

Coordinated Regulation of Shrinkage-induced Na/H Exchange and Swelling-induced [K-Cl] Cotransport in Dog Red Cells

Further Evidence from Activation Kinetics and Phosphatase Inhibition

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ABSTRACT Hypertonic shrinkage of dog red cells caused rapid activation of Na/H exchange and rapid deactivation of [K-Cl] cotransport. Hypotonic swelling caused delayed deactivation of Na/H exchange and delayed activation of [K-Cl] cotransport. Okadaic acid stimulated shrinkage-induced Na/H exchange and inhibited swelling-induced [K-Cl] cotransport. The data are compatible with the kinetic model of Jennings and Al-Rohil (1990. *J. Gen. Physiol.* 95:1021–1040) for volume regulation of [K-Cl] cotransport in rabbit red cells and suggest that in dog red cells Na/H exchange and [K-Cl] cotransport are controlled by a common regulatory system. The proposal of Jennings and Schulz (1991. *J. Gen. Physiol.* 96:799–817) that activation/deactivation of volume-sensitive transport involves phosphorylation/dephosphorylation of a regulatory protein is supported by these observations.

INTRODUCTION

Na/H exchange and [K-Cl] cotransport can be reversibly activated and deactivated in dog red cells. In a previous report we presented evidence that these two systems are reciprocally linked, so that when one is turned on, the other is turned off. For example, activation of Na/H exchange and suppression of [K-Cl] cotransport occur when cells are shrunken, or when they are loaded with Mg or Li ions. Conversely, suppression of Na/H exchange and activation of [K-Cl] cotransport occur when cells are swollen, depleted of Mg ions, or hemolyzed and resealed (Parker, McManus, Starke, and Gitelman, 1990). The reciprocal behavior of these two transport systems suggests that they are controlled by a separate entity or system that acts as a

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volume-sensitive regulator. A similar type of interaction between swelling- and shrinkage-induced transporters had been previously suggested in experiments with duck red cells (Starke and McManus, 1990).

Recently Jennings and Al-Rohil (1990) reported experiments in rabbit red cells which showed that the responses of [K-Cl] cotransport to volume perturbation were kinetically asymmetric. When cells were swollen by a sudden reduction in the tonicity of the bathing medium, there was a 10-min time lag before [K-Cl] cotransport was fully activated. In contrast, when preswollen cells were osmotically shrunken, the cotransporter was deactivated at a rate too rapid to measure. Jennings and Al-Rohil proposed a model for the on/off kinetics in which the rate of interconversion between active and inactive states of the transporter was described by the sum of forward and reverse rate constants, as outlined in the Methods section of this paper.

The stimulus for the experiments presented here was the notion that if Na/H exchange and [K-Cl] cotransport are indeed reciprocally regulated, and if the two systems respond oppositely to a perturbation of cell volume, then perhaps the Na/H exchange deactivation might exhibit the same delayed response to cell swelling as [K-Cl] cotransport activation. Conversely, the activation of Na/H exchange by cell shrinkage might proceed as rapidly as the inactivation of [K-Cl] cotransport.

A second objective of the present studies was to test the hypothesis of Jennings and Al-Rohil (1990) and Jennings and Schulz (1991) that protein phosphorylations mediate the activation and deactivation of transporters with changes in cell volume.

A partial account of our findings has been presented in abstract form (Parker, Colclasure, and McManus, 1991).

