BBA 75923

EFFECT OF CELL VOLUME ON POTASSIUM TRANSPORT IN HUMAN RED CELLS

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(Received January 3rd, 1972)

SUMMARY

The influx of K^+ into fresh human red cells is shown to be reversibly dependent on cell volume. With a 10 % decrease in cell volume, K^+ influx increases by an average of 19 %. The effect persists in the presence of cardiac glycosides which are more effective inhibitors of K^+ influx in shrunken cells than in normal ones; furthermore, increases in extracellular K^+ inhibit the action of cardiac glycosides more in shrunken than in normal cells. It is suggested that the volume dependent flux change is due to a conformational change in a receptor on the cell surface. This hypothesis is consistent with observed changes in measurable characteristics of the transport mechanism.

INTRODUCTION

Cation fluxes depend on cell volume in dog and cat red cells, as shown in dog cells by Parker and Hoffman[1] and in cat cells by Sha'afi and Hajjar[2]. Romualdez *et al.*[3] have studied the metabolic requirements of the volume effect in the dog and have put forward a working hypothesis that attributes the reversible volume effect to reversible conformational changes of a receptor site on the cell membrane. Their hypothesis is based upon a selective change of Na^+:K^+ selectivity such that the Na^+ selectivity of the external receptor is enhanced as the cell shrinks, and the inwardly directed Na^+ electrochemical potential gradient then drives more Na^+ into the cell. In the case of the dog, the process is apparently a passive one since both Na^+ and K^+ movements are down an electrochemical potential gradient. We have looked for a similar effect in human red cells and have found a marked dependence of K^+ influx on human red cell volume which is compatible with a volume induced conformational change in a receptor on the surface of the cell.

The rationale of our experiments was based on the view that a surface receptor site undergoing a conformational change should show a sensible alteration in all of its measurable qualities. Cardiac glycosides have been shown by Solomon *et al.*[4] and Glynn[5] to inhibit K^+ uptake, presumably by interaction with a surface site, a process which can be effectively inhibited by increases in extracellular K^+ concentration. We have found that both these properties are altered by changes in red cell volume, a finding which is consistent with a volume induced conformational change in the site.

EXPERIMENTAL RESULTS

All experiments were carried out on fresh human red cells at 37 °C and fluxes were determined using the technique and equations given by Solomon[6]. Table I shows the effect of osmolality changes on $^{42}K^+$ flux in duplicate experiments. Similar flux increases may be produced either by the addition of sucrose or an osmotically equivalent amount of NaCl. Removal of NaCl from the normal suspension medium reverses the direction and decreases the $^{42}K^+$ influx. In four other experiments under the same conditions the addition of 50 mM sucrose increased the $^{42}K^+$ influx by an average of 19 %, though the fractional increase was quite variable, ranging from a low of 1.09 to a high of 1.52. Restoration of the volume to its normal value reverses the effect.

The effect does not appear to be linear with cell volume since, in one experiment, the addition of 25 mM sucrose produced 75 % of the effect of 50 mM sucrose, whereas further increase in sucrose concentration to 100 mM did not increase the $^{42}K^+$ influx above that found at 50 mM.

TABLE I

EFFECT OF CELL VOLUME ON K^+ INFLUX

Condition	*K^+ influx* (mM/l cell per h)*	*Influx relative to control*	*Relative cell volume*
Control	1.36	1.0	1.0
+50 mosM sucrose	1.59	1.17	0.90
+50 mosM NaCl	1.51	1.11	0.91
−50 mosM NaCl	1.18	0.87	1.11

* Influxes are given in terms of original cell volume.

Streeten and Solomon[7] showed that K^+ influx into human red cells could be described in terms ot the equation, $\Phi_{in} = [K^+_{out}]/(0.697 + 0.329\ [K^+_{out}])$ in which Φ_{in} is influx in mM/l cell per h and $[K^+_{out}]$ is concentration in the medium in mM. As Glynn[8] has shown, an additional term is required to account for the passive K^+ leak which is evident at high K^+ concentrations. However, this term does not make a significant contribution to Φ_{in} at extracellular K^+ concentrations below 17 mM, the highest concentration used in these experiments.

