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George B. Segel, … , William Simon, Marshall A. Lichtman

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Research Article

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GEORGE B. SEGEL, WILLIAM SIMON, and MARSHALL A. LICHTMAN, *Departments of Pediatrics, Medicine, Biomathematics, and Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642*

ABSTRACT Phytohemagglutinin (PHA) or concanavalin A treatment of lymphocytes causes an increase in membrane permeability so that the leak rates of Na and K increase 1.5- to 2-fold. Active Na and K transport increase proportionately in response to the increased membrane permeability. We have examined the role of lymphocyte Na concentration in sustaining the increased Na and K transport observed after PHA treatment. Cell Na concentration increases from 14.8 to 20.5 mmol/liter cell water in PHA-treated lymphocytes ($P < 0.001$). Four lines of evidence suggest that the 5–6 mmol/liter cell water increase in lymphocyte Na accounts for the increase in active Na and K transport in mitogen-treated lymphocytes. First, PHA does not increase directly the maximal Na, K-ATPase activity of isolated lymphocyte membrane vesicles. Second, when the Na concentration is increased by 6 mmol/liter cell water in unstimulated lymphocytes, Na and K transport increase nearly twofold. Third, the cell Na concentration (15 mmol/liter cell water) is near the K_m for Na activation of the Na, K-ATPase in lymphocyte membranes. The ATPase activity thus, is capable of increasing as the cell Na rises above normal. Fourth, if lymphocytes are incubated in a medium containing a low Na concentration, K transport does not maintain the internal K concentration and the fall in cell K is accentuated in PHA-treated lymphocytes. These studies indicate that the adaptive acceleration of Na and K transport in mitogen-treated lymphocytes is mediated by a small increase in cell Na.

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INTRODUCTION

When human blood lymphocytes are stimulated with plant lectins such as phytohemagglutinin (PHA),[1] there is an immediate increase in the plasma membrane permeability (1). As a result of this alteration, K leaks from the cell at an increased rate (2–4). At the same time, active K transport increases proportionately (3, 5). Although Na flux has not been studied as extensively, one report has shown an increase in radioactive Na exodus from prelabeled lymphocytes treated with concanavalin A (6).

Opinions differ regarding the genesis of the stimulus for increased active cation transport. It has been suggested that PHA acts by stimulating the Na, K-ATPase so as to increase the transduction of energy for transport (6, 7). This hypothesis seems unlikely in that it requires an equivalent effect, over a wide range of PHA concentrations, on membrane permeability on the one hand, and on transport ATPase activity on the other. Moreover, in detailed studies of the Na, K-ATPase activity of lymphocyte membrane vesicles, we could not identify an increase in ATPase activity when PHA was added either to isolated membrane vesicles, to the cells from which membranes were prepared, or to both (8).

An alternative hypothesis is that lectin treatment leads to a change in the lymphocyte internal monovalent cation concentrations. This perturbation stimulates the Na, K-pump and the result is a new steady state at a pump rate about twice normal. However, in several studies of lectin-treated lymphocytes, no deviation of K concentration has been identified (3, 5, 9, 10).

[1] *Abbreviation used in this paper*: PHA, phytohemagglutinin.

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Because of the difficulties of measuring cation content in lectin-treated cells, Na could not be measured accurately. We have surmounted this technical difficulty and in the current studies, we have examined: (*a*) the Na concentration of lectin-treated lymphocytes; (*b*) the relationship of the Na and K transport rate to the Na concentration in lymphocytes; and (*c*) the kinetic parameters of Na activation of the Na, K-ATPase of lymphocyte membrane vesicles.