METHODS

Venous blood from four healthy mongrel dogs was drawn into heparinized vacuum tubes within 15 min of the start of each experiment. After centrifugation the plasma and buffy coat were discarded and the cells were washed as described in the figure legends. The composition of preincubation and incubation media is shown in Table I. In the experiments shown in Figs. 1–4, cells were preincubated for 15 min at 37°C in hypo- or hypertonic media, then centrifuged at room temperature and the supernate removed. The cell pellet was resuspended in a prewarmed (37°C) flux medium containing radioisotope. At each sampling time during the test incubation, a portion of the suspension was decanted into an ice-cold tube and immediately centrifuged (4°C) for 5 min. After removal of the supernate, the cells were washed twice in ice-cold isotonic medium, after which they were weighed and extracted with perchloric acid for

TABLE I
Wash and Incubation Media

	NaCl	KCl
	<i>mM</i>	
Hypotonic medium	80–90	4
Hypertonic medium	180–220	4
Isotonic medium	140	4

All solutions contained 5 mM glucose and were buffered with 10 mM hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) adjusted to pH 7.4 at 37°C by titration with tris(hydroxymethyl) aminomethane (Tris) base (HEPES-Tris).

determination of radioactivity, as previously described (Parker et al., 1990). The uptake of Na or K was estimated by dividing the cell radioactivity by the specific activity of the incubation medium.

Figs. 6 and 7 show experiments with the protein phosphatase inhibitor, okadaic acid, obtained from Moana Bioproducts, Inc., Honolulu, HI (lot 106).

Data describing the time course of activation/deactivation of transporters after abrupt changes in cell volume were analyzed by fitting them to the equation of Jennings and Al-Rohil (1990):

$$U = J_1 t + (J_1 - J_0)(T)(\exp[-t/T] - 1) \quad (1)$$

where U is the amount of ion taken up by the cells in millimoles per kilogram dry weight, J_0 is the steady-state flux before the volume perturbation, J_1 is the steady-state flux after the volume perturbation (both fluxes expressed as millimoles per kilogram dry weight per minute), t is the incubation time in minutes, and T is the relaxation time (equal to the reciprocal of the sum of the forward and reverse rate constants for the interconversion of the transporter between resting and active states).

RESULTS

The purpose of the experiments shown in Figs. 1–4 was to examine the time course of swelling-induced K flux and shrinkage-induced Na flux after activation or deactivation by an abrupt change in extracellular osmolality. Half the cells were preincubated in a hypertonic medium, and half in a hypotonic one, for 15 min. At the beginning of the flux period both aliquots of cells were resuspended in an isotope-containing medium that was either hypotonic (Figs. 1 and 4) or hypertonic (Figs. 2 and 3). Thus, in each experiment, half the cells (controls) were resuspended in the same medium to which they had previously been exposed, and the other half (test cells) were subjected to a sudden change in tonicity, and therefore cell volume. In Figs. 1 and 4 test cells were abruptly swollen after being shrunken during preincubation, while the control cells remained swollen during both the preincubation and test incubation. In Figs. 2 and 3 test cells were abruptly shrunken after being swollen during preincubation, while the control cells remained shrunken during both the preincubation and test incubation.

Fig. 1 shows that the delay in swelling-induced activation of [K-Cl] cotransport reported in rabbit red cells by Jennings and Al-Rohil (1990) also occurs in dog red cells. Cells were preincubated in hypo- or hypertonic media, then centrifuged and resuspended in a hypotonic medium that contained ^{86}Rb . Isotope uptake in the hypotonically preincubated control cells (*swell-swell*) was linear with time. If 2.5 min are added to each sampling time to correct for the time it took to separate cells from media, the course of isotope entry can be extrapolated through the origin. The hypertonically preincubated, abruptly swollen cells (*shrink-swell*) showed a gradual increase in isotope uptake rate before reaching a steady state. The lines through the points in Fig. 1 represent a fit of the equation derived by Jennings and Al-Rohil (1990) (see Methods). The curve describing the gradual activation of [K-Cl] cotransport in preshrunken cells has a T of 8 min, comparable to the value of 10.5 min for rabbit cells (Jennings and Al-Rohil, 1990).

