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CATION MOVEMENTS ACROSS MOUSE RED BLOOD CELLS *

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Summary

This paper describes some features of the Na and K transport systems in red cells obtained from B_{10}.A mice. When mouse erythrocytes were incubated in a plasma-like control medium, the scillaren-sensitive Na efflux was 3.6 ± 0.4 mmol/l red blood cells per h while the scillaren-sensitive K influx was 3.1 ± 0.3, values not significantly different from each other. Scillaren had no significant effect on either Na influx or K efflux. There was a large (approx. 3 mmol/l red blood cells per h) scillaren-sensitive, Na-Na exchange diffusion component present under K-free conditions. When K was present in the incubation medium, this exchange system was suppressed.

Introduction

Very little is known concerning the transport properties of mouse red blood cells, especially with regard to electrolytes. Mouse erythrocytes were first used in comparative non-electrolyte permeability studies by Jacobs [1]. He measured the rate of penetration of glycerol, ethylene glycol, and erythritol by determining the time required to produce 75% hemolysis at 20° C in 0.02 M NaCl plus 0.3 M solutions of the above compounds. He found that red cells of mice, as well as those of rats, guinea-pig and human, were highly permeable to glycerol, while those of sheep and ox were not. The most notable peculiarity of mouse cells was that they were at least two orders of magnitude more permeable to erythritol than were human or guinea-pig cells. Electrolyte permeability studies have not heretofore been performed on mouse red cells.

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The present study was undertaken to characterize the transport processes responsible for Na and K movements across the red cell membrane of B_{10}.A/SgSnAo mice. The results indicate that under normal conditions (i.e., when $[Na]_o = 140$ mM and $[K]_o = 4$ mM) the ratio of glycoside-sensitive Na efflux to K influx is unity. In addition to an (Na, K)-pump, a scillaren-sensitive Na-Na exchange system was also observed. This exchange mechanism becomes apparent and is inhibited by scillaren only upon removal of external K. Under these conditions, the magnitude of Na-Na exchange is larger than that of the scillaren-sensitive pump fluxes, possibly reflecting an increase in the turnover rate of the $(Na^+ + K^+)$-ATPase enzyme.

Methods

The mice (*Mus musculus*, strain B_{10}.A/SgSnAo) used in this study were all adult, ranging in age from 4 months to 1.5 years. Blood was obtained by cardiac puncture from a mouse slightly anesthetized with either halothane or chloroform. Blood was withdrawn into a heparinized, 3 ml plastic syringe fitted with a 25-gauge needle. All of the inbred mice used in this study were obtained from the stocks of Dr. D.B. Amos, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC. For uniformity only male mice were used. The quantity of blood that could be obtained from a single mouse varied from 0.75 to 1.2 ml (yielding 0.3 to 0.5 ml of packed cells), depending upon the size of the animal. In any given experiment, blood from a single mouse was used. Pooled samples were not employed.

The blood was immediately transferred to a clean, siliconized 15 ml glass centrifuge tube and washed three times with 30 to 45 vols. of ice-cold artificial mouse plasma solution (control solution; the composition of which is given in Table I). Supernatant and buffy coat were removed by aspiration. Between washes the cells were centrifuged for 5 min at $1470 \times g$. After the last wash, the cells were transferred to a 25 ml Erlenmeyer flask and suspended in control medium to a final hematocrit of 5—8%. The cells were then incubated for 3 h at 37° C with constant gentle agitation in a water bath. Both unidirectional influx and efflux experiments commenced after this initial 3 h preincubation period.

Unidirectional Na and K influx measurements

After the initial preincubation period, the cells were divided into two groups and placed in separate 15-ml centrifuge tubes. Both groups of cells were then washed four times with 60 vols. of ice-cold control solution. One group served as control; the other was the experimental group. The wash solutions were always identical to the media used in the subsequent flux measurements.

