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Effect of Sodium Transport Inhibition on Active Phosphate Transport by Toad Bladder

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Summary. The relationship between active Na transport (estimated by the short-circuit (SCC)) and active inorganic phosphate (P_i) transport was studied in the toad bladder. When SCC was inhibited by amiloride, ouabain, or removal of K from the serosal bathing solution, active P_i transport was totally inhibited. When Na was replaced isotonically by choline in either the mucosal bathing solution or both the mucosal and serosal bathing solutions, there was no measurable SCC or active P_i transport. These experiments are compatible with the hypothesis that active P_i transport occurs only in the presence of active Na transport.

Active transport of inorganic phosphate has recently been demonstrated in the urinary bladder of the toad, *Bufo marinus*. Parathyroid hormone inhibits bladder phosphate transport but has no detectable effect on sodium transport. On the other hand, increasing phosphate concentration in the solutions bathing the bladders increases phosphate transport without a detectable effect on sodium transport (Sellers *et al.*, 1977). In the present studies the effects of inhibition of sodium transport on bladder phosphate transport were examined.

Materials and Methods

Urinary hemibladders of the toad, *Bufo marinus*, of Colombian or Texas origin were mounted in plastic chambers as previously described. Two contiguous areas of the same hemibladder were studied. Both sides of the bladders were bathed in 4 ml of amphibian Ringer's solution containing (in mM) 90 NaCl, 25 $NaHCO_3$, 3 KCl, 1 $CaCl_2$, 0.5 KH_2PO_4, 0.5 $MgSO_4$, and 5.5 glucose. This solution was gassed with 97% O_2 and 3% CO_2, maintaining a pH of approximately 7.6. Osmolality of this solution was approximately 230 mosmol/Kg H_2O. Short-circuit current (SCC) was measured by modification of the method of Ussing and Zerahn (1951). The cross-sectional area of each bladder area studied was 3.2 cm^2.

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Bladders with initial potential differences (PD) less than 10 mV were discarded. Bladders were kept short-circuited continuously except for brief periods during which PD was measured. PD and SCC were measured every 15 min beginning at least 1 hr before the flux periods.

When the short-circuit current was stable, 10 μCi of carrier-free $KH_2{}^{32}PO_4$ were added to the mucosal bathing solution of one bladder area and to the serosal bathing solution of the contiguous bladder area. After 1 hr to allow for isotopic equilibration, the bathing solutions were sampled, and control phosphate flux was measured for two consecutive 45-min periods.

Following the two control periods, the bathing solutions were altered as described below. After 1 hr, phosphate flux was measured for two additional 45-min periods. We have previously reported that net phosphate flux does not change in control bladders during this time (Sellers *et al.*, 1977). These data are repeated in Table 1 for comparison.

The 50-μl samples were added to 7 ml of scintillation counting mixture (Toluene-Triton X-100, 3:1 with 4 g Omnifluor per liter) and counted in a Packard Model 3375 Liquid Scintillation Counter (Packard Instrument Company, Chicago, Ill.).

Net phosphate flux was calculated as the difference between the mucosal-to-serosal flux and the serosal-to-mucosal flux. Phosphate flux is expressed as nanomoles/cm^2/hr. For each bladder, the mean of the two control periods was compared with the mean of the two experimental periods. The data were evaluated using Student's *t*-test and are presented as the mean ± SE.

In a small number of experiments the concentration of isotope on the recipient side exceeded 0.6% of the concentration on the source side at the time of the first sample. These high initial recipient concentrations were usually associated with visible holes in the bladders at the end of the experiment. For this reason, all experiments in which such a high initial recipient side isotopic concentration occurred were discarded.

Short-Circuit Current Inhibition by Amiloride, Ouabain, or Removal of Potassium

Following the two control periods, the short-circuit currents of the bladders were altered by (i) addition of 10^{-4} M amiloride to the mucosal solutions, (ii) addition of 5×10^{-5} M ouabain to the serosal bathing solutions, or (iii) rinsing both bladder sides with a modified Ringer's solution in which KCl and KH_2PO_4 were replaced isotonically by NaCl and NaH_2PO_4. The bathing solutions were gassed with 97% O_2–3% CO_2. In the experiments with no potassium in the bathing solutions, ^{32}P was added as in control experiments. One hour later, phosphate fluxes were measured for two 45-min periods.

Removal of Sodium from the Bathing Solutions

Following the two control periods, the bathing solutions were altered by the following:

1) Both the mucosal and serosal sides of the bladders were rinsed three times with a modified Ringer's solution in which choline chloride and choline bicarbonate were substituted isotonically for NaCl and $NaHCO_3$, respectively.

2) The mucosal sides of the ox bladders were rinsed three times with choline Ringer's solution as described above. The serosal sides were rinsed three times with amphibian Ringer's solution as in controls.