Formally, K^+ influx may be described in Michaelis–Menten terms according to the following reaction scheme* which assumes the back reaction of the second step to be negligible, so $k_{-2} = 0$.

$$K^+_{out} + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} K^+R \xrightarrow{k_2} R + K^+_{in} \qquad (1)$$

R stands for the receptor sites which are treated formally in terms of a chemical concentration. Since $[R]/[K^+R]$ enters the equation for the dissociation constant only as a ratio, it makes no difference what units are used to express the concentration

* This reaction scheme provides a formal description of one step, presumably the first, in the kinetics of K^+ entrance. Normally the driving force exercises its effect either through the step which destroys R at the inner membrane surface or that which synthesizes R at the outer surface. Hence Eqn 1 contains no information about the coupling between energy and transport.

of these two constituents and any parameter relating the concentration to the membrane can be used. In the alternative absorption isotherm treatment, R would be interpreted as the unoccupied fraction of receptor sites, θ, and $[R]/[K^+R]$ would equal $\theta/(1-\theta)$. The steady-state flux may be expressed linearly according to the following equation,

$$[K^+_{out}]/\Phi_{in} = K_{dis}/\Phi_{max} + [K^+_{out}]/\Phi_{max} \quad (2)$$

in which K_{dis}, the dissociation constant of the K^+–receptor complex, is given by,

$$K_{dis} = [K^+_{out}]\,[R]/[K^+R] = (k_{-1} + k_2)/k_1 \quad (3)$$

and $\Phi_{max} = k_2\, c_R$; c_R is the total concentration of receptor sites, filled and empty.

Fig. 1, which is typical of four experiments, shows the expected linear relationship between $[K^+_{out}]/\Phi_{in}$ and $[K^+_{out}]$. Shrinking the cells decreases the slope of the line but does not change the intercept. Since the slope $= (k_2\, c_R)^{-1}$ the volume must exercise its effect by changing either the rate constant or the total concentration of receptor sites, or both. Since the intercept in Fig. 1, K_{dis}/Φ_{max}, is independent of cell volume $(k_{-1} + k_2)/k_1 k_2 c_R$ remains constant. If $k_{-1} \ll k_2$, $K_{dis}/\Phi_{max} = 1/k_1 c_R$. Thus K_{dis}/Φ_{max} can remain invariant provided the volume change affects k_2 alone and does not alter k_1 or c_R. The requirement that $k_{-1} \ll k_2$ means that the forward reaction is the dominant one and that the K^+ receptor complex dissociates much more frequently in the direction leading to K^+ influx than in returning the K^+ to the external solution. It also seems much more reasonable for the volume change to alter the characteristics of the receptor site as expressed in a change in the frequency of dissociation of the site in the forward reaction direction, rather than to effect a change in the total number of receptor sites, which would appear to be a much more complex operation.

Calculations based on Fig. 1 show that reducing the cell volume by 10% increases the maximum influx from 1.59 to 2.02 mM/l cell per h as a result of an increase in K_{dis} from 1.37 to 1.81 mM/l. The meaning of K_{dis} may be understood more easily in other terms, since $[K^+R]/[R] = [K^+_{out}]/K_{dis}$. At a medium concentration of 5 mM K^+, the number of filled sites is 3.65 times the number of empty ones for normal cells and only 2.85 times the empty ones for shrunken cells. Fewer

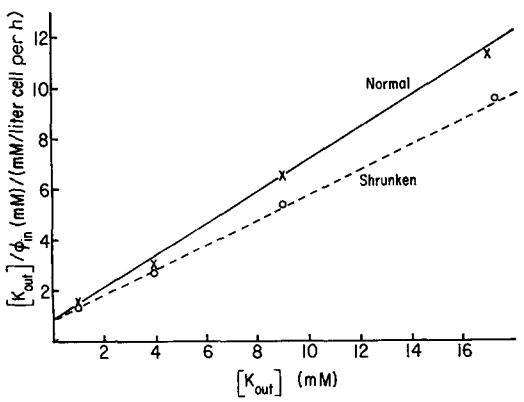

Fig. 1. Relation of K^+ influx to K^+ in the medium plotted according to Eqn 2.

sites are filled in the shrunken cells because the forward dissociation rate of the filled site is greater. If one takes 100 sites per cell as a reasonable estimate (see ref. 9), then 78.5 % of them are filled in normal cells and 74.0 % in shrunken cells. In four experiments of a similar kind on normal cells the mean value for Φ_{max} was 1.99 ± 0.23 mM/l cell per h and for K_{dis}, 1.58 ± 0.17 mM/l. In shrunken cells $\Phi_{max} = 2.51 \pm 0.30$ mM/l cell per h and $K_{dis} = 2.04 \pm 0.18$ mM/l.