After the final wash, the cells were tightly packed and most of the supernatant removed by aspiration. Four 25 ml Erlenmeyer flasks, each containing 1.25 ml of solution (control and experimental, with and without $5 \cdot 10^{-4}$ M scillaren, a cardiac glycoside) plus radioactive tracer (8 μCi/ml ^{22}NaCl; 80 μCi/ml ^{42}KCl) were prewarmed at 37° C. The cells were transferred to these flasks with large-tipped, glass micropipettes, the final hematocrit being 2—5%. Four to five 250 μl samples were taken at appropriate intervals (10—20 min).

Samples were immediately pipetted into 15-ml siliconized glass centrifuge tubes (either conical or round bottom). These tubes contained 2 or 4 ml, respectively, of the organic ester, dibutylphthalate [2], and 10 ml of ice-cold, isotonic $MgCl_2$ saturated with $MgCO_3$. These tubes were previously chilled and kept submerged in ice during the course of the experiment. The cells, upon centrifugation, passed through the phthalate leaving the incubation medium behind. The 250 μl of cell flux suspension were thus diluted some 40-fold by the $MgCl_2$ solution, and most non-cellular Na, K, and tracer (since they remained in the aqueous top layer) were removed by simply aspirating the aqueous layer off. The inside of the tube was then washed three times with distilled water. After the last wash, the tube was lightly spun so that all of the residual fluid clinging to the sides would be layered on top of the phthalate, and then easily removed along with most of the phthalate by aspiration. Cell pellets prepared in this fashion trap very little extracellular sodium and potassium. The mean values of extracellularly trapped Na and K (expressed as the percentage of the total measured Na (or K) which is extracellular) were 1.16 ± 0.24% (n = 23) and 0.002 ± 0.004% (n = 23), respectively. These numbers were calculated on the basis of $[Na]_o$ = 140 mM and $[K]_o$ = 4 mM. Under these conditions, corrections for trapped tracer and/or ions were not made. Centrifugation was accomplished by using either a clinical centrifuge (spun at 1470 × g for 7 min) or a Lourdes Instrument Co. high-speed centrifuge spun at 10 000 × g for 4 min.

The cells were then lyzed by the addition of 3 or 4 ml of 15 mM $LiNO_3$ to the centrifuge tubes using a Krogh-Key syringe system, i.e., a glass syringe fitted within a metal frame containing an adjustable screw located immediately behind the plunger. This system provides an easy means for rapid, reproducible delivery of a large number of constant fluid volumes. The volume of hemolyzing solution delivered was determined by weight just prior to use. Each tube was then counted for radioactivity, after which time the lysate was analyzed for Na, K and hemoglobin. Two, usually three, aliquots of flux supernatant were secured for determination of external specific activity and ion concentrations. The external specific activity was found always to be constant throughout an influx experiment.

Na or K influx was computed for each individual flux period by using the following equation:

$$J_{ion}^{in} = \frac{\Delta C_i}{(\Delta t)[SA_o - SA_i(t_{1/2})]}$$

where J_{ion}^{in} represents the total unidirectional ion influx (in mmol ion/l red blood cells per h), Δt is the duration of the flux period ($t_2 - t_1$) in hours, SA_o is the specific activity of the ion in the external bathing medium (in cpm/mmol ion), $SA_i(t_{1/2})$ is the specific activity of the ion inside the cells (in cpm/mmol ion) at the midpoint of the flux period, i.e.,

$$SA_i(t_{1/2}) = \frac{SA_i(t_1) + SA_i(t_2)}{2}$$

and finally, ΔC_i (in Δcpm/l red blood cells) is the increase in intracellular counts during the flux period.

Unidirectional Na and K efflux measurements

The protocol for tracer efflux experiments was essentially the same as that for influxes, except for the following modifications. Cells were loaded with tracer (10 μCi/ml for ^{22}Na and 100 μCi/ml for ^{42}K) during the 3 h preincubation period. After washing four times with tracer-free, ice-cold media, the cells were suspended at a hematocrit of 2—5% in flux flasks containing 1.7 ml of tracer-free medium at 37° C. At each sampling, two aliquots of cell suspension were removed: one 250 μl sample was treated in a manner identical to that described for the influx procedure, and another 100 μl sample was used for supernatant radioactivity and chemical determinations. This latter sample was pipetted into a microcentrifuge tube which contained 75 μl of dibutylphthalate, and immediately spun for 1 min. The cells were thus isolated from the supernatant and the supernatant sample could then be saved for later analysis without fear of contamination.

