The Role of Potassium in Active Transport of Sodium by the Toad Bladder

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ABSTRACT Studies were carried out on the isolated urinary bladder of the toad, *Bufo marinus*, in order to explain the dependence of active sodium transport on the presence of potassium in the serosal medium. Attempts to obtain evidence for coupled sodium-potassium transport by the serosal pump were unsuccessful; no relation between sodium transport and uptake of K^{42} from the serosal medium was demonstrable. Rather, the predominant effect of serosal potassium appeared to be operative at the mucosal permeability barrier, influencing the permeability of this surface to sodium. The mucosal effects of serosal potassium were correlated with effects on cellular cation content. When sodium Ringer's solution was used as serosal medium, removal of potassium resulted in significant decrease in tissue potassium content, commensurate increase in tissue sodium content, and marked depression of mucosal permeability and sodium transport. When choline replaced sodium in the serosal medium, removal of potassium resulted in only slight alterations of tissue electrolyte content, and effects on mucosal permeability and sodium transport were minimal.

INTRODUCTION

Among the concepts prominently advanced to explain how most animal cells maintain high concentration gradients of sodium and potassium ions, despite appreciable leakage of both, is that of coupled sodium-potassium transport. Thus it has been suggested that the high potassium and low sodium content of red blood cells (1, 2), nerve cells (3), and muscle cells (4, 5) is the consequence of associated transport of potassium into cells and of sodium out of cells, both processes possibly sharing a single carrier system (2, 6).

Koefoed-Johnsen and Ussing, in a unifying generalization, have extended this concept to polar tissues, pointing out that appropriately situated highly selective membranes could operate in conjunction with such coupled sodium-potassium exchange to effect net transport of ions across cells from one surface to the other (7). Thus they have proposed that active transport of sodium ions

by the epithelial cells of the frog skin from its outer to its inner surface may involve diffusion through the outward-facing cell membrane, selectively permeable to sodium, followed by active transport across the inner border by action of a "sodium pump." The latter process is thought to be associated with active uptake of potassium, which later leaks out of the cell through the inner border, selectively permeable to potassium. Since, according to this scheme, each sodium ion which is ejected is replaced by a potassium ion, there is no separation of electrical charge by the pump; rather, the electrical potential generated by the frog skin is accounted for as the sum of a sodium diffusion potential at the outer surface and a potassium diffusion potential at the inner surface of the cell.

The histologically simpler toad bladder, comprising a single layer of epithelial cells supported by a fine layer of connective tissue, resembles the frog skin in several important functional characteristics (8). In particular the toad bladder shares the ability of the frog skin to transport sodium actively from its outer (urinary or mucosal) surface across its inner (body or serosal) surface. As in the case of the frog skin, this process is dependent on the presence of potassium in the fluid bathing its inner surface (9). The experiments to be described were carried out in order to test the possibility of coupled sodium-potassium transport in this representative polar tissue.

METHODS

The techniques employed here have been described previously (8, 10).

In vitro studies were carried out on paired halves of the urinary bladder of the toad, *Bufo marinus*. The membranes were mounted in lucite chambers, as described by Ussing and Zerahn (10), and bathed on each side by 15 ml of appropriate medium. Potassium concentration of serosal medium was 3 to 4 meq per liter, except where otherwise specified. Low concentrations of serosal potassium were employed in the studies of Table III in order to minimize non-specific K^{42} labeling unassociated with sodium transport. Although short-circuit current values were depressed by the low potassium media, they remained appreciable throughout these short experiments.

In the mucosal Na^{22} labeling experiments tissues were exposed to isotope for a period of 60 minutes. The half-time of equilibration of tissue with mucosal isotopic sodium has been previously shown to range from 1.0 to 4.6 minutes (11).

Sodium transport was evaluated by measurement of short-circuit current (8). Previous studies have demonstrated that when either sodium Ringer's solution or choline Ringer's solution containing usual concentrations of potassium is used as serosal medium, short-circuit current is equivalent to the net transfer of sodium from the mucosal to the serosal medium (8, 11). Although precise quantification is difficult with the low rates of sodium transport associated with absence of potassium from the serosal medium, isotope flux studies following adequate periods for equilibration indicate again that short-circuit current approximates net sodium transport (12).