The time course of deactivation of [K-Cl] cotransport is presented in Fig. 2. The preincubation of cells in hypo- vs. hypertonic media was the same as for the

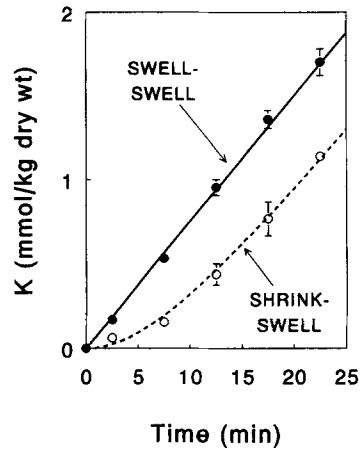


FIGURE 1. Activation of swelling-induced K flux. See Table I for details of media composition. Cells were washed twice with isotonic medium and divided into two lots. Half were resuspended in 15 vol of 180 mM NaCl hypertonic medium and half in 80 mM NaCl hypotonic medium, and then incubated for 15 min at 37°C. Both suspensions were centrifuged at room temperature, the supernates were removed, and the cell pellets were resuspended in 10 vol of 80 mM NaCl hypotonic medium prewarmed to 37°C. ^{86}Rb was then added to each flask at a final concentration of 1

$\mu\text{Ci/ml}$, a zero time sample was taken, and the suspension was sampled at 5-min intervals thereafter. Open circles and dashed line indicate hypertonic preincubation (*shrink-swell*); closed circles and solid line indicate hypotonic preincubation (*swell-swell*). The dashed line represents a fit of Eq. 1 with J_0 and J_1 equal to 0 and 0.075 mmol/kg dry weight per min, respectively, and $T = 8$ min. To correct for the time it took to separate cells from medium, 2.5 min were added to each sampling time. The solid line represents Eq. 1 with $T = 0$ min. Each point represents mean \pm SD for cells from four different dogs.

experiment shown in Fig. 1, but the flux measurements were carried out in a hypertonic medium to promote deactivation of [K-Cl] cotransport. The rates of ^{86}Rb entry in shrunken cells were almost unmeasurable at these time intervals (note the difference in vertical axis scale between Figs. 1 and 2), and there was no difference

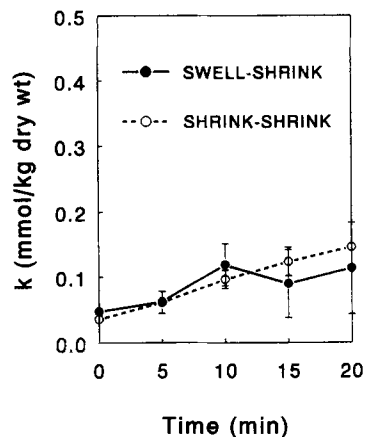


FIGURE 2. Deactivation of swelling-induced K transport. See Table I for details of media composition. Cells were washed twice with isotonic medium and divided into two lots. Half were resuspended in 15 vol of 190 mM NaCl hypertonic medium and half in 80 mM NaCl hypotonic medium, and then incubated for 15 min at 37°C. Both suspensions were centrifuged at room temperature, the supernates were removed, and the cell pellets were resuspended in 10 vol of 190 mM NaCl hypertonic medium prewarmed to 37°C. ^{86}Rb was then added to each flask at a final concentration of 1

$\mu\text{Ci/ml}$, a zero time sample was taken, and the suspension was sampled at 5-min intervals thereafter. Open circles and dashed line indicate hypertonic preincubation (*shrink-shrink*); closed circles and solid line indicate hypotonic preincubation (*swell-shrink*). The lines were drawn to connect the data points. Each point represents mean \pm SD for cells from four different dogs.

between the preswollen and preshrunk cells. Deactivation of [K-Cl] cotransport is therefore faster than can be measured by the techniques used here.

The time courses for activation and deactivation of Na/H exchange with cell shrinkage are shown in Figs. 3 and 4, respectively. The objectives, strategy, procedures, incubation times, and sampling times were exactly as described in the ^{86}Rb experiments (Figs. 1 and 2), although the tonicities of both hypo- and hypertonic media were set slightly higher. ^{22}Na entry into shrunken cells (Fig. 3) was ~ 10 times faster than in swollen cells (Fig. 4), as indicated by the different scales on the vertical axes.

