Sodium-Independent Active Potassium Reabsorption in Proximal Tubule of the Dog

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ABSTRACT Prior studies of proximal tubule reabsorption have failed to distinguish conclusively between a separate active K+ transport system and K+ movement linked to Na+ reabsorption. To attempt to dissociate movement of K+ from Na+ and Ca++, recollection micropuncture experiments were performed in proximal tubules of intact and thyroparathyroidectomized (TPTX) dogs under two different conditions known to inhibit Na+ reabsorption: saline expansion to 5% body wt, and 5 mg/kg acetazolamide. A control hydropenic group was also studied. Tubular concentrations of K+, Na+, and Ca++ were measured by electron probe analysis. During initial collections, mean±SEM tubular fluid/plasma $(TF/P)_{K+}$ was 1.07 ±0.05, 1.05±0.05, and 1.00±0.03 in intact hydropenic ($n=7$), saline ($n=6$), and acetazolamide ($n=8$) groups; fractional reabsorption (FR) of K+ in proximal tubules was 0.35, 0.39, and 0.31 respectively. After saline, $(TF/P)_{inulin}$ fell from 1.81 to 1.34 ($P<0.01$); $(TF/P)_{K+}$, $(TF/P)_{Na+}$, and tubular fluid/ultrafiltrate, $(TF/UF)_{Ca++}$ did not change, so that FR of all three ions fell proportionately. After acetazolamide, however, despite a 24% inhibition of FR of Na+ and Ca++, $(TF/P)_{K+}$ fell to 0.85±0.04 ($P<0.005$) so that FR of K+ was unchanged at 0.34.

In three corresponding groups of TPTX dogs, similar results were obtained. Acetazolamide ($n=7$) inhibited FR of Na+ and Ca++ by 41%, but $(TF/P)_{K+}$ fell from 1.03±0.03 to 0.89±0.04 ($P<0.005$) so that FR of K+ was unchanged (0.36–0.34).

A separate uphill transport system for K+ in proximal tubules is therefore unmasked by acetazolamide, a drug which selectively inhibits Na+ (and Ca++) reabsorption. Saline, on the other hand, inhibits net reabsorption of all three ions, probably by increasing passive backflux via intercellular channels.

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INTRODUCTION

More than two decades ago, Berliner, Kennedy, and Orloff postulated on the basis of renal clearance studies that filtered potassium (K+) is extensively reabsorbed in the proximal tubule, and that most of the K+ in the final urine is the result of secretion by the distal nephron (1). Micropuncture studies have, in general, confirmed this hypothesis, and mechanisms of distal K+ transport have been elucidated by Giebisch (2). However, the characteristics of the K+ reabsorptive process in the proximal tubule remain unclear. Potassium transport could be linked or coupled in some way to reabsorption of sodium (Na+) or fluid, as appears to be the case with calcium (3) and phosphate (4), or there might be an independent K+ reabsorptive pathway.

By the use of electron microprobe analysis (EPA) [1] of tubular fluid we have evaluated the interrelationships between ions within the proximal tubule under two different conditions that inhibit proximal tubular Na+ reabsorption: saline loading and acetazolamide administration. Our results indicate that K+ can be reabsorbed by an uphill transport system dissociated from the reabsorption of Na+ and Ca++.

METHODS

Mongrel female dogs, weighing 15–25 kg and starved for 12 h but not water-restricted, were anesthetized with intravenous sodium pentobarbital (20 mg/kg), intubated, and ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.) Surgical preparation of animals and recollection micropuncture technique were performed as previously described from this laboratory (4). Four to seven surface proximal tubules were punctured in each dog,

[1] *Abbreviations used in this paper:* C, clearance; EPA, electron microprobe analysis; FE, fractional excretion; FR, fractional reabsorption; In, inulin; P, plasma; PTH, parathyroid hormone; TF, tubular fluid; TPTX, thyroparathyroidectomized; UF, ultrafiltrate.

TABLE I

*Effect of Continued Hydropenia, Saline Infusion, or Acetazolamide on Ureteral Urine and Proximal Tubule of Intact and TPTX Dogs**

