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The Kinetics of Sodium Transport in the Toad Urinary Bladder

III. The Role of Potassium

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Summary. The kinetics of sodium movement in the toad urinary bladder were determined as a function of the potassium concentration in the medium. Results indicated that (1) there is a linear relationship between net sodium transport and the measured rate coefficient for the serosal sodium efflux pump; this relationship is independent of serosal [K]; (2) changing potassium concentrations has no effect on sodium entry or exit at the mucosal border of the epithelium; (3) reduction in serosal [K] inhibits the serosal sodium pump, but elevation in $[K]_S$ up to 20 mM has no effect on the pump in the steady state; (4) changes in $[K]_S$ caused reciprocal changes in sodium pool size.

In the isolated urinary bladder of the toad, transepithelial sodium transport is the only energy-dependent ionic transfer mechanism under normal conditions. That is, when the bladder is bathed in identical media, the current required to clamp the spontaneous transepithelial potential difference at zero is carried across the bladder by sodium alone (Leaf, Anderson & Page, 1958). Although it seems clear from electrophysiological data (Gatzy & Clarkson, 1965; Leb, Hoshiko & Lindley, 1965) that the characteristics of the mucosal- and serosal-facing membranes lining the cells are quite different, useful information regarding fluxes across these boundaries has, until recently, been impossible to obtain. However, it is indeed possible to measure the fluxes of either sodium or potassium across these boundaries by utilization of an isotope washout technique and compartmental analysis (Finn & Rockoff, 1971; Finn & Nellans, 1972). In addition, we have been able to measure a moiety of sodium within the toad bladder epithelium which behaves as though it is the "transport pool". One characteristic of this system is that it appears to behave as a sodium-potassium exchange system, as originally suggested by Koefoed-Johnsen and Ussing (1958).

A number of studies has been conducted using cells separated from the epithelium by scraping in an attempt to gain further information with regard to the sodium transport system (Lipton & Edelman, 1971; MacKnight, DiBona, Leaf & Civan, 1971; Handler, Preston & Orloff, 1972), but these studies suffer the serious drawback that they require, of course, disruption of the epithelial system. To the extent that such disruption alters the function of the cells, and to the extent that the very properties of the intact epithelium depend upon the existence of oriented cells with tight junctions, such techniques may not yield useful or correct information about normal physiology. We have, therefore, continued our studies in an attempt to define the characteristics of the intact toad bladder epithelium.

The role of potassium in the sodium transport system is not entirely clear. Although it has been suggested that removal of potassium causes a decrease in the permeability of the mucosal border (Essig & Leaf, 1963), the studies on which this conclusion was based were open to a number of criticisms, and it has subsequently been suggested that removal of potassium must also affect the active transport step itself (Finn, 1970), as it does in a number of other well-studied systems (Hodgkin & Keynes, 1955; Hoffman, 1966). We therefore studied the effects of changes in potassium concentration on sodium transport kinetics with three questions in mind: (1) what is the role of potassium in the sodium transport system, (2) what is (are) the site(s) of action of potassium, and (3) are our current model and method adequate and correct?

Materials and Methods

The toads used in these studies were *Bufo marinus*, originating in Colombia, S.A., and obtained from the Pet Farm, Miami, Florida or the Tarpon Zoo, Tarpon Springs, Florida. The toads were kept in moistened San-i-cel (Paxton Processing Company, Paxton, Illinois) prior to use. The animals were pithed and the bladder dissected free and placed in Ringer's solution (composed, in mM, of NaCl 109, KCl 2.5, $CaCl_2$ 1.0, $NaHCO_3$ 2.4, and glucose 5.5; the pH was approximately 7.8 when gassed with air. When the potassium concentration was changed it was substituted for sodium on a mole-for-mole basis). The bladders were mounted in a chamber in which the media were stirred by rapidly rotating impellers, with separate entrance and exit ports on each side, as previously described (Finn & Rockoff, 1971), and the transepithelial potential automatically clamped at zero. No bladder was studied unless the spontaneous transepithelial potential difference was 40 mV or more. Following the establishment of a constant short-circuit current, ^{24}Na was added to the mucosal medium and washout studies were performed as described previously. Briefly, the tracer is allowed to remain in the chamber for at least 45 min, during which time nonradioactive Ringer's is continually pumped through the serosal chamber. At the conclusion of this loading period, nonradioactive Ringer's is pumped through both chambers at a rate of 38.2 ml/min per chamber (nominal pump rate) for 1 min. Previously, we have shown that this procedure removes virtually all of the loading solution from the chamber (Finn & Rockoff, 1971). After 1 min, the

flow rate is abruptly reduced to 7.8 ml/min per chamber, and all effluent is collected into test tubes mounted in a fraction collector. Fluid is collected from each side for 30-sec periods for 30 min and subsequently counted. The count rates are corrected for decay and analyzed by the methods of compartmental analysis.