Sodium and potassium content of media and tissues was determined with a Baird-

Atomic flame photometer (Cambridge), and chloride was measured by the method of Sanderson (13).

K^{42} was counted in a Baird-Atomic well scintillation counter (Cambridge) and Na^{22} in an Auto-gamma spectrometer (Packard Instruments Company, La Grange, Illinois).

K^{42} was prepared on the reactor of the Massachusetts Institute of Technology and provided by Iso/Serve, Inc. (Boston). Na^{22} was obtained from Nuclear Science and Engineering Corporation (Pittsburgh).

Water content of the blotted toad bladder was considered to be equal to the loss in weight occurring after drying at 95°C for 24 hours.

Tissue uptake of radioactivity has been expressed as percentage labeling of tissue or tissue water,

$$\frac{\text{Tissue concentration of radioactivity}}{\text{Medium concentration of radioactivity}} \times 100.$$

In order to express sodium transport in units comparable to percentage tissue labeling despite variable degrees of stretching in mounting the tissues, short-circuit current is normalized in terms of 100 mg of bladder rather than in terms of unit area of preparation. The statistics of the measurements are not affected significantly by this convention.

Experimental determinations in paired half-bladders were compared by partial analysis of variance.

RESULTS

Previous work in this laboratory has demonstrated that active transport of sodium across the epithelium of the toad bladder involves penetration of a highly selective mucosal permeability barrier, presumably a passive process, followed by transport against an electrochemical potential gradient at the serosal surface (11, 14). Removal of potassium from the sodium Ringer's solution bathing the serosal surface of the bladder reversibly abolishes sodium transport (9). Dependence of sodium transport on the presence of potassium in the serosal medium suggested a requirement for potassium by the serosal pump, and existence of a coupled sodium-potassium pump seemed likely in view of the above-cited studies in several types of tissue (1–7).

A correlation between rates of potassium uptake and sodium efflux has been demonstrated in several types of non-polar tissue when sodium content (and sodium efflux) are altered by variation of incubation media (2–4). Such correlation has been cited as evidence for coupling of sodium and potassium transport in these tissues. Table I summarizes results of analogous experiments with the toad bladder. Both surfaces of the control half-bladder were exposed to sodium Ringer's solution, while choline replaced sodium in the media of the experimental half-bladder. After a suitable interval for equilibration,

tracer amounts of K^{42} were added to the serosal medium and a 30 minute period was allowed for tissue labeling. As is shown in Table I, removal of sodium from the media bathing both surfaces of the toad bladder, with consequent marked decrease in short-circuit current, does in fact result in highly significant decrease in the 30 minute uptake of K^{42} from the serosal medium, in keeping with the above-cited observations in non-polar tissues.

TABLE I

NET SODIUM TRANSPORT AND 30 MINUTE UPTAKE OF SEROSAL K^{42}; EFFECT OF ABSENCE OF SODIUM FROM THE SOLUTION BATHING BOTH SURFACES OF THE TOAD BLADDER

	Sodium transport (μa/100 mg) Solution		K^{42} labeling (per cent) Solution	
	Sodium Ringer's	Choline Ringer's	Sodium Ringer's	Choline Ringer's
	207	23	383	199
	142	0	255	157
	280	2	418	161
	405	18	599	171
	57	0	344	173
	118	19	435	166
	156	60	596	415
Mean	195	17	433	206
$\Delta \pm$S.E.M.	-178 ± 44		-227 ± 40	
p	<0.01		<0.01	

Each surface of the control half-bladder was exposed to 15 ml of sodium Ringer's solution (8). Choline replaced sodium in the media bathing the experimental half-bladder. Following a period of 5 to 25 minutes for equilibration, tracer amounts of K^{42} were added to the serosal medium. Sodium transport is evaluated by the average short-circuit current during the 30 minutes of exposure to K^{42}.

