqn. 2 is given the symbol m.

Many of the flux measurements were made on cells which, because they were incubated in solutions of differing tonicity had different volumes. Cell water content was used as an index of cell volume and was determined by drying a sample of packed cells to constant weight in a 70 °C vacuum oven. The cell water content, C_w, was calculated as the wet weight minus the dry weight, divided by the wet weight. Flux measurements were corrected for differences in relative cell volume by relating the flux to the cell solid and thus, presumably, to the membrane surface area:

$$m_0 = m_x\left(\frac{1-C_{w_0}}{1-C_{w_x}}\right) \tag{2}$$

where m_0 is the flux corrected to the original or isotonic cell volume; m_x is the flux measured at a cell volume other than isotonic; C_{w_0} is the cell water content with the cells in an isotonic solution; C_{w_x} is the cell water content with the cells in an anisotonic solution.

RESULTS

Fig. 1 shows the time course of ^{24}Na and ^{42}K entry in dog red blood cells at different volumes. Na approaches specific activity equilibrium most rapidly when the cells are shrunken, whereas the movement of K is fastest in the most swollen cells. Attempts to fit the data to the steady-state, two-compartment model of Eqn. 1 showed marked deviations from predicted kinetics, particularly in the case of Na (Fig. 1a). This may be due in part to the net gain by the cells of NaCl and water during the

incubation period [8]. The kinetic characteristics of the early time points were considered sufficiently good, however, to permit the use of Eqn. 1 in obtaining an estimate of the initial rate of influx. Eqn. 2 was used to correct the fluxes to original cell volume,

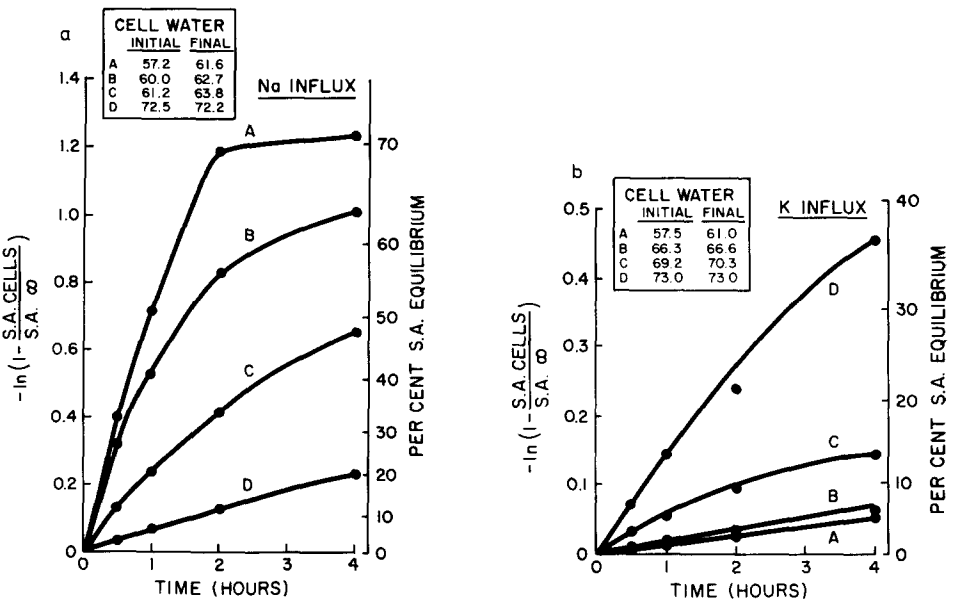

Fig. 1. The approach of ^{24}Na (a) and ^{42}K (b) to specific activity equilibrium in solutions of differing tonicity over an incubation period of 4 h at 37 °C. Fresh cells were washed and suspended in four solutions with NaCl concentrations ranging from 80 to 220 mM. All solutions contained 5 mM KCl, 17 mM Tris and 10 mM glucose (pH 7.5 at 25 °C). The left-hand ordinate represents the left-hand term of Eqn. 1 with the sign changed. The right-hand ordinate gives the relative specific activity (S.A.) of the cells to the medium expressed as a percentage. The inset box shows the cell water content at the beginning and end of the 4 h flux period.

