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A MODEL FOR THE CONTROL OF POTASSIUM TRANSPORT IN PHA-STIMULATED HUMAN BLOOD LYMPHOCYTES

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INTRODUCTION

When human blood lymphocytes are stimulated with plant lectins such as phytohemagglutinin (PHA) there is an immediate increase in the plasma membrane permeability (1). As one manifestation of this alteration, K^+ leaks from the cell at approximately twice the rate observed in unstimulated lymphocytes (2). At the same time, active K^+ transport increases proportionately (3).

Opinions differ regarding the pathogenesis for the stimulus to increased active K^+ transport. It has been suggested that PHA acts by stimulating the Na^+, K^+-ATPase so as to increase the transduction of energy for transport (4,5). This hypothesis is strained 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, conflicting evidence has been provided on the activity of the Na^+, K^+-ATPase isolated from lectin-treated lymphocytes. Some investigators have reported an increase in Na^+, K^+-ATPase activity when plant lectins were added to cells or membranes in vitro; others have found no increase in its activity. In part the divergent findings are a reflection of inadequate assay systems for lymphocyte ATPase activity.

An alternative hypothesis is that the increased permeability of the membrane leads to a rise in cell Na^+ and a fall in the cell K^+. These alterations in cell ion concentration stimulate the pump to translocate the Na^+ and K^+ at a higher rate. Such a hypothesis has considerable support from studies of red cells. However, repeated studies of cell K^+ concentration in PHA treated cells, in which the active transport rate is about twice control cells, have found values equivalent to those of untreated cells (3,7, 8,9). Perhaps, the sensitivity of the pump to changes in cell cation concentration is such that lymphocyte K^+

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transport increases when cell monovalent cations change by only a small quantity.

In order to test these two alternative hypotheses we have prepared lymphocyte membranes with a high degree of purity and have characterized in detail the kinetic characteristics and substrate specificity of their Na^+, K^+-activated phosphohydrolases. The assay used has proven highly reproducible when adenosine triphosphate (ATP) hydrolysis is assessed either by release of radioactive phosphorus from $AT^{32}P$ or by colorimetric measurement of inorganic phosphate released from ATP (6). Using this assay, we have been able to reexamine the effects of PHA on the lymphocyte Na^+, K^+-ATPase.

In addition, we have correlated the rate of active K^+ transport with the concentration of lymphocyte K^+ in order to determine the relationship of pump rate with internal monovalent cation concentration. By studying the restitution of intracellular K^+ concentration after exposure of cells to cold temperature, the instantaneous rate of K^+ transport could be calculated as the intracellular cation concentration was restored to a steady state.

RESULTS

Studies on the lymphocyte plasma-membrane ATPase

Plateletpheresis residues provided the means to harvest human lymphocytes in amounts equivalent to thousands of milliliters of blood so that large quantities of plasma membranes could be prepared (10). We added lymphocyte homogenates to sucrose gradients and isolated membrane vesicles of high purity as judged by electron microscopy and enzyme assays. These membrane vesicles had a mean increase in 5' nucleotidase activity of about 30-fold and minimal mitochondrial and nuclear contamination.

We studied the substrate specificity and the kinetics of the cofactor and substrate requirements of the Mg^{++} and the Na^+, K^+-ATPase activities. This was done so that we could study the effects of lectins on these enzymes. Mean Mg^{++}-ATPase activity was 1.9 nmol Pi, μg $protein^{-1}$, 30 min^{-1}. Mg^{++} activated the hydrolysis of ITP, UTP, GTP and TTP and was shown by competition studies to be an enzyme with broad substrate acceptance. The Na^+, K^+-activated phosphatase did not hydrolyze ADP, AMP, ITP, UTP, GTP or TTP and thus was specifically an ATPase (Table 1). Mean Na^+, K^+-ATPase activity was 1.5 nmol, μg $protein^{-1}$, 30 min^{-1}. This activity was completely inhibited by the cardiac glycoside, ouabain. No difference in Na^+, K^+-ATPase activity was

TABLE 1

SUBSTRATE SPECIFICITY OF LYMPHOCYTE MEMBRANE PHOSPHATASE ACTIVITY

Substrate	Mg^{++} Activation	K^+ Activation in Presence of Na^+ and Mg^{++}				
		K^+ Concentration				
		1mM	2mM	5mM	10mM	20mM
ATP	2.7	.61	1.4	1.8	1.8	1.7
ADP	1.4(0.50)	.34(.04)	.54(.24)	.37(.07)	.54(.24)	.51(.21)
AMP	.13	.13	.13	0	0.034	0
GTP	3.0	0	0	0	0	0
ITP	2.0	.24	.24	.068	.034	.034
UTP	3.8	0	0	0	0	0
TTP	2.6	.035	0	0	0	0
Glycerol-P	0	0	0	0	0	0
p-phenyl-P	0	0	0	0	0	0

The data are expressed as nmol Pi released, $\mu g\ protein^{-1}$, $30\ min^{-1}$. All substrates were present at a concentration of 4 mM. The assay was run in the presence of 4 mM Mg^{++} 120 mM Na^+.