Unidirectional tracer efflux was calculated from the following equation:

$$J_{ion}^{out} = \frac{\Delta C_i}{(\Delta t)[SA_i(t_{1/2})]}$$

where the symbols have the meanings defined earlier. The specific activity of the external compartment (SA_o) was in all experiments at least two orders of magnitude smaller than the specific activity of the internal compartment, hence corrections for back flux of tracer were unnecessary. Hemolysis corrections were not made; in most flux experiments visual evidence of hemolysis could not be detected. Since SA_i decreased with time, a midpoint specific activity was computed and used assuming that SA_i varied linearly with time.

Many experiments have been performed while the cells were not in a steady state with respect to the ion being studied. However, the above equations were still employed in calculating the flux. Over the time interval in which the flux was being followed, changes in internal cation composition were small (usually less than 5%), and hence the cells could be considered to be in a 'quasi steady state'. Indeed, in these experiments, unidirectional fluxes computed using non-steady-state flux equations [3] showed no systematic difference when compared to the fluxes computed using the above equations.

Solutions were all made up from reagent grade chemicals. In experiments in which the K concentration was varied, an equivalent amount of Na was either added or removed. In experiments in which the Na concentration was reduced, an osmotically equivalent amount of $MgCl_2$ was substituted. The composition of all solutions used in these experiments is presented in Table I.

Scillaren was added to the desired experimental solution to give a final concentration of $5 \cdot 10^{-4}$ M. Scillaren was obtained as a mixture of scillaren A and B (2 parts A to 1 part B) from ICN Pharmaceuticals, Inc., Plainview, NY. Scillaren was chosen in lieu of the more commonly used red cell active transport inhibitor, ouabain, for the following two reasons: (a) rodent tissue appears to have a very high dissociation rate constant for the ouabain binding reaction, hence rodent tissue is comparatively insensitive to the effects of ouabain [4—7], and (b) in parallel inhibitor experiments, $5 \cdot 10^{-4}$ M scillaren

TABLE I

COMPOSITION OF MEDIA

All solutions had an osmolality of 300 ± 5 mosM/kg and a pH 7.4 at 37°C. All values are expressed in mM.

Substance	Control	K-Free	Na-Free	Na, K-Free
Sodium	141.5	141.5	—	—
Potassium	4.5	—	4.0	—
Chloride	140.0	140.0	202.0	202.0
Calcium	1.5	1.5	1.5	1.5
Magnesium	1.0	1.0	101.0	101.0
Phosphate	2.5	2.5	—	—
Glycylglycine	27.0	27.0	27.0	23.5
D-Glucose	10.0	10.0	10.0	13.5
Adenine	1.0	1.0	—	—
Adenosine	—	—	1.0	1.0
Penicillin (mg/l)	100.0	100.0	—	—
Streptomycin sulfate (mg/l)	50.0	50.0	50.0	50.0

was always found to inhibit both K influx and Na efflux to a greater extent than $1 \cdot 10^{-3}$ M ouabain.

Osmometry, pH, chemical analysis of Na, K, and Cl, hematocrit and absorbance measurements were performed as described previously [8]. The ATP levels of perchloric acid-extracted murine erythrocytes were determined by a fluorimetric assay technique [9,10].

Results

Composition and transport of Na and K in mouse red cells incubated in control medium

Composition. The stability of mouse red cells was evaluated by washing and incubating the cells in standard control medium, then determining their water content, and the concentrations of adenosine triphosphate (ATP) and cations. These values were then compared to those measured in fresh cells suspended in plasma. Fig. 1 shows that fresh B_{10}.A mouse red cells contained 0.663 ± 0.001 gm H_2O/gm cells, 1.6 ± 0.3 mmol ATP/l red blood cells, 116 ± 2 mmol/K l red blood cells, and 8.7 ± 0.3 mmol Na/l red blood cells. These values did not change significantly during 4 h of incubation at 37° C in control medium.