GLOSSARY OF SYMBOLS

α'_{Na}	Time constant for Na diffusion, h^{-1}
α'_{K}	Time constant for K diffusion, h^{-1}
β	Constant which relates the difference in internal and external K concentration to the diffusion rate, h^{-1}
K or Na	Internal potassium or sodium concentration, mmol/liter cell water
K_{ext} or Na_{ext}	External K or Na concentration, mmol/liter
K(t) or Na(t)	Internal K or Na concentration at time (t), mmol/liter cell water
K(∞) or Na(∞)	Internal K or Na concentration at equilibrium, mmol/liter cell water
λ_K	Time constant for K recovery, h^{-1}
λ_{Na}	Time constant for Na recovery, h^{-1}
N	Cell count, number of cells
Ψ_{in}	Total active K influx, mmol·liter cell water^{-1}·h^{-1}
Ψ_{out}	K diffusion out of the cell, i.e., efflux, mmol·liter cell water^{-1}·h^{-1}
t	Time, h
θ_{in}	Na diffusion into the cell, i.e., influx, mmol·liter cell water^{-1}·h^{-1}
θ_{out}	Total active Na efflux, mmol·liter cell water^{-1}·h^{-1}

METHODS

Isolation of lymphocytes. Lymphocytes were prepared from the mononuclear cell-rich plateletpheresis residues of healthy donors (11). The mononuclear cells were separated on a step gradient of 32.8% (wt/vol) sodium metrizoate: 9% (wt/vol) Ficoll (1:2.4 vol/vol) (12). The cells from the gradient interface were resuspended at a concentration of 5×10^6 cells/ml in tissue culture medium 199 that contained Earle's salts with 20 ml/dl fetal calf serum to which penicillin (100 U/ml) and streptomycin (100 μg/ml) were added, and incubated at 37°C under air and 7% CO_2 to allow cell equilibration, and adherence of monocytes to the incubation flask (10).

The cells were harvested, washed and resuspended in TC-199 with fetal calf serum that contained penicillin and streptomycin, and incubated in air with 7% CO_2 at 37°C. The cell suspensions contained 93±1.5% (mean±SE) lymphocytes and the proportion of cells excluding trypan blue dye was >95%.

Measurement of lymphocyte Na and K concentration. For measurement of cell Na and K, 15 μl of [^{14}C]sucrose containing 1.5 μCi (1.7 mCi/mg sp act) were added to 10-ml samples of lymphocyte cell suspensions that contained $4\pm1 \times 10^6$ cells/ml. The cells were sedimented and the supernate removed so that the residual volume was <0.05 ml. The cell pellet was washed twice without dispersion with 15 ml Hepes-buffered 142 mM choline chloride, pH 7.3. This maneuver permitted removal of the Na-rich supernate without altering the Na and K within the cells (1, 3). The tubes were recentrifuged to insure sedimentation of any disturbed lymphocytes; the supernatant fraction was removed and the cell pellet was resuspended in 2.8 ml of 15 mM LiCl. The cells were disrupted by sonication at 25 W for 30 s. 500 μl of the sonicated cell suspensions were transferred to 10 ml Bray's solution for determination of β-radioactivity, and 20 μl of the original supernate were added to 480 μl of 15 mM LiCl and transferred to 10 ml Bray's solution for measurement of β-radioactivity. Cell Na and K content were calculated from the Na and K in the cell pellet measured by flame photometry minus the Na and K trapped in the pellet as determined by the [^{14}C]sucrose space. Ion content was converted to concentration with direct measurements of cell volume as described (3). Cell volume was identical in untreated and PHA-treated lymphocytes during the first 4 h after PHA treatment (9). The percentage of cell water by weight was 78±0.4 g/100 g cells.

Calculation of active Na and K transport. Active Na and K transport can be quantified by measuring the rate of increment in cell Na or decrement in cell K when the Na, K-pump is inhibited by incubating cells in medium containing ouabain (4 μM) or in medium without external K. This concentration of ouabain maximally inhibits Na and K transport as does the absence of external K. The influx of Na and the exodus of K under these conditions follow first-order kinetics (2, 3) and can be calculated as follows:

$$\text{For Na: } \alpha'_{Na} = -\ln[(Na_{ext} - Na_{int}(t))(Na_{ext} - Na_{int}(0))^{-1}]/t$$

$$\text{For K: } \alpha'_{K} = -\ln[(K_{int}(t) - K_{ext})(K_{int}(0) - K_{ext})^{-1}]/t,$$

where α'_{Na} and α'_{K} are the time constants (h^{-1}) for Na influx and K efflux respectively.

Na influx, θ_{in}, and in the steady-state active Na efflux, θ_{out}, equals α'_{Na} (Na_{ext}-Na_{int}), where θ_{in} and θ_{out} are expressed as millimole·liter cell water^{-1}·h^{-1}.

