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COMPARTMENTATION AND EXCHANGE OF POTASSIUM IN THE TOAD BLADDER*

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SUMMARY

1. The urinary bladder of *Bufo marinus* was studied to determine the principal barrier to K^+ diffusion across the bladder.

2. The K^+ content is 37.6 μequiv/g wet weight of whole bladder. The K^+ content of the serosal portion of bladder is 13.1 μequiv/g wet weight. The K^+ content of the epithelial cells is calculated to be 24.5 μequiv/g wet weight.

3. Exchange between bladder K^+ and $^{42}K^+$ in the mucosal bath was determined at 2, 6 and 24 h. In 6 h the exchange approaches a steady-state value representing 1.12 μequiv/g of whole bladder.

4. The exchange between bladder K^+ and $^{42}K^+$ in the serosal bath was determined at intervals from 0.08 to 6 h. There is a complex rate of exchange which is not at a steady state at 6 h. At 1 h the exchange is not accounted for by exchange with serosal tissue K^+ alone.

5. The data indicate that the major barrier to diffusion of K^+ is near the mucosal side of the epithelial cell.

INTRODUCTION

The urinary bladder of *Bufo marinus* has been studied extensively regarding its role in Na^+ metabolism[1–5]. Recently we reported that this structure is also capable of K^+ excretion[6]. We therefore undertook studies of the K^+ content of the bladder and the exchange characteristics of this K^+ in order to be able to interpret the observation of K^+ excretion with regard to the single intracellular transport compartment model used for Na^+ transport in this tissue.

METHODS

Equipment, toads, and Ringer solution were as previously reported[6]. Labeled Ringer solution was made to a final concentration of about 3.0 mequiv/l using $^{42}K_2CO_3$. The unlabeled Ringer solution in each experiment was made with a matching K^+ concentration.

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Hemibladders dissected from pithed toads were cannulated and allowed to equilibrate with Ringer solution for 15–30 min before exposure to the labeled solution. All exposures were with labeled solution on either the mucosal or the serosal side of the bladder, and unlabeled solution on the respective opposite side.

In exposures longer than 10 min, the unlabeled Ringer solution was changed frequently in order to minimize backlabeling from the flux of isotope coming into the solution. The labeled solution was changed in long experiments so that the specific activity would not change more than 2 %.

At the end of the exposure period, the bladders were drained, then carefully blotted on filter paper to remove excess moisture. An aliquot of the exposure bath was obtained to determine the specific activity of K^+. The bladders were weighed, counted for isotope content and the K^+ content determined by extracting them with water and analyzing an aliquot of the extract. Eleven bladders were ashed by the method of HALD[7] and the K^+ content of the ash determined. These showed no more K^+ than would be expected from their water content and thus established the efficiency of the extraction procedure.

The K^+ content of serosal tissue was determined on tissue obtained by scraping the epithelial cells from whole bladders which had been incubated in collagenase (2 mg/ml) in Ringer solution. In addition the K^+ content was determined on toad parietal peritoneum, which is serosal tissue.

CALCULATIONS

All counting data were subject to standard corrections for background and decay. The specific activity of the bladders and baths was expressed in counts/min per μequiv K^+. The ratio of specific activity of the bladder to the specific activity of the bath was calculated. The ratio times the K^+ content of the bladder in μequiv/g wet weight gives a virtual exchange value (E) in units of μequiv/g wet weight of bladder. E is then that quantity of K^+ in the bladder which would be at the same specific activity as the bath in order to account for all the radioactivity found in the bladder.

RESULTS

Analysis of serosal tissue and whole bladder

The average K^+ content of the serosal tissue obtained from five bladders was 20.1 $\pm$ 2.5 μequiv/g wet weight (average $\pm$ 1 S.E.) of scraped serosal tissue. The average K^+ content of five pieces of serosal tissue from parietal peritoneum was 18.0 $\pm$ 2.4 μequiv/g wet weight. These values are not significantly different from each other ($P > 0.40$).