After completion of a washout experiment, the potassium concentration in the serosal medium was changed (the sequence of changes in potassium concentration was varied in a random fashion), a new steady state of short-circuit current was achieved and another washout study was performed. Each bladder was studied at least twice and most often three times at different potassium concentrations. At the completion of each set of experiments, the bladder was punched out of its assembly with a cork borer, dried to constant weight at 80 °C, and the dry weight obtained.

Results

In these experiments potassium concentrations from 1 to 20 mM were used. We have previously shown (Finn, Handler & Orloff, 1967) that when the potassium concentration is reduced to zero, the p.d. and short-circuit

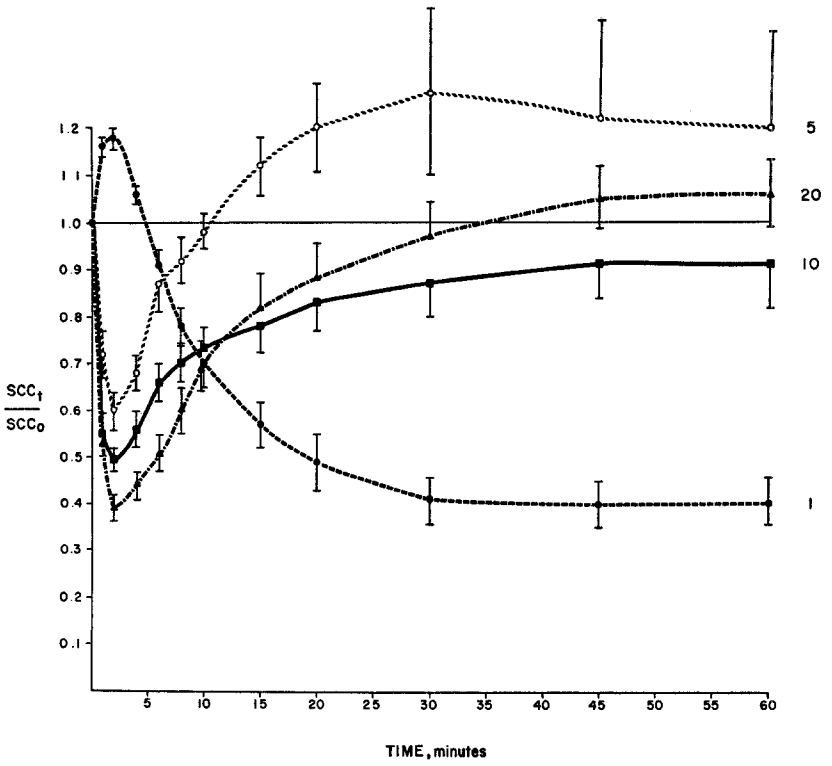

Fig. 1. Effect of changes in serosal potassium on short-circuit current. These data are obtained on the same preparations shown in Table 1. The currents are normalized to the value at zero time, when [K] was abruptly changed to the value shown at the right of each line. Bars represent SEM. Washout studies were done in each case after short-circuit current became stable

Table 1. Effects of alterations

	J_{net}	k_{S1}	A_1	k_{M1}	J_{1M}
					$K=2.5$ *vs.*
	0.557±0.058	0.052±0.006	14.16±1.46	0.308±0.024	4.93±0.55
Δ_{C-E}					
p_{diff}					
					$K=2.5$ *vs.*
	0.632±0.086	0.047±0.010	16.19±2.57	0.288±0.036	5.59±1.15
Δ_{C-E}					
p_{diff}					
					$K=2.5$ *vs.*
	0.448±0.036	0.036±0.005	18.72±2.62	0.337±0.019	6.84±1.13
Δ_{C-E}					
p_{diff}					
					$K=2.5$ *vs.*
	0.432±0.076	0.046±0.008	15.72±2.9	0.255±0.021	4.32±0.77
Δ_{C-E}					
p_{diff}					