K efflux, ψ_{out}, and in the steady-state active K influx, ψ_{in}, equals α'_{K} (K_{int}-K_{ext}), where ψ_{in} and ψ_{out} are expressed as millimole·liter cell water^{-1}·h^{-1}. The derivation of these calculations has been published (3, 13).

Correlation of K influx with lymphocyte Na and K concentration. Lymphocyte cell suspensions ($4\pm1 \times 10^6$ cells/ml) were divided into 10-ml aliquots and incubated under air and 7% CO_2 for 4 h in the absence of external K or for 48 h at 4°C. Cell viability after these treatments was >95%. After incubation, the medium was adjusted to 5 mM K or the cell suspensions were transferred to a 37°C water bath. The restoration of the lymphocyte Na and K concentrations was measured during 90 min of incubation.

The recovery of cellular ions to equilibrium concentrations in terms of internal K and Na may be described by the following series of equations:

K recovery (dk/dt) in terms of internal K

$$K(t) = K(\infty) - [K(\infty) - K(0)]e^{-\lambda_K t} \quad (1)$$

$$\left.\frac{dK}{dt}\right|_t = \lambda_K[K(\infty) - K(0)]e^{-\lambda_K t}, \quad (2)$$

where K(t) is the K concentration at any time t; K(0) is the initial K concentration when the cells are transferred from 4°

to 37°C or 5 mM external K is added; and K(∞) is the equilibrium concentration of K after 90 min incubation. dK/dt is the rate of K recovery of any time t.
From Eq. 1

$$e^{-\lambda_K t} = \frac{K(\infty) - K(t)}{K(\infty) - K(0)} \quad (3)$$

and $\lambda_K(h^{-1})$ is the slope when $-\ln [K(\infty) - K(t)/K(\infty) - K(0)]$ is plotted against time. λ_K represents the time constant for recovery of lymphocyte cellular K.
From Eqs. 2 and 3

$$\left.\frac{dK}{dt}\right|_t = \lambda_K[K(\infty) - K(t)]. \quad (4)$$

From Eq. 4, one can determine dK/dt, i.e., K recovery at any K concentration.

K recovery (dK/dt) in terms of internal Na

Cell Na and K are altered reciprocally when active cation transport is blocked by studying cells at 4°C, in the presence of ouabain or in the absence of external K. Studies in red cells in which Na and K can be altered independently (14) indicate that the cell Na exerts the dominant effect on transport at high intracellular K concentrations (15). To describe dK/dt in terms of lymphocyte Na we used the following relationships:

$$K(t) = C - Na(t) \quad \text{and} \quad K(\infty) = C - Na(\infty), \quad (5)$$

where C ≅140 mM.
From Eqs. 4 and 5

$$\left.\frac{dK}{dt}\right|_t = \lambda_K[Na(t) - Na(\infty)]. \quad (6)$$

From Eq. 6, one can determine dK/dt at any Na concentration.

Na recovery (dNa/dt) in terms of internal Na

$$Na(t) = [Na(0) - Na(\infty)]e^{-\lambda_{Na}t} + Na(\infty). \quad (7)$$

$$\left.\frac{dNa}{dt}\right|_t = -\lambda_{Na}[Na(0) - Na(\infty)]e^{-\lambda_{Na}t}. \quad (8)$$

From Eq. 7

$$e^{-\lambda_{Na}t} = \frac{Na(t) - Na(\infty)}{Na(0) - Na(\infty)}. \quad (9)$$

$\lambda_{Na}(h^{-1})$ is the slope when $-\ln [Na(t) - Na(\infty)/Na(0) - Na(\infty)]$ is plotted against time. λ_{Na} represents the time constant for recovery of lymphocyte Na.