The content of 20.1 μequiv/g wet weight of scraped tissues is 13.1 $\pm$ 2.17 μequiv/g wet weight of whole bladder. The K^+ content of whole bladder averaged 37.6 $\pm$ 0.98 μequiv/g wet weight. The K^+ content of the epithelial cells was calculated to be 24.5 μequiv/g. We were unsuccessful in attempts to analyze the scraped epithelial cells for K^+ content.

Mucosal exchange experiments

E was determined on bladders exposed to $^{42}K^+$ in the mucosal bath. Average values were 0.62 μequiv/g wet weight for two bladders at 2 h, 1.12 $\pm$ 0.08 μequiv/g wet weight for seven bladders exposed 6 h and 1.12 $\pm$ 0.07 for seven bladders exposed 24 h.

Serosal exchange experiments

The ratio of the specific activity of the bladder to the specific activity of the exposure media when ^{42}K-labeled bath is placed on the serosal side of the bladder and the resultant values of E are shown at varying times of exposure in Table I. It is evident from these values that the exchange from the serosal side has not reached a steady state in 6 h.

TABLE I

^{42}K-UPTAKE BY TOAD BLADDER WITH LABEL IN SEROSAL BATH

Bladders were exposed to ^{42}K-labeled Ringer solution as described in the text. The ratio of specific activity of K of bladder to specific activity of K of bath is reported. The calculation of E is described in the text. The values are averages of the number of observations indicated by parentheses. The value following the $\pm$ is 1 S.E.

Time (h)	*Ratio of specific activities*	*E (μequiv/g wet wt. whole bladder)*
0.08	0.087 (4)	3.27 $\pm$ 0.47
0.17	0.173 (7)	6.51 $\pm$ 1.13
0.50	0.369 (7)	13.86 $\pm$ 0.82
1.00	0.448 (7)	16.83 $\pm$ 0.93
2.00	0.450 (6)	16.93 $\pm$ 0.96
3.00	0.506 (8)	19.02 $\pm$ 0.44
4.00	0.655 (5)	24.63 $\pm$ 0.41
6.00	0.772 (2)	29.03 $\pm$ 0.17

DISCUSSION

These studies were undertaken because of the finding in our laboratory of K^+ excretion by the toad bladder *in vivo*[6]. Briefly summarized, we isolated the bladder by suturing the cloacal orifice closed. This was done by direct vision, using a pediatric nasal speculum to dilate the anus and gain access to the bladder orifice. This preparation makes use of the well-known difference between the amphibian genitourinary system and the mammalian system. In the amphibian, the ureters do not enter the bladder directly as in the mammal. Instead, the ureters or mesonephric ducts enter the cloaca on the opposite side from the bladder orifice. Urine must first enter the cloaca, and then enter the bladder through the cloacal orifice. The surgical procedure described then makes the bladder a closed sac which is not accessible to urine from the kidney. In addition, catheters were placed in the ureters and delivered through the anus to the outside to decrease the likelihood of renal urine entering the bladder.

Before closing the bladder as described above, it was emptied and then filled with "mock" urine of known K^+ concentration. The total K^+ contained in the "mock"

urine increased after a 24-h period. The amount of increase in K^+ was greater if the toad was given KCl solution by stomach tube. The final concentration of K^+ in the "mock" urine exceeded the concentration of K^+ in the plasma, thus the K^+ movement was not down a concentration gradient.

Because of this evidence that the toad bladder excretes K^+, we undertook these studies to determine the relative permeabilities of the serosal and mucosal plasma membranes of the epithelial cells to K^+. Such data will be necessary in understanding the mechanism of K^+ transport across the bladder.

From the exchange data it is obvious that the mucosal side of the cell is less permeable to K^+ than the serosal side. It is interesting to note that the K^+ which exchanges on the mucosal side of the cell is greater than would be predicted if the barrier to K^+ diffusion on this side were the same as the barrier to inulin diffusion.

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