The Mg^{++} activated enzyme hydrolyzed both γ and β phosphorus of ATP when assayed by the chemical measurement of Pi. The Mg^{++} activated ATPase activity when corrected for ADPase was 2.2 (2.7-0.5).

ADP is stated to contain no more than 1% ATP. With the use of the luciferase assay, we measured 5% ATP by molarity or 0.2 mM ATP/4 mM ADP. Thus ATPase, rather than ADPase, activity could explain most or all of the apparent ADPase activity. Corrected values for the ADPase, subtracting the ATPase activity resulting from contaminating ATP are shown parenthetically. ATP measured by luciferase assay was 0.0% in AMP, 1.0% in ITP and 0.7% in GTP. That contamination did not influence significantly the results shown.

TABLE 2

EFFECT OF PHA ON LYMPHOCYTE MEMBRANE ATPase

	Untreated Cells		PHA Treated Cells	
	Mg^{++}	Na^+,K^+	Mg^{++}	Na^+,K^+
No PHA in assay	2.3±.29	1.5±.03	1.7±.06	1.2±.07
PHA (8 µg/ml) in assay	2.1±.22	1.4±.09	1.6±.07	1.0±.07

The data are expressed as the mean ± SE ATPase activity (nmol Pi, µg protein^{-1}, 30 min^{-1}) from 3 populations of human blood lymphocytes. In these studies lymphocytes were incubated in the absence (untreated cells) or presence (PHA treated cells) of 8 µg/ml PHA in a manner analogous to that used for mitogenic or K^+ transport studies. Membranes prepared from untreated and PHA treated cells were then assayed for ATPase activity in the absence or presence of PHA.

observed when either a colorimetric or radioisotopic assay was used to measure Pi release. The Km of the enzyme for K^+ was ∿1.0 mM and the Km for Na^+ was ∿15 mM.

No stimulation of membrane Na^+, K^+-ATPase was observed when either lymphocytes or lymphocyte membranes were treated with mitogenic concentrations of PHA (Table 2). (see reference 6 for further details)

Correlation of K^+ with Lymphocyte K^+ Concentration

After exposure to phytohemagglutinin, human blood lymphocytes have an alteration in membrane permeability, and K^+ leaks from the cell at an accelerated rate. Active K^+ influx increases proportionately and cell K^+ is maintained at a concentration that is very similar to unperturbed lymphocytes (3,9). We have examined the relationship of the active transport rate to lymphocyte K^+ concentration in order to see if the pump rate can be nearly twice that of untreated lymphocytes in the presence of an apparently normal internal K^+ concentration.

Lymphocyte cell suspensions (3 ± 1 x 10^6 cells/ ml) were divided into 10 ml aliquots and incubated under air and 7% CO_2 at 4°C. After 48 hours, the cell suspensions were transferred to a 37°C water bath for 90 min. Lymphocyte K^+ concentration fell when cells were stored at 4°C. Under

these conditions, active K^+ transport is inhibited more than is diffusion and there is a net movement of K^+ from cells to medium. We measured K^+ concentration at various times t ($K^+(t)$) during exposure to 4°C and recovery at 37°C. The decrement in lymphocyte K^+ concentration after 48 hours incubation at 4°C was about 40 mmol/l cell water. When cells were restored to 37°C the cellular K^+ increased to normal values. The equilibrium K^+ concentration in six lymphocyte populations was 95 ± 9% (SD) of the value before incubation at 4°C.

The restoration of cellular K^+ concentration follows an inverted exponential curve for which a mathematical model was constructed. The equations permitted calculation of a time constant to represent the recovery. The time constant was calculated from the exponential recovery of the lymphocyte K^+ concentration from 84 mM ($K^+(0)$) to 121 mM ($K^+(\infty)$). The strength of the correlation of the exponential function $-\ln (K^+(\infty)-K^+(t))(K^+(\infty)-K^+(0))^{-1}$ with time was $r^2 = 0.99 \pm 0.006$ (SD). The derivation of this formula and the mathematical model derived from these studies have been presented elsewhere (11). The time constant measured in seven populations of human blood lymphocytes was 3.05±0.44 (SD) h^{-1}.

The time constant can be used to calculate a transport rate at any cell K^+ concentration (Table 3). This component of K^+ transport represents the fraction of transport contributing to the recovery of cell K^+, dK^+/dt. The rate of active K^+ transport necessary to balance the passive diffusion of K^+ out of the cell at each K^+ concentration, Ψ_{out}, must be added to this rate to determine the total active K^+ transport, Ψ_{in}. Total lymphocyte K^+ influx in resting lymphocytes is 19 mmol·l cell water^{-1}·h^{-1}. A decrease of 5% in K^+ concentration from 121 to 115 mmol/l cell water was associated with a two-fold increase in the total K^+ influx to 36 mmol·l cell water^{-1}·h^{-1}. Further decreases in cellular K^+ concentration were associated with more striking increases in lymphocyte K^+ influx. (Table 3).