That this decrement in tissue K^{42} labeling is not, however, the consequence of depression of sodium transport *per se* can be shown by experiments in which sodium is removed only from the mucosal medium, while the serosal surface of the bladder is exposed to standard sodium Ringer's solution. Under these circumstances, although removal of mucosal sodium results in marked depression of sodium transport, no effect on 30 minute K^{42} uptake is demonstrable (Table II).

There remains the possibility that sodium transport might promote uptake of potassium into a superficial pool, small in magnitude relative to tissue pools not involved in coupled exchange, and hence undemonstrable in 30 minute studies. For this reason the effect of variation of the rate of sodium transport on 5 minute uptake of K^{42} was studied. As above, sodium transport was altered by variation of the sodium content of only the mucosal medium

while the serosal surface of the bladder was exposed to sodium Ringer's solution. In order to accentuate the difference in sodium transport by the two tissues, vasopressin was added to the serosal medium of the control half-bladder (15). As is shown in Table III, despite significant differences in sodium transport, again no effect on K^{42} uptake was demonstrable.

TABLE II

EFFECT OF ABSENCE OF MUCOSAL SODIUM ON NET SODIUM TRANSPORT AND 30 MINUTE UPTAKE OF SEROSAL K^{42}

	Sodium transport (μa/100 mg) Mucosal solution		K^{42} labeling (per cent) Mucosal solution	
	Sodium Ringer's	Choline Ringer's	Sodium Ringer's	Choline Ringer's
	130	24	362	395
	380	56	612	219
	119	11	350	260
	163	19	362	371
	54	18	377	431
	38	24	353	386
	165	21	302	306
	354	60	405	412
	92	17	243	221
	243	34	350	297
Mean	174	28	372	330
$\Delta \pm$S.E.M.	-146 ± 33		-42 ± 41	
p	<0.001		<0.3	

Both tissues were exposed to sodium Ringer's solution on their serosal surfaces. The mucosal medium of one half-bladder was sodium Ringer's solution, while that of the paired half-bladder was choline Ringer's solution. Following a period of 5 to 25 minutes for equilibration, tracer amounts of K^{42} were added to the serosal medium. Sodium transport is evaluated as in Table I.

Previous K^{42} uptake studies in sodium Ringer's solution have demonstrated a "fast" pool of some 20 per cent of total intracellular potassium with a half-time of equilibration of about 10 minutes (12). An approximate value for potassium influx may therefore be calculated from the percentage of tissue labeling in 5 minutes, neglecting slight back diffusion occurring during this period. The quotient of associated increments in potassium and sodium transport then gives a value for the coupling ratio; as is shown in the last column of Table III, such values are much less than 1.

Finding no evidence for rigid coupling, the possibility was considered that the predominant effect of serosal potassium was operative not at the pump site, but at the mucosal boundary, influencing the permeability of this surface to sodium. In order to evaluate this possibility a study was made of the in-

fluence of serosal potassium on tissue labeling with radioactive sodium from the mucosal medium. Paired half-bladders were exposed on their mucosal surface to sodium Ringer's solution containing Na^{22}. The serosal surface of the control half-bladder was exposed to sodium Ringer's solution, while that of the experimental half-bladder was exposed to sodium Ringer's solution lacking potassium. As can be seen in Table IV, removal of potassium from