Fig. 3 shows that cells shrunken during both preincubation and test periods (*shrink-shrink*) have a rapid initial rate of Na entry that appears to become progressively slower at each sampling interval, presumably due to the effect of backflux. By

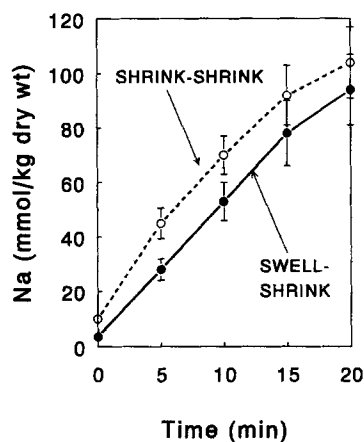


FIGURE 3. Activation of shrinkage-induced Na transport. See Table I for details of media composition. Cells were washed twice with isotonic medium and divided into two lots. Half the cells were resuspended in 15 vol of 220 mM NaCl hypertonic medium and half in 90 mM NaCl hypotonic medium, and then incubated for 15 min at 37°C. Both suspensions were centrifuged at room temperature, the supernates were removed, and the cell pellets were resuspended in 10 vol of 220 mM NaCl hypertonic medium prewarmed to 37°C. ^{22}Na was then added to each flask at a final

concentration of 1 $\mu\text{Ci/ml}$, a zero time sample was taken, and the suspension was sampled at 5-min intervals thereafter. Open circles and dashed line indicate hypertonic preincubation (*shrink-shrink*); closed circles and solid line indicate hypotonic preincubation (*swell-shrink*). The lines were drawn to connect the data points. Each point represents mean \pm SD for cells from four different dogs.

the end of the flux period ^{22}Na specific activity in the cells is 25–30% that of the external medium. Cells swollen during the preincubation period and then abruptly shrunken during the test period (*swell-shrink*) showed consistently lower values for Na uptake during the early time points, but the rate of isotope uptake did not increase with time. Thus, whereas the turning on of [K-Cl] cotransport with abrupt cell swelling showed a time lag (Fig. 1), the turning on of Na/H exchange with abrupt cell shrinkage had occurred by the time the first sample was taken (Fig. 3). Previous studies with other cell types have shown a characteristic time lag between the initial osmotic shrinkage and activation of Na/H exchange, ranging from 20–30 s (Cassel, Whiteley, Zhuang, and Glaser, 1985; Grinstein, Rothstein, and Cohen, 1985) to 5–25 min (Siebens and Kregenow, 1985). The data shown in Fig. 3 suggest that the activation delay for abruptly shrunken dog red cells was too rapid to measure by the present methods.

Fig. 4 shows the time course for deactivation of shrinkage-induced Na transport. The preincubations were carried out with the same solutions used in Fig. 3. At the beginning of the test period, both the preshrunk and preswollen cells were resuspended in a hypotonic medium in order to turn off the Na/H exchanger. Fig. 4 shows a linear time course for Na uptake into the preswollen cells (*swell-swell*); the specific activity of the cell Na rose to only 2–3% of that in the external medium by the end of the test period, and the curve reflects the absence of appreciable backflux. Preshrunk cells showed a nonlinear time course for Na uptake after transfer to the hypotonic test medium (*shrink-swell*). The rapid initial flux decelerated during the first 10 min and became linear as the Na uptake rates of preswollen and preshrunk cells approached the same value.