Unidirectional Na and K fluxes. Table II presents a summary of all the unidirectional Na and K fluxes performed in the presence and absence of $5 \cdot 10^{-4}$ M scillaren in control medium. Scillaren had little effect upon Na influx or K efflux, but significantly inhibited both K influx and the Na efflux. The mean glycoside-sensitive K influx and Na efflux were 3.1 and 3.6 mmol/l red blood cells per h, respectively. These fluxes are not significantly different from each other ($P > 0.2$). The results obtained from simultaneous measurements of net Na and K movements in the presence and absence of scillaren were consistent with predictions based upon the unidirectional experiments. In addition, the net movements of Na and K from red cells incubated in K-free medium were similar to those observed in the presence of scillaren. Scillaren, also,

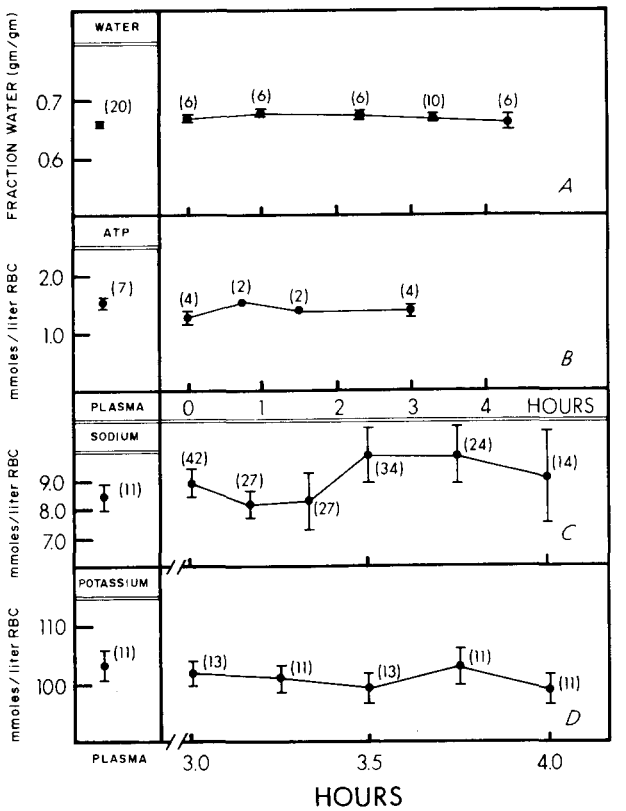

Fig. 1. Water fractions (A), ATP levels (B), and Na^+ (C) and K^+ (D) concentrations in mouse erythrocytes at various times during incubation at 37°C in control medium. Values for intracellular water fractions, ATP, Na, and K concentrations obtained from cells suspended in plasma are also presented for comparison. Each point represents the mean value of (N) determinations, while the vertical bars indicate 1 S.E. The composition of the control medium is given in Table I. RBC, red blood cells.

had no significant effect upon the net changes in intracellular cation composition of mouse red cells exposed to K-free solutions.

Unidirectional Na fluxes. Table III presents a summary of all the unidirectional Na influx and efflux experiments performed in the presence and absence of external K (with and without scillaren). Removal of external K resulted

TABLE II

UNIDIRECTIONAL Na AND K FLUXES ACROSS MOUSE RED BLOOD CELLS INCUBATED IN CONTROL MEDIUM

Results are expressed in mmol/l red blood cells per h ± S.E.

	Total flux	$+5 \cdot 10^{-4}$ M scillaren	Scillaren-sensitive flux
Na influx	3.8 ± 0.4	4.2 ± 0.5	+0.3 ± 0.3
Na efflux	5.9 ± 0.5	2.2 ± 0.3	−3.6 ± 0.4
K influx	3.6 ± 0.2	0.5 ± 0.1	−3.1 ± 0.3
K efflux	5.1 ± 0.5	4.5 ± 0.5	−0.6 ± 0.1

TABLE III

UNIDIRECTIONAL Na INFLUX AND EFFLUX IN MOUSE RED BLOOD CELLS INCUBATED IN THE PRESENCE AND ABSENCE OF EXTERNAL K

Flux values given in mmol/l red blood cells per h ± S.E.