Total active K transport (Ψ_{in})

$$\Psi_{in} = \frac{dK}{dt} + \Psi_{out}, \quad (10)$$

where Ψ_{out} represents K diffusion out of the cell and

$$\Psi_{out} = \beta(K(t) - K_{ext}) \quad (11)$$

and $\Psi_{in} = \Psi_{out}$ at equilibrium, therefore

$$\Psi_{in}(\infty) = \beta(K(\infty) - K_{ext}). \quad (12)$$

Combining Eqs. 10, 11 and 12, thereby eliminating β

$$\Psi_{in}(t) = \frac{dK}{dt} + \Psi_{in}(\infty)\left[\frac{K(t) - K_{ext}}{K(\infty) - K_{ext}}\right]. \quad (13)$$

From Eq. 6

$$\Psi_{in}(t) = \lambda_K[Na(t) - Na(\infty)] + \Psi_{in}(\infty)\left[\frac{K(t) - K_{ext}}{K(\infty) - K_{ext}}\right]. \quad (14)$$

Eq. 14 expresses total active transport in terms of the internal Na concentration during the recovery phase (dK/dt) and the K diffusion (Ψ_{out}).

Measurement of lymphocyte plasma membrane ATPase. Lymphocyte suspensions were disrupted at 4°C in a Dounce homogenizer and the plasma membranes isolated with a sucrose step gradient (30–40%) and high speed centrifugation at 54,450 *g*. The membrane fraction is located just above the interface of the 30 and 40% sucrose layers. These membranes show a 30-fold enrichment of 5′-nucleotidase and <5% of the succinate dehydrogenase activity of the cell homogenate (8).

Na,K-ATPase activity was measured as the increment over the Mg ATPase when Na and K were added to the assays. This was equivalent to the decrement in total ATPase activity in the presence of ouabain (8).

RESULTS

Na and K transport in untreated and PHA-treated lymphocytes. Initially, we examined the relationship of the rate of active Na transport to the rate of active K transport in human blood lymphocytes. Active Na and K transport were calculated from the decrement in cell K and increment in cell Na after 4 h incubation without external K or in the presence of ouabain. The mean K influx in five populations of lymphocytes was 17 and the mean Na efflux was 16 mmol·liter cell water^{-1}·h^{-1} (Table I). Thus, Na and K are transported against their respective concentration gradients in a ratio of ≅1:1.

We studied the active Na and K transport in PHA-treated lymphocytes by measuring the increase of Na and decrease of K in the presence of ouabain (Fig. 1). The active transport rate of K in untreated cells, 16 mmol·liter cell water^{-1}·h^{-1}, was similar to the active transport rate of Na, 14 mmol·liter cell water^{-1}·h^{-1}. When lymphocytes were treated with PHA, 8 μg/ml (Burroughs Wellcome Co., Research Triangle Park, N. C.) active Na and active K transport increased to 23 and 26 mmol·liter cell water^{-1}·h^{-1}, respectively. Cell Na and K concentrations were unchanged in untreated cells during 4 h of observation. The addition of PHA did not alter the K concentration (3, 9, 10); however, the Na concentration increased (see below).

Lymphocyte Na and K concentration in the absence

TABLE I
Active Na and K Transport in Human Lymphocytes

Experiment	Sodium		Potassium	
	α'_{Na}	Efflux (θ_{out})	α'_{K}	Influx (ψ_{in})
1	0.11	14	0.11	14
2	0.14	18	0.15	19
3	0.084	11	0.10	13
4	0.16	20	0.17	21
5	0.11	14	0.13	17
Mean±SE	0.12±0.01	16±2	0.13±0.01	17±2

α', the time constant, is expressed as h^{-1} and transport (θ_{out} and ψ_{in}) as millimole·liter cell water^{-1}·h^{-1}.
Experiments No. 1–4 were performed in the absence of external K; experiment No. 5, in the presence of 4 μM ouabain.

and presence of PHA. The presence of an increase in cell Na in PHA-treated lymphocytes observed in Fig. 1, suggested that Na might be the stimulus for the increase in active Na and K transport which occurs after PHA treatment. We studied, therefore, the concentration of Na and K 90 min after PHA treatment, when Na and K transport are persistently elevated (Table II). Quintuplicate measurements were made in 10 populations of human blood lymphocytes.