The left-hand side of the Table gives the control values obtained ([K] = 2.5 mM) in each series. The right-hand side gives the values obtained in the same preparations following the indicated change in [K]. Each value is given as mean ± SEM. Data refer only to the first compartment (comp. 1; *see* Finn & Rockoff, 1971), where the J_{ij} or k_{ij} represent the flux (μEquiv × min^{-1} × 100 mg dry wt^{-1}) or rate coefficient (min^{-1}) for transport rate into comp. *i* from comp. *j*, and A_1 is the pool size (μEquiv × 100 mg dry wt^{-1}).

current reverse, and chloride appears to carry all of the current across the bladder in the mucosal-to-serosal direction. It was in order to avoid this complication that the lowest potassium concentration used in the present studies was 1 mM.

Reduction of serosal potassium concentration to 1 mM results in a transient increase in the short-circuit current and in a subsequent fall to a new steady-state value, as has been described previously (Fig. 1) (Sullivan, Tucker & Scherbenske, 1971; Mendoza, 1972). The values for the steady-state sodium kinetics are shown in Table 1. These data are from the faster of the two sodium compartments previously described (Finn & Rockoff, 1971); as shown then, none of the changes described in the experimental conditions, nor any of those described in this paper, had any

in medium K on Na kinetics

J_{net}	k_{S1}	A_1	k_{M1}	J_{1M}
$K=1.0$ (n=11)				
0.138±0.032	0.020±0.003	16.78±2.05	0.289±0.038	5.26±1.10
0.419±0.051	0.032±0.007	−2.63±0.94	0.019±0.043	−0.32±1.00
<0.01	<0.02	<0.02	NS	NS
$K=5.0$ (n=7)				
0.629±0.147	0.054±0.008	15.38±1.47	0.319±0.040	5.68±0.46
0.003±0.138	−0.007±0.014	0.82±1.22	−0.031±0.069	−0.09±1.12
NS	NS	NS	NS	NS
$K=10.0$ (n=6)				
0.399±0.083	0.043±0.014	15.47±2.42	0.333±0.039	5.85±0.98
0.048±0.104	−0.007±0.011	3.25±0.66	0.003±0.028	0.99±0.72
NS	NS	<0.01	NS	NS
$K=20.0$ (n=7)				
0.405±0.076	0.055±0.013	13.78±2.22	0.289±0.014	4.27±0.58
0.027±0.031	−0.008±0.009	1.95±0.61	−0.034±0.024	0.05±0.34
NS	NS	<0.01	NS	NS

M=mucosal medium, S=serosal medium. Values for the remaining J_{ij}(J_{M1}, J_{S1}, J_{1S}) are not given because they are calculated from the data given ($J_{ij}=k_{ij}A_i$, and $J_{net}=J_{1M}-J_{M1}=J_{S1}-J_{1S}$). Δ_{C-E}=difference between control and experimental values. p_{diff} represents the probability that these differences could occur by chance alone. NS=not significant. Mean SCC in all controls is equivalent to 21±3 μAmps×cm^{-2}. p.d. was 72±6 mV.

effect on the parameters of the slower pool. As indicated in this Table, there is a highly significant reduction in net sodium transport, in the rate coefficient for sodium efflux at the serosal side (the pump rate coefficient), and a significant increase in the sodium transport pool size. There is no change in the entry of sodium from the mucosal solution into the transport pool, nor is there any change in the rate coefficient for efflux at the mucosal side. Thus, reduction in potassium concentration affects net sodium transport solely by its effect on the rate coefficient for the pump.

The rise in the sodium transport pool following the reduction in the potassium concentration in the solution is, of course, not surprising, since one might expect that a reduction in the rate of egress of sodium from the pool into the serosal solution should lead to a rise in the content of the

sodium transport pool provided there is no effect of potassium change on the entry step at the mucosal boundary. As we have previously shown (Finn, 1973) a similar reduction of the serosal potassium concentration results in a decrease in the size of the potassium pool, and the magnitude of this change is approximately equal to the rise in sodium pool content seen here.

Studies were next done to test the effect of elevation in potassium concentration on the sodium transport kinetics. Previous observations, both by us (Finn, 1973; Finn & Krug, 1973) and by others (Sullivan *et al.*, 1971; Mendoza, 1972) have suggested that a rise in external potassium concentration may result in an elevation in sodium transport as measured by the short-circuit current. Although we have seen such a rise on several occasions, the overall steady-state change in short-circuit current was not significant, as shown in Table 1 and Fig. 1, even when serosal [K] was raised to as high as 20 mM.