Flux	$[K]_o$ (mM)	Total flux	$+5 \cdot 10^{-4}$ M scillaren	Scillaren-sensitive flux
In	4	3.7 ± 0.4	4.6 ± 0.4	+0.9 ± 0.7
	0	7.4 ± 0.9	3.7 ± 0.2	−3.7 ± 0.9
Out	4	6.2 ± 1.3	3.4 ± 0.5	−2.9 ± 1.3
	0	8.5 ± 1.7	1.6 ± 0.5	−6.9 ± 2.2

in a 3.7 ± 1.1 mmol/l red blood cells per h increase in Na influx; this entire increase was inhibited by scillaren. Upon removal of external K, Na efflux also increased by 2.3 ± 1.1 mmol/l red blood cells per h. The magnitude of these increases in the two oppositely directed unidirectional Na fluxes subsequent to the removal of external K were not significantly different from each other ($P > 0.4$). These results are not in agreement with those reported for human cells by Garrahan and Glynn [11] or for rat cells by Beauge and Ortiz [12]. These authors found that upon removal of external K, Na influx increased but Na efflux decreased by one-third to one-half control levels. In summary, for mouse red cells suspended in media containing 140 mM Na:

(i) Both Na influx and efflux are increased to the same extent by removal of external K.

(ii) The increments in Na influx and efflux observed when external K is removed are scillaren-sensitive.

Incubation of mouse erythrocytes in Na-free solutions

(a) Net fluxes. The normalized changes which occurred in the intracellular Na and K concentrations in mouse red cells incubated in Na-free media in the presence and absence of $5 \cdot 10^{-4}$ M scillaren are depicted in Fig. 2. When the red cells from seven mice were incubated in Na-free media, they lost 2.6 ± 0.5 mmol/l red blood cells of their internal sodium after 1 h. Scillaren ($5 \cdot 10^{-4}$ M) effectively blocked the $[Na]_i$ loss which occurred in Na-free medium (Fig. 2B). In the presence of scillaren and in Na-free medium, red cell internal Na content remained virtually unchanged after 1 h. Fig. 2A shows that there was no significant loss of K from mouse red cells incubated for 1 h in Na-free medium in the presence or absence of scillaren.

(b) Unidirectional Na effluxes. Table IV presents a summary of all the unidirectional Na efflux measurements made on mouse red cells incubated in Na-free solutions plus and minus $5 \cdot 10^{-4}$ M scillaren. Removal of external Na resulted in a 2.4 ± 0.4 mmol/l red blood cells per h decrease in Na efflux. In the presence of scillaren, Na efflux was independent of external Na concentration. Thus, about half of the unidirectional Na outflux occurs through a system which is dependent upon the presence of external Na and is inhibited by scillaren.