The lymphocyte K concentration in untreated cells was the same as that in PHA-treated cells (126 mmol/

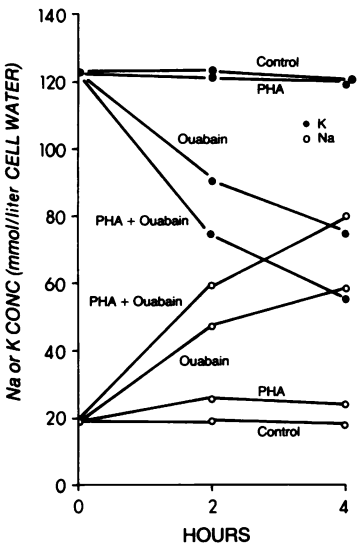

FIGURE 1 Na and K concentrations of human blood lymphocytes during 4 h incubation in the absence and presence of PHA and ouabain. Open circles represent Na concentrations and closed circles K concentrations. The rate of active transport can be calculated from the rate of fall in K concentration and rise in Na concentration (see Methods).

TABLE II
Lymphocyte Na and K Concentration in Untreated and PHA-Treated Cells

Experiment	Sodium			Potassium		
	Control	PHA	Δ	Control	PHA	Δ
1	16.7	19.7	+3.0	126	126	0
2	20.5	27.0	+6.5	132	135	+3
3	19.5	21.7	+2.2	119	120	+1
4	10.1	18.2	+8.1	128	126	−2
5	12.9	17.4	+4.5	121	123	+2
6	21.6	27.7	+6.1	129	127	−2
7	13.3	17.1	+3.8	128	128	0
8	12.0	18.2	+6.2	119	117	−2
9	6.0	17.9	+11.9	131	132	+1
10	15.2	20.5	+5.3	125	125	0
Mean	14.8	20.5	+5.8	126	126	+0.1
SE	1.6	1.2	0.9	0.9	1.7	0.6

The data are expressed as millimole per liter cell water. The values represent the mean of five replicates in each cell population. PHA was present at a concentration of 8 μg/ml.

liter cell water). The results of cell K content are similar to those we have found previously (3, 9, 10). In contrast, there was an increase in cell Na after PHA treatment in each of the 10 populations studied. The mean cell Na concentration was 14.8 mmol/liter cell water in untreated cells and 20.5 mmol/liter cell water in PHA-treated cells ($P < 0.001$).

Recovery of lymphocyte Na and K after pump inhibition. To determine whether the 6-mM increase in lymphocyte Na was responsible for sustaining the increased rate of Na and K transport after PHA treatment, we measured transport in untreated lymphocytes at various Na and K concentrations. Lymphocyte Na concentration was increased and K concentration decreased when cells were incubated in the absence of external K. After 4 h incubation, we observed a 48±4.9 mM (mean±SE) increase in cell Na and a 50±4.6 decrease in cell K. When external K was restored to the incubation, the cell K rose and cell Na fell to their original values (Fig. 2). The equilibrium cation concentrations reached 97±3% of their initial values. The restoration of both cell Na and K followed an exponential curve for which a mathematical model was constructed. The equations described in Methods permit calculation of a time constant λ, which represents the recovery of either Na or K. Fig. 3 illustrates the calculation of λ from the data in Fig. 2. λ_{Na} and λ_K were measured in five populations of human blood lymphocytes (Table III). λ_{Na} was 3.2±0.3 h^{-1} and λ_K was 3.0±0.2 h^{-1}. The strength of the correlation of the exponential functions $-\ln [(K(\infty) - K(t))(K(\infty) - K(0))^{-1}]$ or $-\ln [(Na(t) - Na(\infty))(Na(0) - Na(\infty))^{-1}]$ with time is

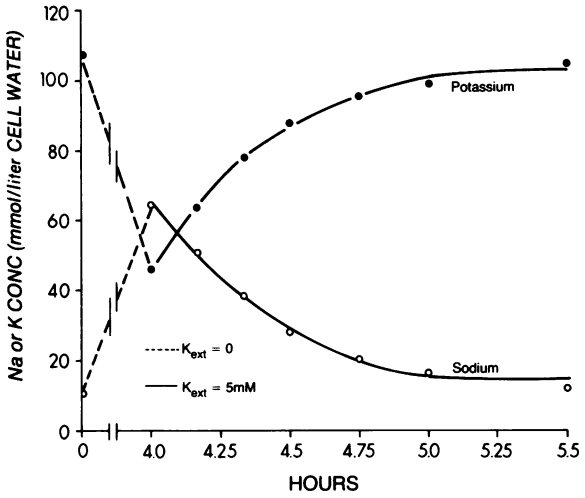

FIGURE 2 Recovery of lymphocyte Na and K concentration. Cell Na and K concentrations were measured at 0 and 4 h in the absence of external K and at intervals during 1.5 h after external K was adjusted to 5 mM. The reaccumulation of cell K and decrease in cell Na were exponential functions with time and fit a mathematical model presented in Methods.