A conformational change in the receptor site should affect the interaction of the site with the cardiac glycosides. These drugs are believed to inhibit K^+ uptake by inhibiting the formation of the K^+–receptor complex, perhaps through the agency of a cardiac glycoside sensitive ATPase[10]. Fig. 2 shows the result of one experiment in which the dose response to ouabain was measured in normal and shrunken cells. The curves were drawn by eye to correspond to the general shape of the curves which Solomon *et al.*[4] showed to be characteristic of ouabain inhibition. The point for the control has been arbitrarily assigned to a concentration of $1 \cdot 10^{-9}$ M ouabain where inhibition is virtually zero. Shrinking the cells displaces the dose response curve upwards but at the same time shifts the 50 % inhibition point. This point is calculated as the ouabain concentration which reduces K^+ influx to a value halfway between the control flux and that observed with $1 \cdot 10^{-6}$ M ouabain. If the figures are taken from the curve in Fig. 2, shrinking the cells increases the 50 % inhibition concentration from $1.1 \cdot 10^{-7}$ to $2.0 \cdot 10^{-7}$ M ouabain; if the data is taken from linear interpolation between the data points at $1 \cdot 10^{-8}$ and $1 \cdot 10^{-7}$ M ouabain, the increase is from $1.6 \cdot 10^{-7}$ to $2.3 \cdot 10^{-7}$ M.

It was thought that greater changes might be observed in the behavior of glycosides whose affinity for the receptor site was less than that of ouabain and so studies were carried out with digitoxigenin and strophanthidin. The results in Table II are expressed in terms of the percentage inhibition of control influx with no allowance for the fraction of the influx that is insensitive to cardiac glycosides. The effect of shrinking the cells is much larger with either of the other inhibitors than with ouabain and varies in detail with the molecular architecture of the inhibitor as can be seen from a comparison of results at $1 \cdot 10^{-6}$ and $1 \cdot 10^{-7}$ M.

Solomon *et al.*[4] and Glynn[5] have shown that the cardiac glycoside inhibition may be suppressed by increasing extracellular K^+ though, as Glynn has pointed out,

TABLE II

EFFECT OF CELL VOLUME ON GLYCOSIDE INHIBITION OF K^+ INFLUX

Inhibitor	*Inhibitor concn (M)*	*% Inhibition*		*Difference in % inhibition*
		Normal	*Shrunken**	
Ouabain	$1 \cdot 10^{-6}$	77.2	81.0	3.8
	$1 \cdot 10^{-7}$	64.0	66.2	2.2
	$5 \cdot 10^{-8}$	52.0	54.0	2.0
Digitoxigenin	$1 \cdot 10^{-6}$	67.5	70.8	3.3
	$1 \cdot 10^{-7}$	35.2	53.6	18.4
Strophanthidin	$1 \cdot 10^{-6}$	60.0	67.9	7.9
	$1 \cdot 10^{-7}$	38.6	57.9	19.3

* Cells shrunken in 50 mosM sucrose.

the kinetics may not be explained in terms of simple competitive inhibition. In Fig. 3, the effects of increased K^+ concentration are compared in the presence of $1 \cdot 10^{-6}$ M ouabain and $1 \cdot 10^{-6}$ M digitoxigenin. In both instances, the addition of extracellular K^+ is more effective in decreasing the cardiac glycoside effect in the shrunken cells than in the normal ones. The effect is much larger with digitoxigenin and becomes most apparent at the higher concentrations of K^+. With strophanthidin the effect is only apparent at the highest K^+ concentration. Thus, changes in cell volume also alter the nature of the competition between K^+ and the cardiac glycoside. The differences observed with glycosides of different architecture are entirely consonant with expectations based on a conformational change in the receptor site.