The bathing solutions above were gassed with 97% O_2 and 3% CO_2. ^{32}P was added to the mucosal solution of one bladder area and to the serosal solution of the contiguous bladder area. The pH of both mucosal and serosal solutions was 7.6. After 1 hr, phosphate fluxes were measured for two 45-min periods. In a small number of experiments, following the last flux period, bladders were rinsed three times with amphibian Ringer's solution, and SCC was measured.

Results

Active transport of inorganic phosphate (P_i) was significantly inhibited by amiloride, ouabain, and potassium-free bathing solution (Table 1). Short-circuit current (SCC) was inhibited 75% by ouabain, 100% by amiloride, and 100% by potassium-free bathing solution.

After Na was replaced by choline in the serosal and mucosal bathing solutions, no statistically significant P_i transport occurred (Table 1). Na

Table 1

Method of SCC inhibition		Phosphate flux (nanomoles/cm²/h)		*P* Control *vs.* Experimental
		Control	Experimental	
None	$M \rightarrow S$	0.49 ± 0.11	0.64 ± 0.17	<0.01
($n=21$)	$S \rightarrow M$	0.20 ± 0.03	0.29 ± 0.03	<0.02
	Net	0.29 ± 0.11	0.35 ± 0.17	>0.5
		$P<0.025$	$P<0.05$	
Amiloride	$M \rightarrow S$	0.32 ± 0.07	0.34 ± 0.04	>0.5
($n=19$)	$S \rightarrow M$	0.18 ± 0.05	0.43 ± 0.12	<0.005
	Net	0.14 ± 0.05	-0.09 ± 0.07	<0.025
		$P<0.01$	$P>0.1$	
Ouabain	$M \rightarrow S$	0.37 ± 0.05	0.32 ± 0.05	>0.2
($n=24$)	$S \rightarrow M$	0.19 ± 0.03	0.43 ± 0.09	<0.005
	Net	0.18 ± 0.04	-0.11 ± 0.10	<0.005
		$P<0.001$	$P>0.2$	
Potassium-free	$M \rightarrow S$	0.76 ± 0.11	0.69 ± 0.09	>0.5
Ringer's solution	$S \rightarrow M$	0.37 ± 0.07	0.61 ± 0.10	<0.025
($n=20$)	Net	0.38 ± 0.13	0.08 ± 0.12	<0.005
		$P<0.005$	$P>0.5$	
Choline-For-Na	$M \rightarrow S$	0.44 ± 0.009	0.56 ± 0.09	>0.1
M and *S* ($n=21$)	$S \rightarrow M$	0.25 ± 0.04	0.45 ± 0.09	<0.005
	Net	0.19 ± 0.08	0.11 ± 0.10	>0.4
		$P<0.025$	$P>0.2$	
Choline-For-Na	$M \rightarrow S$	0.39 ± 0.11	0.31 ± 0.06	>0.4
$n=29$	$S \rightarrow M$	0.13 ± 0.02	0.41 ± 0.06	<0.001
	Net	0.26 ± 0.11	-0.10 ± 0.08	<0.005
		$P<0.025$	$P>0.2$	

n=number of bladders. Phosphate flux was measured for two control flux periods of 45 min each which began one hr after the addition of 10 μCi of $KH_2\ ^{32}PO_4$ to the source sides. The mean flux for these two periods was calculated for each bladder. SCC was then inhibited by addition of amiloride to the mucosal solutions or ouabain to the serosal solutions, removing potassium from mucosal and serosal solutions, or removal of Na from mucosal and serosal solutions or only mucosal solutions. One hour later, phosphate transport was measured for two additional 45-min flux periods. The mean flux for these two periods was calculated for each bladder. Data are expressed as mean ± SE.

transport as measured by SCC was completely inhibited. When Na was replaced by choline in the mucosal bathing solutions only, P_i transport was significantly inhibited (Table 1). In these experiments SCC fell to 0, and at the end of the experiments there was no measurable Na in the mucosal solutions. When the bladders in both experiments were rinsed with Na-containing Ringer's solution, SCC returned to normal levels. P_i transport was not measured after the Na-containing Ringer's solution was added.

In all experiments there was a statistically significant increase in $S \rightarrow M$ phosphate flux with no significant change in $M \rightarrow S$ phosphate flux.

This contrasts with the control experiments in which net phosphate flux was not significantly changed although both $M \rightarrow S$ and $S \rightarrow M$ phosphate flux rose significantly.

Discussion

The relationship between sodium (Na) and phosphate (P_i) reabsorption in the mammalian kidney has been studied for many years. Evidence supporting a link between Na and P_i reabsorption in the mammalian nephron has been reported by a number of investigators (Wen, 1974; Baumann *et al.*, 1975; Hoffman, Thees & Kinne, 1976; Ullrich *et al.*, 1977).

