Relationship of Na Transport and K Excretion by the Urinary Bladder of *Bufo marinus*[1] (35234)

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In vivo studies by Kallus and Vanatta (1) indicate that the toad bladder excretes K. Leaf *et al.* (2) showed that the toad bladder reabsorbs Na. The present experiments were designed to test whether the K excretion was dependent on the sodium reabsorption. The rate of Na reabsorption was varied by lowering the Na concentration in the mucosal medium, and by stimulating the Na pump with vasopressin.

Materials and Methods. The source and the routine care of toads, instrumentation, solutions, and the source of isotopes were as previously described (1). Pitressin (from Parke, Davis and Co.) was used as vasopressin. Calomel electrodes immersed in Ringer solution were used in measuring the potential difference (PD) across the membrane with agar bridges between the electrode bath and the incubation chambers. Asymmetry of the electrodes and bridges was checked before and after each experiment and was less than 1.0 mV.

All toads were maintained half-immersed in 40 m*M* KCl for 5 days prior to sacrifice. In addition each toad was given 8–10 ml of 120 m*M* KCl by stomach tube, in divided doses during the last 48 hr.

Toads were pithed and their hemi-bladders were mounted between glass chambers. The serosal chamber volume was 2 ml and the mucosal chamber, 8–10 ml. In the experiments with sodium-free Ringer solution, pooled plasma from K loaded toads was labeled with ^{42}K and used as the serosal bath.

The basic pattern of all experiments was to determine the serosal to mucosal (S→M) flux of K during seven successive 15-min periods. The first two, and last two periods were control observations, and the middle three periods were for the experimental intervention. In 10 experiments the intervention was placing a sodium-free Ringer solution (choline Ringer solution) on the mucosal side of the bladder. In 9 experiments the intervention was placing vasopressin 20 milliunits/ml in the serosal bath.

In the experiments in which a sodium-free Ringer solution was used, Na Ringer solution containing a K concentration matching the labeled plasma was used in the mucosal chamber during the control periods, and the sodium-free Ringer solution likewise contained a matching K concentration. Oxygen was bubbled into the mucosal chamber throughout the experiment, because foaming was a problem if O_2 was bubbled through plasma.

In the vasopressin experiments, Ringer solution was placed on both sides of the bladder, with ^{42}K label in the serosal bath. During the experimental periods vasopressin was in the serosal bath. Between the last experimental period and the following control period, the chamber was rinsed twice with Ringer solution to remove the vasopressin. In these experiments oxygen was bubbled through the serosal chamber throughout the experiment.

In the time control experiments, labeled plasma was in the serosal chamber during periods I and II and a Na Ringer solution containing a matching K concentration was in the mucosal chamber. During periods III, IV, and V, the serosal bath was a labeled Na Ringer solution.

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TABLE I. Effect of Na Depletion from the Mucosal Side of the Toad Bladder on the ^{42}K S→M Flux.

Flux in nEq/100 mg of bladder × min; PD in mV.

	Ringer bath		Na-free choline Ringer bath			Ringer bath	
Time period:	I	II	III	IV	V	VI	VII
Flux	17.94[a] ±6.04	19.12 ±5.94	19.69 ±6.17	20.25 ±6.36	20.26 ±6.22	24.37 ±8.83	24.13 ±8.21
PD	17.0[a] ±4.8	15.9 ±4.6	5.9 ±2.2	2.1 ±1.0	1.3 ±0.9	16.0 ±4.7	14.0 ±4.5

[a] Av of 10 expts. ± SEM.

The flux was determined by draining the mucosal chamber into a counting tube 1 min before the end of the flux period, and refilling the chamber with Ringer solution. The chamber was then drained at the end of the period, and refilled with the appropriate solution to start the next period, except when the serosal solution had to be changed.

All bladders were equilibrated with the labeled serosal solution, and the control mucosal solution for 30 min before the first control flux period. The mucosal chamber was washed as described above for the end of a flux period, to end this equilibration period and begin the first flux period.

Standard counting techniques, and calculations were used in determining the K flux. Counts were to a statistical accuracy of $<1\%$.

Results. A. Effect of low mucosal [Na] on S→M flux of K. In Table I are presented the averages of the fluxes and PD determined during each control and experimental period on 10 bladders. At the end of each experimental period the Na concentration of the mucosal media was determined. The average concentrations were 1.27 ± 0.25, 0.32 ± 0.07, and 0.24 ± 0.07 μEq/ml after periods III, IV, and V, respectively. The PD was 0 during 11 of the 30 experimental periods. The average difference of flux II minus flux III was −0.57 ± 1.0 SEM nEq/100 mg of wet weight × min ($p>0.45$). Similarly flux VI minus flux V gave an average difference of +4.74 ± 3.36 nEq/100 mg of wet weight × min ($p>0.30$).

B. Effect of vasopressin on S→M flux of K. In Table II are reported the averages of the fluxes and PD determined during each control and experimental period on 9 bladders. Also shown are the averages of fluxes on 7 time control bladders which received no

TABLE II. Effect of ADH on the S→M Flux of ^{42}K Across the Toad Bladder.

Flux in nEq/100 mg of bladder × min; PD in mV.