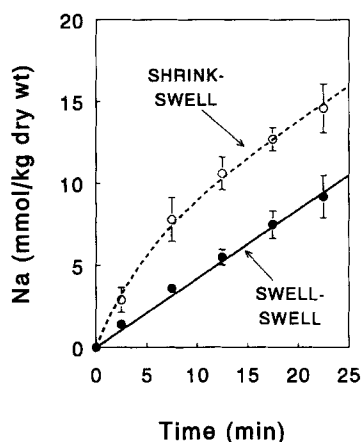


FIGURE 4. Deactivation of shrinkage-induced Na transport. See Table I for details of media composition. Cells were washed twice with isotonic medium and divided into two lots. Half were resuspended in 15 vol of 220 mM NaCl hypertonic medium and half in 90 mM NaCl hypotonic medium, and then incubated for 15 min at 37°C. Both suspensions were centrifuged at room temperature, the supernates were removed, and the cell pellets were resuspended in 10 vol of 90 mM NaCl hypotonic medium prewarmed to 37°C. ^{22}Na was then added to each flask at a final

concentration of 1 $\mu\text{Ci/ml}$, a zero time sample was taken, and the suspension was sampled at 5-min intervals thereafter. Open circles and dashed line indicate hypertonic preincubation (*shrink-swell*); closed circles and solid line indicate hypotonic preincubation (*swell-swell*). The dashed line represents a fit of Eq. 1 with J_0 and J_1 equal to 1.52 and 0.42 mmol/kg dry weight per min, respectively, and $T = 5$ min. To correct for the time it took to separate cells from medium, 2.5 min were added to each sampling time. The solid line represents Eq. 1 with $T = 0$ min. Each point represents mean \pm SD for cells from four different dogs.

The data points in Fig. 4 were fitted by Eq. 1 (Methods). The computed relaxation time for the swelling-induced deactivation of Na transport was ~ 5 min, comparable to the relaxation time for the swelling-induced activation of K transport (Fig. 1).

It is important to point out that although the brief preincubations in hypo- and hypertonic media activate [K-Cl] cotransport and Na/H exchange, respectively, the driving forces for *net* movement of ions under these circumstances are small, and there is no discernible change in cell water content after the 15-min preincubation periods. Fresh cells washed in HEPES-buffered 140 mM NaCl had $65.08 \pm \text{SD } 0.52\%$ water by weight. Cells preincubated in HEPES-buffered 80 or 220 mM NaCl for 15 min at 37°C and then washed twice with 140 mM NaCl had, respectively, 64.84 ± 0.58 and $65.10 \pm 0.43\%$ water ($n = 8$). Furthermore, if there were a significant gain

in solute, for example, after a 15-min preincubation in hypertonic NaCl, the consequences for cell volume would be such as to minimize the observed effects on the kinetics of activation and deactivation of the two transporters when transferred to hypotonic media.

Jennings and Al-Rohil (1990) showed that the activation of swelling-induced K transport was prolonged by treating cells with phosphatase inhibitors, such as vanadate and fluoride. In the experiment shown in Fig. 5, two aliquots of cells were preincubated in hypertonic media to activate Na/H exchange in the presence and absence of vanadate. At the beginning of the flux period each lot of cells was abruptly swollen. The vanadate-pretreated cells showed a slower rate of deactivation of Na transport than the control cells. Similar results were obtained with fluoride (not shown).

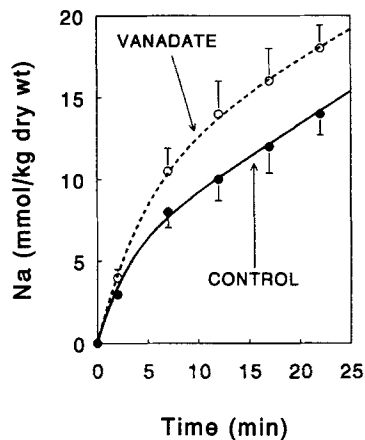


FIGURE 5. Effect of vanadate on the deactivation of shrinkage-induced Na transport. See Table I for details of media composition. The cells were washed twice with isotonic medium and divided into two lots. Each lot was preincubated for 15 min at 37°C in 15 vol of 220 mM NaCl hypertonic medium, with or without 0.5 mM sodium vanadate. Both suspensions were centrifuged at room temperature, the supernates were removed, and the cell pellets were resuspended in 10 vol of 90 mM NaCl hypotonic medium prewarmed to 37°C. ^{22}Na was then added to each flask at a final concentration