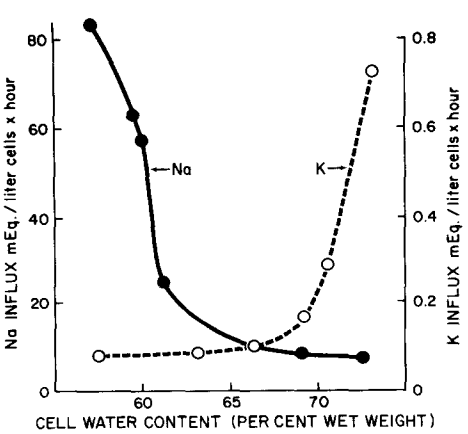

Fig. 2. Influx of ^{24}Na and ^{42}K as functions of cell water content. Data from the first hour of incubation (Fig. 1) were used to calculate values for influx (m_0, Eqn. 2) of Na (left-hand ordinate, solid line) and K (right-hand ordinate, broken line) according to Eqns. 1 and 2.

TABLE I

FLUX OF Na INTO RED BLOOD CELLS FROM NORMAL AND ADRENALECTOMIZED DOGS

Dogs were adrenalectomized 16 days previously and maintained on cortisone acetate (25 mg intramuscularly daily) except for the 24 h preceding the experiments. In experiment 1 cells and plasma from an adrenalectomized dog (A) and a normal dog (N) were incubated at 37 °C for 1 h in the combinations shown while the influx of ^{24}Na was measured. Freezing point determinations indicated that the normal plasma contained 296 mosM/kg while the adrenalectomized dog's plasma had 250 mosM/kg. In Experiment 2 the plasma of a normal dog (dil. N) was adjusted to the same osmolality as that of an adrenalectomized dog by dilution with distilled water. The suspensions were gassed with humidified 95 % O_2/5 % CO_2.

Experiment	Cells	Plasma	Cell water % wet wt.	Na influx mmol/liter cells · h
	N	N	64.9	9.9
1	N	A	68.4	5.0
	A	N	65.3	13.3
	A	A	67.5	5.7
	N	dil. N	65.9	8.4
2	N	A	67.9	7.2
	A	dil. N	66.0	5.3
	A	A	66.4	6.6

and the combined results of the studies shown in Figs 1a and 1b were plotted in Fig. 2 as functions of the initial cell water content. It is apparent from this figure that Na influx shows the greatest rate of increase when the cells are shrunken below their normal volume (65 % cell water), while K influx increases most rapidly as the cells are swollen above their normal volume. The observation (Fig. 1a) that ^{24}Na entry appears to stop short of specific activity equilibrium confirms a similar finding by Elford [9] and suggests that a portion of cell Na is slowly if at all exchangeable with external Na.

Table I confirms the report of Streeten and Moses [5] that red blood cells from adrenalectomized dogs may have a low Na influx, but the data suggest that the permeability change is due to increased cell water content associated with plasma hypotonicity. When cells from adrenalectomized animals were incubated with normal plasma their water content fell, and the Na influx increased. This corrective effect was not observed when the normal plasma was diluted with water so as to be isosmolal with plasma from the adrenalectomized dog.

DISCUSSION

The influence of cell volume on passive Na and K permeability in dog red blood cells is well documented [1–3]. A systematic plot of Na and K flux versus cell water content (Fig. 2) shows that the most striking acceleration of Na movements occurs when cells are shrunken below their normal water content, while K movements are not greatly altered unless the cells are swollen. Elford's failure to find an effect of cell volume on K flux [9] is because his studies were done on cells in iso- to hypertonic media.

The effects of cell swelling and shrinking on Na and K flux are reversible [3, 10] and independent of the osmolality of the medium [3]. Since cell volume influences net as well as isotopic cation movements [1], the mechanism cannot involve a tightly coupled exchange of Na for Na or K for K. Davson and Reiner [11] showed that butanol would abolish the high Na flux seen in shrunken red blood cells from cats, and other agents including phloretin [10] and bisulfite [12] are reported to have a similar effect on dog cells. Metabolic depletion of cells also blunts the volume response [2, 10]. But the nature of the volume signal and the manner in which information about volume causes alterations in membrane permeability are at present obscure.

The decreased Na influx seen in cells from adrenalectomized dogs can be attributed to plasma hypotonicity with resultant cell swelling. Whether adrenalectomy or adrenal cortico-steroids have some other influence on Na permeability is not clear: in our hands, using flux methods detailed above, no in vitro effects of aldosterone or hydrocortisone could be demonstrated in cells from normal or adrenalectomized dogs.

These observations illustrate how important it is that Na-K permeability studies in dog red cells be carefully controlled for the strong influences of cell volume.

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