K^+ Efflux, Influx and Cell Concentration in PHA-Treated Lymphocytes

K^+ efflux and K^+ influx are approximately 19 mmol·l cell water^{-1}·h^{-1} in untreated lymphocytes (Table 4). When lymphocytes are exposed to PHA, K^+ efflux and K^+ influx increase to approximately 39 mmol·l cell water^{-1}·h^{-1}. However, lymphocyte K^+ concentration in untreated and PHA treated lymphocytes was not significantly different (Table 4).

TABLE 3

ACTIVE K^+ TRANSPORT AT NORMAL AND REDUCED K^+ CONCENTRATIONS

Cell K^+ concentration (mmol/l cell water)	dK^+/dt	Ψ_{out}	Ψ_{in}
	(mmol·l cell water^{-1}·h^{-1})		
121	0	19	19
115	18	18	36
110	34	17	51
105	49	16	65

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

TABLE 4

K^+ TRANSPORT IN HUMAN LYMPHOCYTES

	K^+ efflux	K^+ influx	K^+ concentration
	(mmol·l cell water^{-1}·h^{-1})		(mmol/l cell water)
Control	19±2.0(7)	20±2.2(7)	128±23(8)
PHA	40±2.1(7)	38±7.2(7)	124±19(8)

The data represent the mean ± 1 SD. PHA was present at 8µg/ml. Influx values were derived from the rate of $^{42}K^+$ accumulation by the lymphocytes. We have shown that $^{42}K^+$ influx represents active transport (3). Efflux values were calculated from α_e, a diffusion constant, determined from the rate of $^{42}K^+$ exodus from radiolabeled lymphocytes. The number of lymphocyte populations studied is shown in brackets. These results are from reference 3.

DISCUSSION

An increase in Na^+, K^+-ATPase activity is necessary in order to transduce the energy required for increased active K^+ influx. It has been suggested that PHA has a direct effect on the lymphocyte membrane Na^+, K^+-ATPase, increasing its activity to provide the energy needed for enhanced active cation transport. Disagreement exists as to whether a direct effect of lectins on membrane ATPase is demonstrable, however. Increases in membrane Na^+, K^+-ATPase activity of 26% (5) and greater than 100% (4) after lectin stimulation, have been reported. The former study was conducted in membranes prepared from mouse splenocytes preincubated for 2 hours in concanavalin-A; the latter in unpurified membranes from human lymphocytes exposed to PHA or Con-A in the enzyme assay system. Three other laboratories have been unable to demonstrate a positive effect of plant lectins on the Na^+, K^+-ATPase (see reference 6 for review). No stimulation of Na^+, K^+-ATPase was observed when either lymphocytes or lymphocyte membranes were treated with mitogenic concentrations of PHA. Our findings support the latter observation. We could find no evidence for a direct enhancement of lymphocyte membrane ATPase activity by PHA whether the intact cells or the membrane fraction or both were exposed to the lectin.

The magnitude of the active K^+ transport and the activity of the Na^+, K^+-ATPase in intact lymphocytes depends upon the internal concentrations of these cations. Plant lectins can influence the monovalent cation transport system by altering the membrane permeability to cations, imposing the need for increased active transport to maintain homeostasis in the internal cation concentration.

Cation transport is regulated by the cooperative effects of the internal K^+ and internal Na^+ concentrations, although Na^+ effects are dominant. When active cation transport is inhibited (for example, by incubation at 4^oC) the K^+ falls and Na^+ rises in a reciprocal fashion. Upon removal of the inhibition, Na^+ and K^+ return to their steady-state concentrations. Since we have found that lymphocytes treated with ouabain or placed at 4^oC have a close reciprocal alteration in Na^+ and K^+ concentration, we have used the more precise K^+ measurement to show the relationship between the cell cation concentration and active cation transport.

In PHA-treated lymphocytes K^+ transport is doubled at an apparently normal K^+ concentration. In order to maintain a compensatory increase in active transport, cell K^+ concentration should be low and cell Na^+ above normal levels in

PHA-stimulated lymphocytes. However, we could not demonstrate a decrease in lymphocyte K^+ concentration after PHA stimulation at a time when K^+ influx was persistently elevated. The reason for this apparent paradox may be explained by the relationship of monovalent cation transport to monovalent cation concentration. Active Na^+ and K^+ transport in lymphocytes appear to be sensitive to a small increase in Na^+ and decrease in K^+ concentration. A doubling of the K^+ influx occurs at a K^+ concentration that is 95% of control. This increase in K^+ transport is comparable to that observed in PHA-treated lymphocytes.

We were unable to measure with enough accuracy the small change in cell Na^+ that presumably is the major effector of the increased transport rate. Our findings that Na^+ and K^+ change in a reciprocal manner strongly suggest that a 5 to 6 mM fall in K^+ is accompanied by an equimolar increase in cell Na^+. The Na^+ concentration in human lymphocytes is about 10 mM and the Km for Na^+ of the Na^+, K^+-ATPase is 15 mM. A change in Na^+ concentration of 5 to 10 mM will double the rate of the ATPase reaction.

Thus, the increased active K^+ transport in PHA-treated lymphocytes of up to two-times normal may be driven by an alteration in the cell cation concentrations of about 6 mmol/l cell water. The techniques available for measurement of lymphocyte monovalent cation concentration cannot detect a change of this magnitude.

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