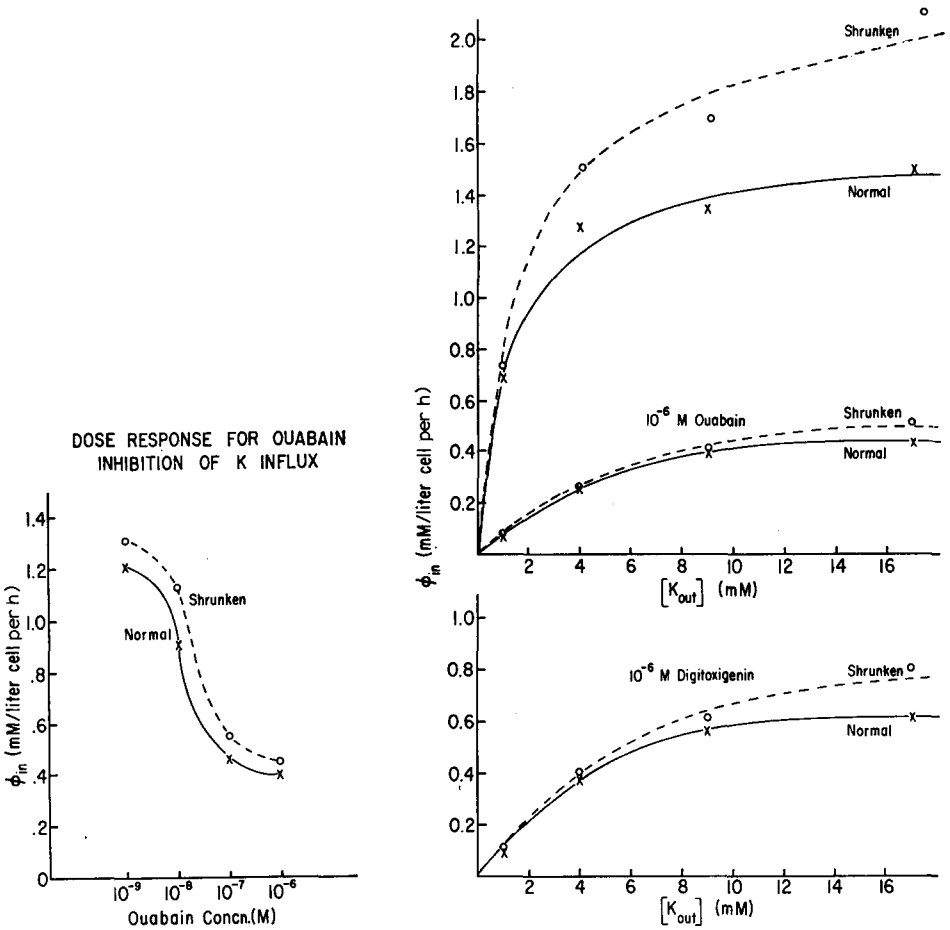

Fig. 2. Dose response curve for ouabain inhibition of K^+ flux in normal and shrunken human red cells. The curves have been drawn by eye, and the control points have arbitrarily been shown at $1 \cdot 10^{-9}$ M ouabain, as described in the text.

Fig. 3. K^+ inhibition of cardiac glycoside effect in normal and shrunken human red cells. The data are from the experiment used in Fig. 1 and the curves for the control values have been computed from the line in Fig. 1. Ouabain and digitoxigenin were added at $1 \cdot 10^{-6}$ M.

DISCUSSION

It is important to know whether the volume effect is primarily dependent on the properties of the surface receptor or whether it is secondary to changes induced in glucose metabolism. The most powerful argument against the second possibility is the simplicity of the kinetics which are consonant with regulation by the rate coefficient of forward dissociation of the K^+–receptor complex. Furthermore, Miller[11] has shown that the rate constant for fructose uptake is not affected by shrinking human red cells with lactose to 0.76 of normal volume. The experiments with the cardiac glycosides also support the view that glucose metabolism is not the primary process governing the volume effect. Whittam and Ager[12] have shown that ouabain, in fresh red cells, affects only about 25 % of the lactate production and that the remaining basal energy production is independent of the presence of the cardiac glycoside. Though basal energy production is unchanged by cardiac glycosides, Table II shows that the volume effect is inhibited by the glycosides. This finding argues that the volume effect is not mediated by the glucose metabolism involved in the basal energy production. Since the remainder of the glucose metabolism is linked to the transport process, this moiety should be affected by the volume effect, presumably as a result of the K^+ transport, rather than as a cause.