Furthermore, as shown in Table 1, elevation of serosal [K] has no significant effect on the fluxes or rate coefficients for sodium movement at either border in the steady state, although there is a clear-cut reciprocal effect on the sodium pool size.

Discussion

In these studies we have explored some of the effects of changes in serosal potassium concentration on sodium kinetics. However, it should be pointed out that all of the washout studies are performed after the short-circuit current reaches a stable value, that is, after 30 min or more following the change in serosal [K]. As shown in Fig. 1, however, there are large transients which peak uniformly at about 2 min. It may be that these transients are related to the sudden change in the potassium gradient across the serosal boundary, such that there is either a net gain (if serosal [K] is elevated) or a net loss (if serosal [K] is lowered) by the cells, accounting for the change in SCC. This does not, of course, account for the subsequent development of a new steady state. Recent evidence (H. Pour-Hassani and A. L. Finn, *unpublished*) indicates that the transepithelial mucosal to serosal sodium flux in fact follows closely the minute-to-minute change in the short-circuit current. Whether such a phenomenon is due to transient effects on the mucosal entry step or the serosal pump step has not yet been determined, since the present kinetic analyses, as stated, are performed when the short-circuit current has become constant.

Under such conditions, we have shown that a decrease in the potassium concentration in the serosal medium brings about a decrease in the unidi-

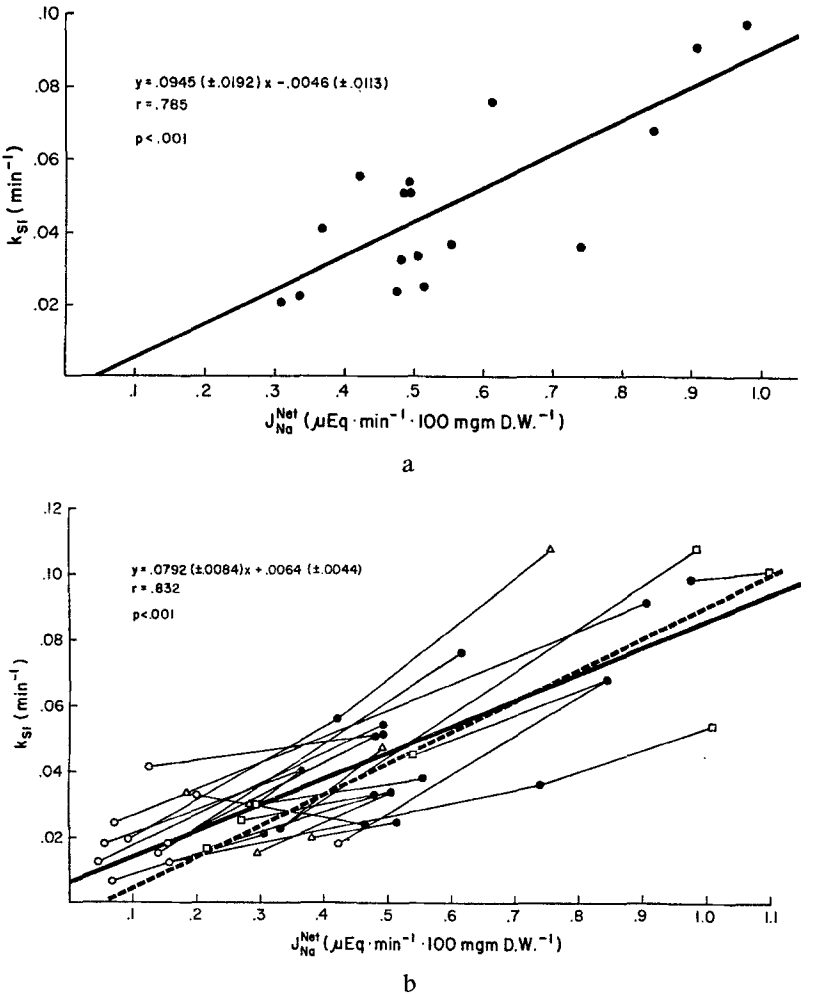

Fig. 2. Relationship between short-circuit current and pump rate coefficient. (*a*) Control values. Each point represents the result of a washout experiment in a single preparation. (*b*) Effect of changes in serosal [K]. The lines connect values obtained in a single bladder. •, control values ([K] = 2.5); these are the same data points shown in *a*. ○, [K] = 1.0, △, [K] = 5, □, [K] = 10. The dotted line is the control line, as shown in *a*; the solid line is the least-squares fit for all the data. The slopes and the intercepts are not significantly different in the two lines