(c) Incubation of mouse erythrocytes in Na- and K-free medium. In order

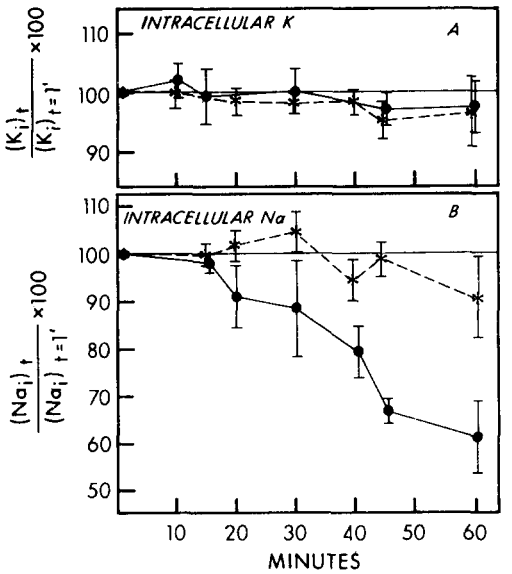

Fig. 2. The effect of scillaren on the intracellular cation composition of mouse erythrocytes incubated in Na-free solutions. Cells were first preincubated in control (Na/K) medium at 37°C for 3 h, washed four times with ice-cold Na-free solution, and finally transferred to a flask containing Na-free medium (———) and to one containing Na-free medium plus $5 \cdot 10^{-4}$ M scillaren (- - - - - -). The cells were incubated at 37°C, samples taken with time, and the intracellular Na and K concentrations determined. The results are expressed as percent of initial value (at $t = 1$ min) vs. time. The initial level of intracellular Na was 5.6 ± 0.3 mmol/l red blood cells, while that for potassium was 104.6 ± 3.0 mmol/l red blood cells. Each point represents the mean value of red cell samples obtained from at least three mice, and the vertical bars indicate 1 S.E.

to explore further the relationships between Na efflux, scillaren and external Na and K, Na efflux from B_{10}.A erythrocytes was measured into solutions nominally free of both Na and K (Table V). In the absence of scillaren, removal of external Na in the absence of external K (Table V, A) reduced Na efflux from 7.5 ± 0.8 to 1.9 ± 0.2 mmol/l red blood cells per h. In the presence of scillaren, removal of external Na in the absence of external K had no effect on Na efflux. Thus, in the absence of external K there was a large efflux of Na which required external Na and was completely inhibited by scillaren. Under these K-free conditions, Na influx was also inhibited by scillaren (Table III). In the presence of external K, Na_o-dependent Na efflux was much less,

TABLE IV

UNIDIRECTIONAL Na EFFLUX FROM MOUSE RED BLOOD CELLS INCUBATED IN THE PRESENCE AND ABSENCE OF EXTERNAL Na

Flux values given in mmol/l red blood cells per h ± S.E.

$[K]_o$ (mM)	$[Na]_o$ (mM)	Total flux	$+5 \cdot 10^{-4}$ M scillaren	Scillaren-sensitive flux
4	140	4.9 ± 0.2	1.4 ± 0.4	3.5 ± 0.4
4	0	2.5 ± 0.4	1.1 ± 0.1	1.4 ± 0.4
Na_o-dependent Na efflux		2.4 ± 0.4	−0.3 ± 0.5	2.1 ± 0.8

TABLE V

Na EFFLUX FROM MOUSE RED BLOOD CELLS INCUBATED IN Na-FREE, K-FREE, AND Na- AND K-FREE MEDIA

(A) The effects of external Na removal upon Na efflux from mouse erythrocytes incubated in K-free medium. Results expressed in mmol/l red blood cells per h. (B) The effects of external K removal upon Na efflux from mouse erythrocytes incubated in Na-free medium.

(A) Experiment number	K-free	K-free + scillaren	Difference	Na-, K-free	Na, K-free + scillaren	Difference
NE21	9.0	1.5	−7.5	2.0	1.3	−0.7
NE24	6.1	0.6	−5.5	2.1	0.8	−1.3
NE25	7.5	0.6	−6.9	1.5	0.7	−0.8
Mean ± S.E.	7.5 ± 0.8	0.9 ± 0.3	−6.6 ± 0.6	1.9 ± 0.2	0.9 ± 0.2	−0.9 ± 0.2
(B) Experiment number	Na-free	Na-free + scillaren	Difference	Na-, K-free	Na, K-free + scillaren	Difference
NE17	2.8	1.1	−1.7	2.2	0.8	−1.4
NE18	3.0	0.7	−2.3	2.2	2.4	+0.2
NE19	5.2	2.4	−2.8	4.7	1.0	−3.7
Mean ± S.E.	3.7 ± 0.8	1.4 ± 0.5	−2.3 ± 0.3	3.0 ± 0.8	1.4 ± 0.5	−1.6 ± 1.1

though also completely inhibited by scillaren (Table IV) and there was no scillaren-sensitive Na influx (Table III). Thus, removal of external K appeared to permit a magnitude of scillaren-sensitive Na-Na exchange substantially in excess of the rate of K-Na exchange observed in the presence of external K.