represented by the value r^2. This provides a measure of how well the observed data fit the mathematical model. A value of 1.0 would indicate a perfect relationship; we calculated a mean value of $r^2 = 0.966$ for Na and 0.988 for K.

The time constant, λ, can be used to calculate a Na or K transport rate at any cell Na concentration (see Methods). This component of transport represents the fraction of transport contributing to the recovery of Na or K. In the case of K, the rate of active K transport necessary to balance the passive diffusion of K out of the cell at each K concentration must be added to this rate

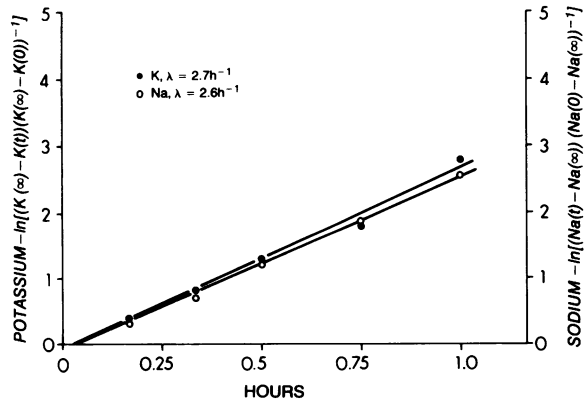

FIGURE 3 The rate constant for recovery of cell Na and cell K. The logarithmic functions of cation recovery are plotted against time. They result in lines with slopes λ_{Na} and λ_K. λ represents the rate of recovery of lymphocyte cell Na or K and can be used to determine either dK/dt or dNa/dt.

TABLE III
Time Constant, λ, for Calculation of Na and K Recovery

Experiment	λ_{Na}*	r^2‡	λ_K	r^2	Type of experiment
1	4.0	0.995	3.7	0.987	K_0§
2	2.6	0.996	2.7	0.991	K_0
3	2.6	0.878	2.5	0.977	4°C
4	3.3	0.968	3.0	0.987	K_0
5	3.3	0.992	3.2	0.999	K_0
Mean	3.2	0.996	3.0	0.988	
±SE	±0.3	±0.02	±0.2	±0.003	

* λ, the time constant for recovery, is expressed as h^{-1}.
‡ r^2 represents the correlation of the measured data with the mathematical model for cation recovery.
§ K_0 indicates experiments where Na and K were altered by cell incubation in the absence of external K, and 4°C indicates experiments where Na and K were altered by exposure to low temperature.

to determine the total active K transport. These data are shown in Table IV. If total lymphocyte K influx in resting cells is 17 mmol·liter cell water^{-1}·h^{-1}, a near doubling of the K influx rate occurs when the Na concentration increases from 15 to 20 mmol/liter cell water. As the lymphocyte Na concentration is increased further within the range of Na studied, the total K influx, ψ_{in}, increases at a rate of 2.8 mmol·liter cell water^{-1}·h^{-1}/mmol rise in cell Na.

Lymphocyte Na and K concentration after PHA treatment in a low Na medium. Since a 6-mM increase in internal Na results in a twofold increase in Na and K transport and since PHA-treated lymphocytes increase their Na by ≅6 mM at a time when transport nearly doubles, we tested whether preventing a rise in cell Na would obliterate the increase in transport after PHA

TABLE IV
Active K Transport at Normal and Increased Cell Na Concentrations

Cell Na (K)	$\frac{dK}{dt}$	ψ_{out}	ψ_{in}
mmol/liter cell water	*mmol·liter cell water^{-1}·h^{-1}*		
15 (126)	0	17	17
20 (121)	15	16	31
25 (116)	30	15.6	45.6
30 (111)	45	15	60
35 (106)	60	14	74
40 (101)	75	13.5	88.5