of 1 $\mu\text{Ci/ml}$, a zero time sample was taken, and the suspension was sampled at 5-min intervals thereafter. Open circles and dashed line indicate cells pretreated with vanadate; closed circles and solid line indicate controls. The lines represent a fit of Eq. 1 with J_0 and J_1 equal to 1.8 and 0.4 mmol/kg dry weight per min, respectively. T was 3 min for the solid line and 5 min for the dashed line. To correct for the time it took to separate cells from medium, 2 min were added to each sampling time. Each point represents mean \pm SD for cells from four different dogs in four paired experiments comparing vanadate with controls.

Recently, Jennings and Schulz (1991) reported that okadaic acid, a protein phosphatase inhibitor (Cohen, Holmes, and Tsukitani, 1990), affects [K-Cl] cotransport in rabbit red cells. The experiments shown in Figs. 6 and 7 examine its effect on dog red cells. When cells were incubated for 10 min in solutions of various osmolalities and then exposed to 300 nM okadaic acid for a further 10 min before measuring Na and K influx (Fig. 6), it was evident that the compound stimulated shrinkage-induced Na flux but inhibited swelling-induced K flux.

Among the effects of okadaic acid reported by Jennings and Schulz (1991) was a further delay of the swelling-induced activation of [K-Cl] cotransport. The experiment shown in Fig. 7 examines the effect of this agent on the kinetics of swelling-

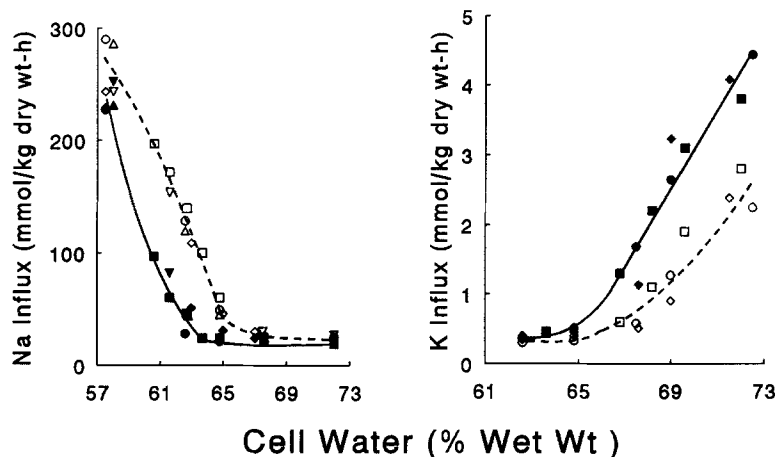


FIGURE 6. Na Influx (*left*) and K influx (*right*) in cells adjusted to different volumes by incubation in media of varying tonicity (NaCl content, see Table I), in the presence (*open symbols*) and absence (*filled symbols*) of okadaic acid. Fresh cells were washed twice with solutions of varying tonicity, resuspended in the same solutions at a cell/medium ratio of 1:10, and incubated for 10 min at 37°C. Each suspension was divided into two equal portions, to one of which okadaic acid was added at a final concentration of 300 nM (Jennings and Schulz, 1991). An equivalent volume of dimethylformamide was added to the controls. All suspensions were then incubated at 37°C for a further 10 min, and then isotope was added and the 30-min flux period was begun. Data for cells from four different dogs are plotted together. Curves were drawn by eye.