Na efflux from red cells incubated in nominally Na- and K-free solutions still displayed a sensitivity to scillaren (approx. 50%). As indicated earlier, this scillaren-sensitive component may represent residual Na-K exchange, or may be an uncoupled Na efflux which manifests itself under these conditions [14].

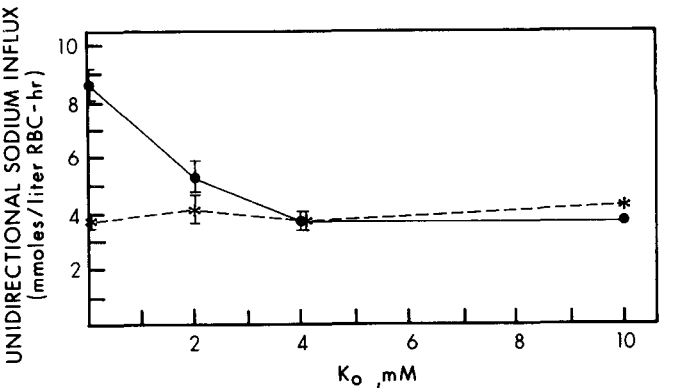

Fig. 3. The influx of Na into mouse red cells in the presence (- - - - -) and absence (———) of $5 \cdot 10^{-4}$ M scillaren as a function of external K concentration. Cells were first subjected to the standard 3 h preincubation period prior to flux determinations. Each point represents the mean of the least three flux experiments, except those at $[K]_o = 10$ mM (single experiment). The bars indicate 1 S.E. In all experiments simultaneous control flasks (i.e., at $[K]_o = 4$ mM) were run. RBC, red blood cells.

(d) The effect of external K on Na influx. Fig. 3 depicts the results of the unidirectional Na-influx measurements made at various external K concentrations in the presence and absence of $5 \cdot 10^{-4}$ M scillaren. The difference between the two curves gives the scillaren-sensitive Na influx at any external K concentration. At $[K]_o$ of 4 mM or greater, Na influx was insensitive to scillaren. At K concentrations less than 4 mM, Na influx increased and the entire increase in influx was inhibitable by scillaren. The magnitude of the Na influx in the presence of scillaren was insensitive to changes in $[K]_o$.

Discussion

In mouse red cells incubated in control medium containing 4 mM K, scillaren-inhibited K influx and Na efflux both equal about 3.5 mmol/l red blood cells per h. Thus, the coupling of Na to K movement through the (Na, K)-pump in this system appears to be 1 : 1. Neither the Na influx nor the K efflux of mouse erythrocytes incubated in control medium was significantly altered by scillaren (Table II). This result is in accord with the view that scillaren affects only the active transport pathways under control conditions. In contrast, Glynn [15] reported that digoxin ($1 \cdot 10^{-5}$ gm/ml) inhibits K efflux from human red cells incubated under similar conditions by some 25%. He attributes this observation to the possibility that a portion of the K efflux occurs through the (Na, K)-pump [16].

When K is removed from the bathing medium, Na influx increases but Na efflux remains virtually unchanged. This increase in Na influx is entirely inhibited by scillaren (Table III). Furthermore, the scillaren-sensitive components of Na influx and Na efflux are not statistically distinguishable in K-free medium. Under K-free conditions, it is likely that scillaren is inhibiting a Na-Na exchange diffusion process [11]. This supposition is made more likely by the observation that removing external Na from an already K-free medium decreases Na efflux by an amount (5.6 ± 0.8 mmol/l red blood cells per h) equivalent to the decrease in Na efflux from cells in K-free medium caused by scillaren (6.6 ± 0.6 mmol/l red blood cells per h) (Table V, A).