TABLE III

RELATIONSHIP OF NET SODIUM TRANSPORT AND 5 MINUTE UPTAKE OF SEROSAL K^{42}

Mucosal solution	Sodium transport	Sodium transport	K^{42} labeling	Serosal potassium concentration	Potassium influx	Δ Potassium influx / Δ Sodium transport
	(μa/100 mg)	(μeq/100 mg/5 min.)	(per cent)	(meq/liter)	(μeq/100 mg/5 min.)	
Choline Ringer's	14	0.044	136	0.56	0.076	0.08
Sodium Ringer's	83	0.26	188	0.50	0.094	
Choline Ringer's	10	0.031	162	0.32	0.052	−0.04
Sodium Ringer's	194	0.60	100	0.32	0.032	
Choline Ringer's	15	0.047	85	0.35	0.030	0.19
Sodium Ringer's	126	0.39	280	0.34	0.095	
Choline Ringer's	15	0.047	143	0.28	0.040	−0.03
Sodium Ringer's	156	0.49	104	0.26	0.027	
Choline Ringer's	73	0.23	179	0.27	0.048	−0.05
Sodium Ringer's	103	0.32	165	0.26	0.043	
Choline Ringer's	38	0.12	143	0.21	0.030	0.00
Sodium Ringer's	107	0.33	150	0.20	0.030	
Mean	27 128		141 164			
Δ±S.E.M.	101±23		23±38			
p	<0.01		<0.5			

Both tissues were exposed to sodium Ringer's solution on their serosal surfaces. After 5 to 20 minutes for equilibration, 1 unit of vasopressin (Parke Davis and Company Pitressin, 20 units per ml) was added to the 15 ml of serosal medium of the "sodium" half-bladder; 7 to 10 minutes later, tracer amounts of K^{42} were added to the serosal medium. The values for sodium transport represent average values during the 5 minutes of exposure to K^{42}.

the serosal medium resulted regularly in a marked diminution in short-circuit current. If diminution of short-circuit current is solely the consequence of inhibition of the serosal pump, it would be anticipated that sodium derived from the mucosal medium would accumulate in the tissue, and Na^{22} labeling would increase. If, on the other hand, the predominant effect of removal of serosal potassium on short-circuit current is mediated at the mucosal surface, depressing the permeability of this surface to sodium, it would be anticipated that labeling with Na^{22} from the mucosal medium would decrease. As is

shown in Table IV, in every instance the half-bladder which had been exposed to potassium-free Ringer's solution was labeled to a lesser degree than the control tissue, the difference being highly significant.

TABLE IV

EFFECT OF SEROSAL POTASSIUM ON NET SODIUM TRANSPORT AND LABELING BY MUCOSAL Na^{22}

Serosal medium, sodium Ringer's solution

	Sodium transport (μa/100 mg) Potassium		Na^{22} labeling (per cent tissue water) Potassium	
	Present	Absent	Present	Absent
	181	0	3.6	1.8
	148	0	3.3	1.7
	210	13	5.2	1.7
	230	0	3.9	1.8
	214	19	4.4	1.9
	446	12	5.5	2.2
	335	12	4.1	2.9
	512	19	8.8	3.5
	640	40	8.7	2.8
	502	72	3.3	2.3
	500	42	7.4	3.1
	817	28	13.2	3.9
Mean	395	21	5.95	2.47
$\Delta \pm$S.E.M.	-374 ± 57		-3.48 ± 0.77	
p	<0.001		<0.001	

Mucosal medium was sodium Ringer's solution. Serosal medium of the control half-bladder was sodium Ringer's solution containing 3.88 meq per liter of potassium. Serosal medium of the experimental half-bladder was sodium Ringer's solution containing no potassium. Prior to addition of Na^{22}, tissues were equilibrated for a period of time sufficient for short-circuit current of the "K-free" half-bladder to fall to less than 20 per cent of its initial value (generally 15 to 60 minutes). The mucosal surfaces of both half-bladders were then exposed to tracer amounts of Na^{22} for 60 minutes. The final potassium concentration of the serosal medium of the experimental half-bladder was measured for each experiment and was always less than 0.24 meq per liter. Sodium transport is evaluated by the final short-circuit current.

The question arises how removal of potassium from the *serosal* medium can influence permeability of the *mucosal* surface of the toad bladder. The serosal surface of the toad bladder is highly permeable to potassium, and, as can be seen from Table V, removal of potassium from the serosal medium results in significant alterations of intracellular electrolyte concentrations, with an average decrement of tissue potassium content of 16.6 meq per liter and increment of tissue sodium content of 17.1 meq per liter. No change in tissue chloride concentration or in tissue water was noted; thus there was no change in total tissue electrolyte content or osmotic activity.