Since active transport of P_i also occurs in the toad urinary bladder (Sellers *et al.*, 1977), the relationship between Na and P_i reabsorption in this system was studied. In the toad bladder, parathyroid hormone (PTH) inhibited P_i transport but had no detectable effect on Na transport as measured by short-circuit current (SCC). In addition, P_i transport in the toad bladder increased with increasing P_i concentration in the bladder bathing solutions, (Seller *et al.*, 1977). The fact that PTH and increased P_i concentration in the bathing solutions altered P_i transport but had no detectable effect on Na transport did not rule out the Na-P_i transport link. Since in the toad bladder the magnitude of P_i transport is less than 1% of the Na transport, a large change in P_i transport could be associated with an undetectable change in the Na transport.

In the present studies, P_i transport was studied after inhibition of Na transport by several different means. When Na transport was inhibited by amiloride, ouabain, or K-free bathing solutions, there was no net transport of P_i. Although it is possible that each method of Na transport inhibition directly affected P_i transport, it seems more likely

that the inhibition of P_i transport was somehow related to the Na transport inhibition. The effects of ouabain on P_i transport in the toad bladder are in agreement with studies in dogs in which infusion of cardiac glycosides into the renal artery produced ipsilateral phosphaturia ((Kupfer& Kosovsky, 1970). To our knowledge, no data are available with regard to the effects of amiloride or K-free solutions on P_i excretion in mammals.

In addition, P_i transport was studied following replacement of Na in the bathing solutions with choline. No P_i transport was demonstrated when Na was replaced by choline in both mucosal and serosal bathing solutions. This inhibition of P_i transport was not caused by death of the bladders since SCC returned to control levels when Na was reintroduced to the solutions. Similarly, no P_i transport was demonstrated when choline replaced Na in the mucosal solutions only. This finding supports the concept that even in the presence of serosal Na, availability of Na at the mucosal surface is necessary for P_i transport.

The mechanism by which inhibition of Na transport interferes with P_i transport in the mammalian nephron is unknown. It is speculated that proximal tubular transport of P_i is driven by a sodium gradient (Ullrich *et al.*, 1977). In the toad bladder it is likely that $S \rightarrow M$ P_i flux occurs by passive movement and that $M \rightarrow S$ P_i flux represents the sum of passive movement and active transport. As reported previously, in control bladders both $M \rightarrow S$ and $S \rightarrow M$ P_i fluxes increased with time without a significant change in net P_i flux (Sellers *et al.*, 1977). Presumably this reflects an increase with time in passive movement of P_i across the toad bladder. Following inhibition of Na transport by ouabain, amiloride, zero K concentration, or by choline substitution, $S \rightarrow M$ P_i flux increased to an extent similar to that in the control experiments while $M \rightarrow S$ flux did not increase significantly. Since a change in passive movement of P_i across the bladder should affect the $M \rightarrow S$ and $S \rightarrow M$ fluxes equally, the increase in $S \rightarrow M$ P_i flux in the absence of a change in $M \rightarrow S$ P_i flux has been interpreted as inhibition of the active P_i transport along with an increase in passive P_i movement. This conclusion is supported by similar findings during inhibition of P_i transport by iodoacetate or anaerobiasis (Sellers *et al.*, 1977). Whether this inhibition of P_i transport is by inhibition of an electrogenic mechanism or by inhibition of an electroneutral cotransport mechanism is unknown.

Much of the evidence in the toad bladder thus far supports the concept that Na transport must be present for P_i transport to occur. In each condition studied, inhibition of Na transport resulted in inhibition

of P_i transport. This does not prove the existence of a Na-P_i linked transport system, but suggests that some dependence of P_i transport on Na transport may exist.

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References

Baumann, K., deRouffignac, C., Roinel, N., Rumrich, G., Ullrich, K.J. 1975. Renal phosphate transport: Inhomogeneity of local proximal transport rates and sodium dependence. *Pfluegers Arch.* **356**:287

Hoffmann, N., Thees, M., Kinne, R. 1976. Phosphate transport by isolated renal brush border vesicles. *Pfluegers Arch.* **362**:147

Kupfer, S., Kosovsky, J.D. 1970. Renal intracellular phosphate and phosphate excretion. *Mt. Sinai J. Med. N.Y.* **37**:359

Sellers, B.B., Hall, J.A., Booth, C.W., Mendoza, S.A. 1977. Active phosphate transport across the urinary bladder of the toad, *Bufo marinus*. *J. Membrane Biol.* **32**:291

Ullrich, K.J., Capasso, G., Rumrich, G., Papavassiliou, F., Kloss, S. 1977. Coupling between proximal tubular transport processes. *Pfluegers Arch.* **368**:245

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited frog skin. *Acta Physiol. Scand.* **23**:110

Wen, S.F. 1974. Micropuncture studies of phosphate transport in the proximal tubule of the dog. *J. Clin. Invest.* **53**:143