Table VI summarizes the properties of Na-Na exchange [17] in five types of mammalian erythrocyte. On the basis of previous work, it appears that at least two types of Na-Na exchange exist—a glycoside-sensitive and a glycoside-insensitive system. In human and rat red cells, the Na-Na exchange process can be observed only in the absence of external K and is glycoside-sensitive. On this basis, Garrahan and Glynn [18] contend that Na-Na exchanges are mediated by the active (Na, K)-pump. To support this claim, they have shown that the kinetics of inhibition of Na-Na exchange by K are identical to the kinetics of activation of the K-Na pump by K_o [18,19]. However, Na-Na exchange does not require hydrolysis of ATP but does require the presence of ATP and ADP [18,19]. Garay and Garrahan [20] have extended these earlier observations and have shown that the maximal rate of Na-Na exchange in human red cells increases linearly with internal K concentration at saturating levels of $[Na]_i$ and that for each turnover of the site, three external Na^+ exchange for three internal Na^+. It is important to point out that there does appear to exist in human cells a small ouabain-insensitive Na-Na exchange

TABLE VI

COMPARATIVE PROPERTIES OF THE Na-Na EXCHANGE SYSTEM IN THE RED CELLS OF DIFFERENT MAMMALIAN SPECIES

LK, low [K]; HK, high [K].

Cell type	Is Na-Na exchange operative under physiological conditions?	When Na-Na exchange is operating, what fraction of total Na efflux is via the exchange pathway?	Is the Na-Na exchange glycoside sensitive?	Does Na influx increase or decrease from control subsequent to K_o removal?	Does Na efflux increase or decrease from control subsequent to K_o removal?	Reference
Human	No *	0.53	Yes	Increase	Decrease	14,20,22
Sheep	Yes	HK = 0.95 LK = 0.88	No	Not measured	Decrease	44
Rat	No	0.77	Yes	Increase	Decrease	12
Bovine	Yes	HK = 0.90	No	Increase	No change **	25,27
Mouse	No	0.49	Yes	Increase	Increase or no change	Present study

* Some authors [21—23] claim that there is a small ouabain-insensitive (15%) Na-Na exchange.

** In LK cells only; HK cells not measured.

system [21—23] of the type described for sheep and ox. This small ouabain-insensitive Na-Na exchange in human cells can be inhibited with phloretin.

In contrast to human and rat red cells, ovine, bovine, porcine and rabbit erythrocytes display Na-Na exchange under normal physiological conditions (i.e., when $[Na]_o = 140$ mM and $[K]_o = 4$ mM). Tosteson and Hoffman [24] using both high (HK) and low (LK) [K] sheep, Sorenson et al. [25] using pig and beef, Rettori et al. [26] using rabbit, and Motais [27] using bovine red cells have shown that a very large fraction of the Na efflux is dependent upon the presence of external Na. Since this process occurs in the presence of external K, and since it is not inhibited by ouabain, Motais [27] believes that this system is not mediated by the (Na, K)-pump.

The data presented in this paper indicate that Na-Na exchange in mouse red cells more closely resembles that observed in human rather than in sheep and bovine red cells. In the presence of external K, removal of external Na reduces Na efflux, and this Na-dependent flux component is completely inhibitable by scillaren (Table IV). However, this component is not Na-Na exchange because there is not inhibition of Na influx by scillaren under control conditions (Tables II and III). It is probable that this Na_o-dependent, scillaren-sensitive component of Na efflux involves K-Na exchange through the pump. Increasing $[Na]_o$ has previously been reported to increase the maximum velocity of the (K, Na)-pump in sheep red cells [24]. In order to settle the point, it will be necessary in future experiments to measure the effect of removing Na_o on scillaren-sensitive K influx. If the proposal that Na_o-dependent, scillaren-sensitive Na efflux occurs through an action of Na_o on the (Na, K)-pump, removal of Na_o should reduce both the scillaren-sensitive K influx and Na efflux.

In the absence of external K, mouse red cells appear to display considerable scillaren-sensitive Na-Na exchange. This observation is consistent with the idea that Na-Na exchange in mouse red cells incubated in the absence of K_o occurs through the (Na, K)-pump. The magnitude of Na-Na exchange under such conditions may be somewhat greater than the magnitude of K-Na exchange which takes place in cells incubated in control medium containing 4 mM K (Table II). This is in contrast to the situation which obtains in human and rat eythrocytes [18,28].

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