ψ_{in} is the total active transport of K into the cell and is equal to dK/dt + ψ_{out}. dK/dt is the recovery rate of intracellular K at various concentrations of cell Na. ψ_{out} represents K diffusion out of the cell.

treatment. We tested this possibility by measuring lymphocyte Na and K concentrations after 3 h of incubation in a medium containing: Tris-HCl, 50 mM; choline-Cl, 90 mM or 10 mM; KCl, 5 mM; NaCl, 10 or 90 mM; $CaCl_2$, 1.5 mM; $MgCl_2$, 1.5 mM; and albumin 10 mg/ml. The low external Na concentration was chosen because it was highly likely to be below the cell Na concentration (Table II).

In medium containing about 90 mM Na, PHA treatment again resulted in an increase in cell Na from 13 to 20 mmol/liter cell water (Table V). A 4% decrease in cell K occurred that was not statistically significant. In low Na medium, cell Na did not increase after PHA treatment. Under these conditions, cell K, 87 mmol/liter cell water, in the PHA-treated cells was nearly 20% less when compared to the control, 106 mmol/liter cell water ($P < 0.02$). The greater fall in cell K in the low Na medium was calculated to represent the effect of a 60% increase in plasma membrane K permeability after PHA treatment. This is consistent with the 50–100% increase in K turnover we have reported in PHA-treated lymphocytes (3).

Na activation of the Na, K-ATPase. If small changes in the internal Na concentration of lymphocytes regulate Na and K transport, the cell Na concentration should fall on the steep portion of the activation curve of the Na, K-ATPase of the lymphocyte membrane. To characterize the Na requirements for the ATPase, we measured the rate of reaction of the enzyme in the presence of 4 mM ATP, 4 mM Mg, 20 mM K and 0–170 mM Na (Fig. 4). Saturation of the Na site occurs at ≅60 mM. A Lineweaver-Burke reciprocal plot of Na activation is shown also. The concentration of Na necessary for half-maximal enzyme activation by this analysis was 15 mM. The maximum rate of enzyme activity was about 1.2 nmol P_i, μg protein^{-1}, 30 min^{-1}.

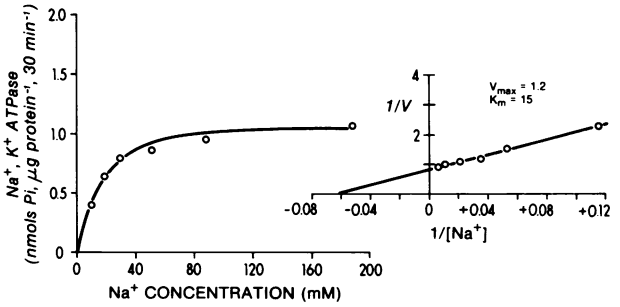

FIGURE 4 Na activation of the Na, K-ATPase. Na, K-ATPase activity is shown on the ordinate and Na concentration is shown on the abscissa. The assay was performed in the presence of 4 mM ATP, 4 mM Mg, and 20 mM K. A Lineweaver-Burke analysis of the effect of Na on the ATPase is shown in the inset.

TABLE V
Lymphocyte Na and K Concentrations in Medium with Low Na Concentration

Experiment	Cell sodium		Cell potassium	
	Control	PHA	Control	PHA
	mmol/liter cell water			
Medium Na = 86±4 mM				
1	13	22	123	121
2	12	15	118	110
3	13	25	101	94
4	15	17	121	119
Mean±SE	13±0.6	20±2	116±5	111±6
Medium Na = 11±0.5 mM				
1	15	13	104	85
2	10	8	112	85
3	10	8	103	83
4	7	8	106	96
Mean±SE	11±2	9±1	106±2	87±3

Measurements were made 3 h after exposure of lymphocyte suspensions to PHA at 8 μg/ml.