	Ringer bath		Ringer bath with ADH			Ringer bath	
Time period:	I	II	III	IV	V	VI	VII
Exptl. flux	16.37[a] ±2.62	18.20 ±2.57	23.11 ±2.86	30.91 ±4.49	31.43 ±3.77	32.22 ±5.51	36.72 ±6.32
Time control flux[c]	13.68[b] ±4.44	17.53 ±5.88	27.99 ±10.17	30.12 ±8.78	49.17 ±14.21		
Av PD exptl.	13.4[a] ±3.4	13.5 ±3.7	23.4 ±5.7	23.3 ±6.5	21.5 ±6.2	13.4 ±3.9	9.9 ±3.9

[a] Av of 9 expts. ± SEM.

[b] Av of 7 expts. ± SEM.

[c] Plasma was used as serosal bath in periods I and II.

vasopressin during the experimental period. Each experimental bladder showed a characteristic increase in PD in response to vasopressin and a return to control values when the vasopressin was removed. It is noted that the flux measurement in both the control and experimental group increased with time. The difference between the control and experimental group was not significant ($p > 0.50$ for periods I to IV and $p > 0.20$ for period V).

In the series of 9 bladders, periods I thru VII were also tested by analysis of variance. The increase with time is shown to be highly significant ($p < 0.005$). It is noted that an increase in flux occurred between periods II and III. Analysis of variance indicates that this increase has a low probability of being due to the application of vasopressin ($p > 0.10$). The correlation between K flux and PD during periods II to VI was tested ($r = 0.033$).

Discussion. The fact that the toad bladder excretes K suggests that the K might move down an electrical gradient produced by the Na pump across this tissue. The fact that Leaf *et al.* (2) reports an agreement between the net Na flux and short circuit current as measured by the method of Ussing and Zerahn (3) is not an argument against this theory. If K did move in response to an electrical gradient, the K would not move when one abolished the electrical gradient by the short circuiting technique, and so this would not affect the agreement between the two measurements.

We chose to use the "chemical short circuiting" technique in which the PD is abolished by decreasing the Na concentration on the mucosal side to near zero. If the net flux into the bladder is in response to the PD, the serosal to mucosal flux would be expected to diminish markedly by this procedure. It can be seen that there is no effect on the K flux.

If the K movement into the bladder were in response to the PD produced by the sodium pump, it would be predicted that the S→M flux would increase in response to vasopressin. The comparison of K flux in the vasopressin-treated bladders, and the time control bladders, as well as the analysis of variance reported make this possibility unlikely. In addition, the fact that the K flux continued to increase after the vasopressin effect on PD was reversed by washing the bladder in periods VI and VII is also against the theory that the K movement is in response to the PD. It cannot be stated with certainty from these data alone that vasopressin might have been the cause of the further increase in K flux during periods VI and VII. However, such a delayed effect of vasopressin seems unlikely since the effect of vasopressin on H_2O permeability is promptly reversed by washing the bladder (5). In addition, such an effect could not be by the mechanism of increasing the PD since the PD returned to control levels during periods VI and VII.

The fact that the K flux increased with time in the experiments with vasopressin, and did not increase as markedly in the experiments in which the Na concentration was varied is of interest. It is probable that the plasma used on the serosal side of the bladder in the low Na experiments supported the physiological processes of the bladder better than the Ringer solution used in the vasopressin experiments. The Ringer solution was necessary in the vasopressin experiments since it would be difficult to be sure that any plasma used was free of vasopressin, oxytocin, or vasotocin, all of which increase Na transport.

The PD recorded in control bladders run somewhat lower than those reported from other laboratories. We have established this to be due to keeping the toads in deionized water. If toads are kept in moist earth, higher PD readings are then obtained which are consistent with those reported from other laboratories.

Ideally, one would like to do these experiments measuring bidirectional K fluxes simultaneously. Such simultaneous fluxes have been reported from this laboratory (3) using ^{42}K and ^{40}K. However, the ^{40}K must be determined by mass spectroscopy, and facilities are available only for a limited number of determinations.

In the report by Kallus and Vanatta (4), the mucosal to serosal flux of K is less than 25% of the serosal to mucosal flux in bladders from toads similarly loaded with KCl. It is

presumed that if the sodium pump were to affect the net K excretion by the toad bladder, it would do so by affecting the serosal to mucosal flux of K rather than the reverse flux. For this reason, these experiments were carried out measuring only the serosal to mucosal flux of K.

These results then indicate that the movement of K into the bladder is not driven by the PD produced by the electrogenic Na pump. It seems unlikely that there is a coupled exchange mechanism with Na exchanging for K in order to accomplish the excretion of K. If this were the case, the K flux should fall when the sodium is virtually absent on the mucosal side of the bladder.

Summary. The serosal to mucosal flux of K in the urinary bladder of *Bufo marinus* is not changed by reducing the mucosal Na concentation to virtually zero. Vasopressin, which is known to increase the rate of sodium reabsorption from the bladder, does not significantly alter the serosal to mucosal K flux. This evidence is against the theory that K excretion in the toad bladder is linked either to Na reabsorption *per se* or to the PD produced across the bladder by the Na pump.

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1. Kallus, F. T., and Vanatta, J. C., Tex. Rep. Biol. Med. **27**, 419 (1969).

2. Leaf, A., Anderson, J., and Page, L. B., J. Gen. Physiol. **41**, 657 (1958).

3. Ussing, H. H., and Zerahn, K., Acta Physiol. Scand. **23**, 110 (1951).

4. Kallus, F. T., Vanatta, J. C, Burke, W. H., and Heatherington, E. A., Physiologist **12**, 264 (1969).

5. Bentley, P. J., J. Endrocrinol. **17**, 201 (1958).

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