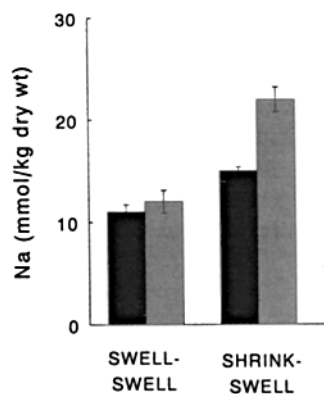


FIGURE 7. Okadaic acid effect on cells that were preswollen (*left-hand bars*) or abruptly swollen (*right-hand bars*). See Table I for details of media composition. Cells were washed twice with isotonic medium and divided into two lots. The cells designated swell-swell were preincubated for 15 min at 37°C in 15 vol of 90 mM NaCl hypotonic medium, with or without 300 nM okadaic acid. The cells designated shrink-swell were preincubated 15 min at 37°C in 15 vol of 220 mM hypertonic medium, with or without

300 nM okadaic acid. All suspensions were centrifuged at room temperature, the supernates were removed, and the cells were resuspended in 10 vol of 90 mM NaCl hypotonic medium prewarmed to 37°C. Cells preincubated with okadaic acid were reexposed to the agent during the test period. ^{22}Na was then added to each flask at a final concentration of 1 $\mu\text{Ci/ml}$, a zero time sample was taken, and the suspension was sampled again 30 min later. Solid bars represent control cells, and hatched bars represent cells exposed to okadaic acid. Results are mean \pm SD for studies on cells from four different dogs.

induced deactivation of Na/H exchange. Na uptake from a hypotonic medium into preswollen cells (*swell-swell*) was compared with uptake into cells that had been preshrunk (*shrink-swell*). As previously shown (Fig. 4), initial Na uptake into preswollen cells was slower than into preshrunk ones. Okadaic acid had little effect on Na flux in cells that had been preswollen (see also Fig. 6), but in cells that were preshrunk and abruptly swollen the delay in deactivation of Na/H exchange was accentuated by the phosphatase inhibitor.

Thus, okadaic acid has two effects: it stimulates the shrinkage-induced and inhibits the swelling-induced pathways at any given cell volume (Fig. 6), and it slows the rate at which cell swelling activates [K-Cl] cotransport (Jennings and Schulz, 1991) and deactivates Na/H exchange (Fig. 7).

DISCUSSION

Jennings and Al-Rohil (1990) have proposed a minimal two-state model for the on/off kinetics of swelling-induced [K-Cl] transport:



in which the rate of interconversion between off (A) and on (B) states is described by forward and reverse rate constants, k_{12} and k_{21} . When cells swell, the ratio k_{12}/k_{21} increases as more and more transporters become activated and K flux increases to a new steady state. The rate at which the system approaches the new steady state is given by the sum of the rate constants, $k_{12} + k_{21}$.

Jennings and Al-Rohil (1990) observed that the rate of approach of [K-Cl] cotransport to the higher steady-state flux after cell swelling (A to B in the above notation) was slower than the rate at which the transporter turned off after cells were taken back to a normal or shrunken volume (B to A). From reasoning based on principles of relaxation kinetics, they concluded that cell swelling must cause a decrease in k_{21} , the deactivation step, rather than an increase in k_{12} , the activation step. Phosphatase inhibitors (e.g., vanadate, okadaic acid) caused a slowing of the rate of increase in [K-Cl] cotransport with cell swelling. They therefore proposed that the forward rate constant, k_{12} , must reflect the rate of dephosphorylation of the regulator controlling transport, and that activation of [K-Cl] cotransport is accomplished by a net dephosphorylation of this system. Thus, the conversion of A to B would represent the action of a phosphatase, and B to A a kinase. Swelling is thought to inhibit the kinase (Jennings and Al-Rohil, 1990; Jennings and Schultz, 1991).

Our results on swelling-induced [K-Cl] cotransport are in total agreement with those of Jennings and Al-Rohil (1990). Moreover, we have shown that Na/H exchange, a shrinkage-activated system, has a slow rate of deactivation (made slower by vanadate and okadaic acid) and a fast rate of activation, which provides further support for the notion that these two systems are governed by a common regulator, perhaps in the manner suggested in Fig. 8.

According to this concept, cell shrinkage would promote an increase in the phosphorylated form (A) of the regulator, leading to activation of Na/H exchange and deactivation of [K-Cl] cotransport. Cell swelling would increase the dephos-

phorylated form (B), causing Na/H exchange to turn off and [K-Cl] cotransport to turn on. The rate at which swelling induces change is slow, and the rate at which shrinkage induces change is fast. The swelling-induced changes are further retarded by inhibition of protein phosphatases.