TABLE V

EFFECT OF SEROSAL POTASSIUM ON ELECTROLYTE CONTENT OF THE TOAD BLADDER

Serosal medium, sodium Ringer's solution

	K (μeq/gm tissue water) Potassium		Na (μeq/gm tissue water) Potassium		Cl (μeq/gm tissue water) Potassium	
	Present	Absent	Present	Absent	Present	Absent
	76.6	57.9	117.1	130.0	88.0	87.0
	63.8	48.8	109.0	117.9	94.6	95.5
	69.9	58.9	109.6	134.7	100.5	98.0
	76.1	43.0	116.5	139.3	92.1	94.9
	78.1	66.7	97.8	115.7	93.0	89.2
	72.7	59.3	106.3	113.6	81.3	86.7
	64.7	45.7	101.3	122.2	84.6	86.0
	73.8	62.5	97.2	118.6	91.4	81.7
Mean	72.0	55.4	106.9	124.0	90.7	89.9
Δ±S.E.M.	−16.6±3.3		17.1±2.4		−0.8±1.64	
p	<0.01		<0.001		>0.6	

The tissues analyzed were those of the first eight experiments of Table IV.

TABLE VI

EFFECT OF SEROSAL POTASSIUM ON ELECTROLYTE CONTENT OF THE TOAD BLADDER

Serosal medium, choline Ringer's solution

	K (μeq/gm tissue water) Potassium		Na (μeq/gm tissue water) Potassium		Cl (μeq/gm tissue water) Potassium	
	Present	Absent	Present	Absent	Present	Absent
	81.8	61.1	38.1	16.9	88.8	102.6
	66.1	68.7	11.0	9.8		
	65.6	59.3	12.6	23.9		
	71.1	60.2	20.9	15.2	80.6	91.5
	77.2	69.4	18.2	23.9	82.0	90.0
	66.5	61.0	18.2	30.1	84.9	73.7
	82.1	69.3	19.4	13.1	82.0	91.9
	78.4	80.3	19.0	14.1	86.3	83.5
	91.2	83.3	35.0	20.8	91.0	94.8
	71.3	73.8	22.0	21.4	95.3	90.2
Mean	75.1	68.6	21.4	18.9	86.4	89.8
Δ±S.E.M.	−6.5±2.4		−2.5±3.3		3.4±3.1	
p	<0.05		>0.4		>0.3	

Conditions as described in Table IV, except for replacement of sodium in the serosal medium by choline.

The tissues analyzed were those of the experiments described in Table VII.

That the alterations in tissue electrolyte content account for the effects of potassium removal on mucosal permeability, and hence sodium transport, is suggested by studies in which the serosal medium contains choline in place of sodium. Under these circumstances removal of potassium from the serosal medium causes only minimal alterations in tissue potassium and sodium content (Table VI), and again no change in chloride or water content. As is

TABLE VII

EFFECT OF SEROSAL POTASSIUM ON NET SODIUM TRANSPORT AND LABELING BY MUCOSAL Na^{22}

Serosal medium, choline Ringer's solution

	Sodium transport (μa/100 mg) Potassium		Na^{22} labeling (per cent tissue water) Potassium	
	Present	Absent	Present	Absent
	359	48	13.2	3.3
	144	96	4.8	3.5
	133	70	3.9	7.9
	255	110	4.8	4.3
	211	151	7.1	5.2
	205	186	6.5	3.9
	203	93	7.7	5.4
	218	139	5.0	4.2
	350	310	6.1	5.1
	434	220	9.5	4.9
Mean	251	143	6.84	4.76
$\Delta \pm$S.E.M.	-108 ± 29		-2.08 ± 1.12	
p	<0.01		>0.05	

Conditions as described in Table IV, except for replacement of sodium in the serosal medium by choline. Prior to addition of Na^{22}, tissues were equilibrated for 15 to 75 minutes, with subsequent exposure to Na^{22} for 60 minutes as in Table IV. The final potassium concentration of the serosal medium of the experimental half-bladder was measured for each experiment and was always less than 0.20 meq per liter. Sodium transport is evaluated as in Table IV.

shown in Table VII, effects on tissue Na^{22} labeling are then not quite significant at the 5 per cent level, and short-circuit current is affected to a much lesser degree than in the presence of serosal sodium.