DISCUSSION

In 1970 it was reported that K uptake increased when human lymphocytes were treated with plant lectins such as PHA (16). Because K exodus was interpreted as being unchanged in those early studies, an increase in K concentration was postulated (16). Measurement of cell K concentration was required to confirm or refute this suggestion. In our initial studies, we observed that the K content was decreased rather than increased after PHA treatment (1). We suspected this to be an artifact since cell volume did not decrease. The decrease in cell K resulted from an exchange of internal K for counter ions in the buffers used to wash the cells prior to K measurement and from disintegration of lectin-treated cells that underwent an aggregation-disaggregation cycle during washing (10). When lymphocytes were prepared for ion measurement without cell dispersion, no alteration in K concentration was observed after PHA treatment (9, 10). Nevertheless, the fact that the membrane was altered after PHA treatment suggested that permeability may be increased, and the normal K concentration of PHA-treated cells demanded that leak increase if pump was increased. Indeed we found that ^{42}K exodus nearly doubled after PHA treatment (2). We subsequently measured K influx, K efflux, and K concentration in the same lymphocyte populations and found that K efflux and K influx increased proportionately and K concentration did not change after PHA treatment (3).

We have shown that the increase in ^{42}K uptake observed after PHA treatment of lymphocytes represents active transport of K into the cell (3). This increase in cation pumping required an increase in the trans-

duction of energy by the Na, K-ATPase. In this regard, we have demonstrated that an increase in ouabain-sensitive ATP production occurs after PHA treatment, which is proportional to the increase in active transport (17).

The increased K transport has been attributed to a direct stimulation of the Na, K-ATPase by two laboratories (6, 7); however, in detailed studies of purified lymphocyte membranes, we could not find an increase in the maximal velocity of the ATPase activity when PHA was added to the membranes, to the cells from which membranes were prepared or to both cells and membranes (8). Further, we could not demonstrate an increase in the affinity of the ATPase for ATP, K or Na after PHA treatment.

An alternative explanation for the increase in ATPase activity and K-Na transport is that the increased membrane permeability leads to an increase in lymphocyte Na concentration. This increase in cell Na could be responsible for the increased pump activity and Na and K transport rates. Na and K transport in red cells is regulated primarily by the internal Na concentration (15, 18). This mechanism for the regulation of pump rate is probably relevant to nucleated cells also (19). Accurate measurement of lymphocyte Na concentration was complicated by the low cell Na concentration relative to the medium Na concentration since PHA-treated lymphocytes cannot be dispersed to wash them free of the suspending medium. We resolved this problem by washing undisturbed cell pellets to rid the cell suspension of most of the residual medium, and used [^{14}C]sucrose to measure the remaining trapped space. When lymphocyte Na was measured in PHA-treated lymphocytes at a time when both Na and K transport were elevated, a significant increase of about 35% was observed. An equivalent decrease in cell K was expected but could not be detected. Such a decrease would represent only a 4% decrement in the cell K which may be beneath the sensitivity of our method. In a previous series of 69 measurements of lymphocyte K after PHA treatment, a 4-mM decrease was observed, although the difference was not statistically significant (10). Moreover, when greater increments in cell Na were induced by several hours of ouabain treatment, by exposure to cold temperature or by incubation in the absence of K, the decrease in K was virtually identical to the rise in Na ($\Delta Na/\Delta K = 0.96$).

Four lines of evidence suggest that an increase in lymphocyte Na sustains the increase in Na and K transport in PHA-treated lymphocytes. First, lymphocyte Na is increased about 35% or 6 mmol/liter cell water after PHA treatment at a time when active transport is increased 1.5- to 2-fold. Second, the cell Na concentration of 14–15 mmol/liter cell water is near the K_m for the Na, K-ATPase of the lymphocyte membrane and thus an increase in cell Na from 15–21 mmol/liter cell water would lead to a significant increase in pump activity. A 6-mM increase in Na heightens the Na, K-ATPase activity ≅35%, whereas active transport nearly doubles. We attribute these differences to factors inherent in the preparation of lymphocyte plasma membranes and measurement of ATPase activity under conditions that do not strictly mimic the intact cells. Third, an increase in Na of 5–6 mmol/liter cell water in unstimulated lymphocytes increases the transport of Na and K the same magnitude as does PHA treatment. Finally, if one reduces cell Na by incubating lymphocytes in a low Na medium, K transport does not maintain the internal K concentration and this is accentuated in PHA-treated lymphocytes in which internal K falls at a greater rate.

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