rectional flux of sodium from the cells to the serosal medium. On the other hand, raising the concentration of potassium in the medium, although it produces a decrease in the size of the sodium transport pool, has no consistent effect on either the entry or exit of sodium in the steady state. To evaluate the nature of the interaction between potassium and sodium transport further, we have, in Fig. 2*a*, plotted the relationship between the

pump rate coefficient, k_{s1}, and net sodium transport, J_{Na}^{Net}, measured as the short-circuit current. The least-squares regression line is shown, and the highly significant correlation between these two independently determined measurements is obvious. Each point represents a single bladder; this relationship strongly supports our contention that we are indeed measuring the correct transport pool, for no other compartment would be expected to yield such results. Furthermore, Fig. 2*b* shows results on the same preparations, both before and after alterations in the serosal potassium concentration. Again, the least-squares fit is shown, and the line is not significantly different from that obtained in the control preparations. Thus, the effect of changes in serosal potassium and hence, as shown above, on sodium kinetics, does not disturb the relationship between the short-circuit current and the pump rate coefficient.

Recent results (Finn, 1973) have indicated that there is present at the serosal border a potassium-potassium exchange system which appears to substitute for the sodium-potassium exchange system when ouabain is present in the bathing medium, and that this system requires the presence of sodium in the mucosal solution. As shown here, on the other hand, alteration in serosal potassium concentration does not affect the steady-state sodium fluxes at the mucosal border. To determine whether there was any component of sodium-sodium exchange present, experiments were performed in which the washout of tracer sodium from the tissue was performed into a medium containing zero sodium concentration. As previously discussed (Finn, 1973), it is possible from these studies to determine the rate coefficient for exit even when there is a step change in the concentration of the ion in the bathing medium. These experiments revealed that there was no evidence for a component of mucosal sodium efflux that was dependent on the medium sodium concentration, at either high or low serosal potassium levels.

Finally, these data lead strong support to the method of determination of the sodium transport pool reported here. As stated above, the strongly positive correlation noted in Fig. 2 is consistent with such a model, since there is no *a priori* reason why the rate coefficient and the net sodium transport measured independently should be so strongly related unless indeed they are measuring the same phenomenon. For instance, if the pool which we were measuring had been, as stated by Zerahn (1969), already subjected to transport, then the measured unidirectional efflux rate coefficient would not be expected to be related to net transepithelial sodium transport. In fact, it should also be added that the changes in the sodium pool were quite similar, although, of course, opposite in direction, to those

seen in the tissue potassium pool following similar changes in potassium (Finn, 1973). It has so far been technically impossible to make measurements of the sodium and potassium transport pool simultaneously, so that a comparison of these two kinds of experiments is not entirely valid. Nonetheless, reduction of external potassium to 1 mM brought about an increase in the sodium pool of about 2.6 μEquiv × 100 mg dry wt^{-1}, and in separate experiments, a decrease in the potassium pool of about 3 μEquiv × 100 mg dry wt^{-1}. Similarly, the increase in potassium concentration to 5 or 10 mM brought about again approximately equal decrements in the sodium transport pool and increments in the potassium pool.

Thus, we have now shown that the sodium efflux at the serosal side is not only dependent upon the sodium concentration on the mucosal side (Finn, 1971), but also on the potassium concentration on the serosal side, that normal uptake of potassium at the serosal border requires the presence of sodium in the mucosal medium (Finn & Nellans, 1972), and, further, that these data are consistent with the operation of a one-for-one linked sodium-potassium exchange system (Finn, 1971; Finn & Nellans, 1972; *present observations*) at the serosal border as originally proposed by Koefoed-Johnsen and Ussing (1958). Two additional findings, however, suggest that this mechanism is not entirely adequate to describe the system. The first is, as discussed above, the existence of a ouabain-dependent potassium-sensitive potassium efflux mechanism at the serosal side (Finn, 1973). The second is recent evidence that ouabain inhibits not only the serosal sodium efflux pump mechanism, but also has an inhibitory effect on sodium uptake at the mucosal medium under certain circumstances (Biber, 1971; Finn, *unpublished data*).

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