Preliminary experiments in our laboratory[13] have shown that a decrease in cell volume also causes an increase in Na^+ influx. Under normal conditions there is no linkage between these two influxes as shown by the observation that ouabain does not inhibit Na^+ influx[14]. Since a decrease in cell volume causes both Na^+ and K^+ influxes to increase it is not likely to be driven by a volume induced change in ATP metabolism.

The question may be asked as to whether the volume effect acts only on those receptor sites which are insensitive to the cardiac glycosides. Such an argument might be tenable because of the relatively slight difference in the ouabain effect in normal and shrunken cells as shown in Fig. 2. However, the data with digitoxigenin and strophanthidin in Table II effectively rule this argument out.

Changes in cell volume produce concomitant changes in cell Na^+ concentration. Since Whittam and Ager[12] have shown that K^+ influx is related to cell Na^+, it might be possible to ascribe our effect on K^+ influx to this cause. However, Fig. 2 and Table II both show that the volume effect persists in the presence of cardiac glycosides which, as Whittam and Ager have shown, abolish the dependence of K^+ influx on cell Na^+. Moreover, Whittam and Ager's data indicate that the K^+ influx is linearly dependent on cell Na^+ up to a concentration of 31 mM Na^+/l cells, whereas shrinking cells beyond 10 % (equivalent to 8 mM Na^+/l cells) produces a negligible additional effect on K^+ influx. Whittam and Ager's data indicates that a 10 % increase in cell Na^+ would cause a 7.5 % increase in K^+ influx. This figure is to be compared to the 17 % increase which accompanies a 10 % shrinkage in cell volume (Table I). The two processes need not produce identical effects since an increase in cell Na^+ concentration causes a change in the amount of Na^+ in the cell relative to hemoglobin and cell membrane constituents, whereas a shrinkage in cell volume has no effect on the concentration of Na^+ relative to the other normal components of the cell.

It is widely believed that the area of the red cell remains constant as the cell swells from its normal biconcave disc shape to a sphere. Fung and Tong[15] point out

that the implicit conclusion that the red cell membrane must therefore be inextensible is erroneous and that local extensions in the cell membrane are necessary for sphering. Furthermore, Fung and Tong[15] present persuasive arguments that the wall thickness and the extensional stiffness of the red cell membrane vary in different portions of the cell membrane. Thus small changes in cell volume may be expected to produce local changes in surface forces which are sufficient to cause deformations in the K^+–receptor site.

It is difficult to determine whether the conformational changes produce an allosteric effect or whether they modify the properties of a single site sensitive to the deformational stresses in the membrane. The argument in favor of an allosteric action rests on the complexity of the kinetics of the cardiac glycoside inhibition. As Glynn[5] pointed out, these kinetics are not compatible with simple competitive inhibition between the glycoside and external K^+ concentration. We have confirmed this finding and extended it to the shrunken cells. This implies that the glycosides act on not only the rate coefficient, k_2, but also on other parts of the system. Hence the volume induced changes in the glycoside $-K^+$ competition argue in favor of involvement of more than one site on the receptor, and, by extension, with an allosteric effect.

It is also interesting that the volume effect is not linear with cell volume and that shrinkage of the cell beyond about 10 % does not result in any further increase in K^+ influx. This argues that the receptor site has reached a fixed conformation when the cell has shrunk to about 90 % of normal, and that below this volume, the site resists further deformation. In dog red cells, Romualdez *et al.*[3] have shown that the volume effect is not induced by a simple change in osmolality, but that the actual cell volume must change, and a similar conclusion has been reached by Sha'afi and Hajjar[2] in cat red cells. The observation that the effect is not linear with cell volume in the human red cell points to a similar conclusion.

The mechanism for regulation of cell volume has long been an interesting puzzle. The present experiments may provide an answer to this question. As the cell shrinks, K^+ and Na^+ influxes increase and provide a force in the proper direction to restore the volume to normal. When the cell swells, the converse process occurs and influx decreases. Regulation appears to be mediated by changes in the conformation of a receptor site within the membrane, which can thus respond directly to signals generated by changes in the cell configuration.

Kregenow[16,17] has reported that changes in the volume of the nucleated red cells of the duck can induce changes in cation fluxes. Furthermore, he has shown that these red cells can use this process to control their volume. His paper has just been published and was not available to us when our work was done.

ACKNOWLEDGEMENT

This study has been supported in part by the National Institutes of Health.

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