The findings in Fig. 6 can also be rationalized by this model (Fig. 8). Inhibition of dephosphorylation of the regulator by okadaic acid would shift the equilibrium between A and B in the direction of A, causing the cells to respond at any particular volume as though they were shrunken compared with controls without the inhibitor. Thus, at any shrunken volume Na/H exchange is stimulated, and at any swollen volume [K-Cl] cotransport is inhibited.

Whereas Grinstein et al. (1985) and Cassel, Katz, and Rotman (1986) have proposed that Na/H exchange is activated by a kinase, in agreement with our model (Fig. 8), Weinman, Steplock, Bui, Yuan, and Shenolikar (1990) find that this transporter is inhibited via the action of cAMP-dependent protein kinase. It is now recognized, however, that there are at least two distinct Na/H exchangers (Burns,

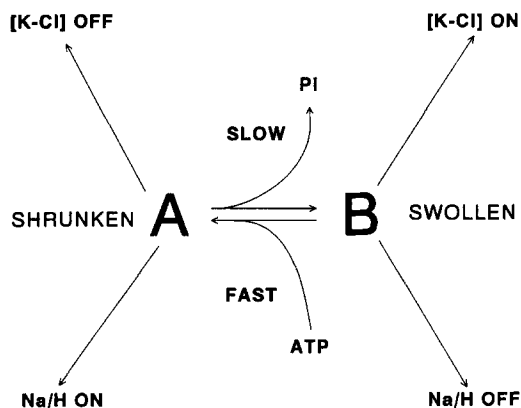


FIGURE 8. Model showing the postulated interconversion of the transport regulator between the A and B states. See text for discussion.

Homma, and Harris, 1990), which might be expected to have different functions and regulatory mechanisms. Altamirano, Breitwieser, and Russell (1988) reported that [Na-K-2Cl] cotransport, which is shrinkage activated in duck red cells (Schmidt and McManus, 1977), is also ATP dependent. Its deactivation after ATP removal from the intracellular perfusate is retarded by vanadate and fluoride. Recently, Lytle and Forbush (1990) presented direct evidence of shrinkage-induced phosphorylation of the [Na-K-2Cl] cotransporter in the shark rectal gland and postulated the existence of a kinase/phosphatase system sensitive to cell volume.

It is possible that there are at least two levels at which such a system might operate (See Cohen, 1985): one at the level of the regulator, as proposed here, and the other at the level of the transporters. Thus, one could envisage a cascade in which the primary volume-sensitive signal (Colclasure and Parker, 1991) acts on a kinase/phosphatase equilibrium at the level of the regulator (Fig. 8), which in turn might affect a second kinase/phosphatase system for which the transporters themselves are substrates. Direct phosphorylation of the transporter has recently been shown to

regulate the activity of Na/H exchange by growth factors (Sardet, Counillon, Franchi, and Pouyssegur, 1990). One could envision many alternative schemata involving phosphorylations and dephosphorylations at different steps of the volume perception–response pathway. It could be, for example, that the limiting step in the action of a phosphatase might be the rate at which an inhibitor of the phosphatase is neutralized by an ATP-dependent reaction (Shenolikar and Nairn, 1991).

Identification of volume-activated kinases and phosphatases and their substrates has not been accomplished in dog red cells, and the notion that reactions of this sort are involved in volume regulation remains inferential. However, these experiments, plus others recently reported (Parker et al., 1990) show a remarkable degree of coordination between swelling-induced and shrinkage-induced responses, which suggests that they share a common regulatory pathway.

The expert technical work of Paula S. Glosson is gratefully acknowledged.

This work was supported by NIH grant DK-11356-24 and a Hematology Research Fund donated by Mr. and Mrs. Thomas J. White, Jr.

Original version received 27 December 1990 and accepted version received 6 May 1991.

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