DISCUSSION

The use of a relatively simple polar tissue for studies of the interrelations of sodium and potassium transport provides certain important advantages over experiments in non-polar tissues. Firstly, the possibility of varying independently the media contacting the opposite surfaces of the toad bladder permits evaluation of the effects on potassium transport of variation of sodium transport, *per se*, as against non-specific effects associated with complete removal of sodium from bathing media. Secondly, spatial separation of the

sites of passive (mucosal) and active (serosal) sodium transport permits an approach to evaluation of the site of major influence of potassium in the over-all transport process.

Although potassium uptake is depressed by complete removal of sodium from the media bathing the bladder, we were not able to demonstrate an influence of net sodium transport on potassium transport, as measured by 30 minute or 5 minute uptake of K^{42} from serosal medium in the presence of serosal sodium. No coupling was demonstrable; if indeed there is coupling of sodium and potassium transport at the pump site, the coupling either must be very loose or must involve only a minute fraction of total tissue potassium content.

Although evidence for linkage of sodium and potassium transport is impressive in several reported instances, such coupling must be a complex and variable process. Thus Hodgkin and Keynes, while citing evidence for coupling of secretory movement of sodium and potassium in giant axons of *Sepia* and *Loligo*, point out that the linkage cannot be rigid (3). Glynn reports both potassium-dependent and potassium-independent active sodium transport in red blood cells (1). The present experiments emphasize again the need for careful interpretation of the evidence for coupling in any given situation, particularly where experimental conditions are likely to produce poorly defined non-specific effects. Further, it is important to consider the possibility in any given instance that potassium dependence of active sodium transport might be a consequence of regulation by potassium of the permeability of a *passive* barrier to sodium movement, as seen in the present studies.

The observation that tissue labeling *decreases* in association with depression of sodium transport when potassium is removed from the serosal medium indicates that depression of mucosal permeability is quantitatively of greater significance than any postulated inhibition of the serosal pump, which would tend to *increase* tissue labeling. Greater depression of short-circuit current than of tissue labeling does not contradict this conclusion, for, lacking knowledge of the extent of mucosal adsorption of sodium, no conclusion can be drawn concerning the fractional decrement in the size of the sodium active transport pool.

The question arises whether potassium has any direct involvement whatever in the operation of the serosal sodium pump in the toad bladder, or whether its influence on sodium transport can be entirely explained on the basis of its mucosal effects. Though the present results suggest that there is no need to invoke an action of potassium at the pump site, the possibility exists that the mucosal effects noted here might be secondary phenomena. MacRobbie and Ussing have reported depression of permeability in the frog skin in association with putative inhibition of the pump by ouabain and/or low serosal pH, and suggest the possibility that any interference with the

pump mechanism may be associated with a decrease in passive permeabilities to ions (16). Preliminary experiments suggest that several metabolic inhibitors may depress permeability of the toad bladder, but to a much lesser extent than does removal of serosal potassium (12).

Although the present experiments do not conclusively rule out potassium dependence of the pump, they suggest that any such postulated dependence cannot be absolute, since net sodium transport after over 60 minutes of exposure to potassium-free choline serosal medium averages 56 per cent of that in the control half-bladders. (Although sodium is moving down a concentration gradient in these experiments, transport appears nevertheless to be active, for flux rates are far higher than are seen with passively permeating ions. Further work is in progress to establish the active nature of this process.)

An important consideration in attempts to relate sodium transport in the frog skin to activity of a coupled sodium-potassium pump has been the evidence for a potassium diffusion potential at the inner surface of this tissue (7). Recent studies in this laboratory indicate that such a potassium diffusion potential is not responsible for the potential jump at the serosal surface of the toad bladder (17).

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