		Clearance				Micropuncture			
		C_{In}	C_{Na}/C_{In} × 100	C_{Ca}/C_{In} × 100	C_K/C_{In} × 100	$(TF/P)_{In}$	$(TF/P)_{Na}$	$(TF/UF]_{Ca}$	$(TF/P)_K$
		ml/min	%	%	%				
Intact control	CP	29±1	0.11±0.02	0.42±0.08	13.6±1.1	1.68±0.05	0.99±0.01	1.10±0.02	1.07±0.05
n = 7	R	32±1	0.06±0.01	0.29±0.04	11.7±1.0	1.70±0.05	0.99±0.01	1.09±0.02	1.06±0.05
		NS	NS	NS	NS	NS	NS	NS	NS
Intact saline	CP	32±2	0.16±0.03	0.31±0.05	11.9±1.3	1.81±0.16	1.01±0.01	1.08±0.03	1.05±0.05
n = 6	R	40±2	2.61±0.79	1.60±0.61	24.5±3.4	1.34±0.05	1.02±0.01	1.13±0.03	1.06±0.02
		$P < 0.005$	$P < 0.025$	$P < 0.05$	$P < 0.005$	$P < 0.01$	NS	NS	NS
Intact	CP	43±4	0.16±0.06	0.49±0.12	14.7±2.2	1.54±0.07	1.04±0.04	1.12±0.02	1.00±0.03
acetazolamide	R	31±2	1.84±0.19	0.48±0.11	61.1±2.3	1.37±0.07	1.07±0.02	1.08±0.02	0.85±0.04
n = 8		$P < 0.005$	$P < 0.001$	NS	$P < 0.001$	$P < 0.005$	NS	NS	$P < 0.005$
TPTX	CP	31±4	0.18±0.10	0.48±0.13	14.1±1.2	1.74±0.07	1.02±0.02	1.10±0.03	1.03±0.04
control	R	29±3	0.12±0.06	0.26±0.07	11.8±1.6	1.73±0.06	1.03±0.02	1.08±0.02	0.99±0.05
n = 7		NS	NS	NS	NS	NS	NS	NS	NS
TPTX	CP	27±4	0.09±0.03	0.60±0.18	13.2±1.4	1.84±0.04	0.98±0.01	1.03±0.03	0.99±0.03
saline	R	34±5	2.09±0.47	1.98±0.48	24.8±1.0	1.36±0.05	0.96±0.01	1.03±0.04	0.94±0.03
n = 6		$P < 0.05$	$P < 0.01$	$P < 0.02$	$P < 0.001$	$P < 0.005$	NS	NS	NS
TPTX	CP	34±2	0.20±0.05	0.78±0.16	12.5±1.9	1.66±0.07	1.00±0.01	1.12±0.03	1.03±0.03
acetazolamide	R	30±4	1.47±0.25	0.55±0.08	47.5±3.2	1.35±0.10	0.99±0.00	1.08±0.03	0.89±0.04
n = 7		NS	$P < 0.01$	NS	$P < 0.001$	$P < 0.005$	NS	NS	$P < 0.005$

* Abbreviations: CP = control period (initial collections); mean±SEM, R = recollections, mean±SEM, NS = $P > 0.05$.

before and after the several experimental procedures to be described. Urine was collected separately from each kidney and clearance values from the left (experimental) side are reported in Results. Blood samples were collected at the beginning, midpoint, and end of each clearance period.

Intact control animals. To evaluate the effect of time and/or the recollection technique upon the measured parameters, seven intact dogs received only the sustaining inulin-saline infusion at 1.0 ml/min. Recollections were begun 45–60 min after completion of initial collections.

Intact saline animals. In six intact dogs, after initial collections, isotonic saline equivalent to 5% of body weight was infused over 20–25 min. Subsequent urinary losses were replaced with an equal volume of isotonic saline. After a steady state of urine volume was achieved (45–60 min), recollections were begun.

Intact acetazolamide animals. In eight dogs, after initial collections, 5 mg/kg acetazolamide was infused intravenously over 2 min, followed by infusion of the drug at 5 mg/kg/h. Incremental urinary losses were replaced with equal volumes of 100 mM sodium bicarbonate solution to prevent acidosis or volume contraction. Recollections were made after attainment of steady-state urine flow.

Thyroparathyroidectomized (TPTX) dogs. Since parathyroid hormone (PTH) inhibits proximal Na+ reabsorption (4), studies were also done in three corresponding groups of TPTX dogs to see if the absence of PTH affected K+ transport. In TPTX control dogs ($n = 7$) and TPTX saline dogs ($n = 6$), surgical TPTX was carried out 2 h before initial collections. In TPTX acetazolamide dogs ($n = 7$), TPTX was performed 2–3 days before the study and exogenous parathyroid extract (Eli Lilly & Co., Indianapolis, Ind.) 100 U was given intramuscularly twice daily until 24 h before the experiment. The protocols were otherwise in every way similar to the corresponding groups of intact dogs.

Blood samples for calcium were ultrafiltered as described before (4), and calcium in serum ultrafiltrate (UF) and urine was measured by a fluorometric technique described by Borle and Briggs (5). Na+, K+, and inulin concentrations of plasma (P) and urine and tubular fluid (TF) inulin concentrations were measured as previously described (4). TF Na+, Ca++, and K+ concentrations were measured by EPA, by a modification (6) of the method described by Lechene (7). 21 urine samples were analyzed for K+ by flame photometry and aliquots of each by EPA over a range of concentration from 2.7 to 9.7 meq/liter. The mean±SEM of the quotient of EPA/flame photometer results was 1.01±0.01. Clearances, fractional reabsorptions, and excretions were calculated by usual formulae (4). Statistical analysis was performed as described before (4).

RESULTS

Intact dogs

Clearance results. (Table I, left). In seven hydropenic control dogs, there was no significant change in inulin clearance (C_{In}), fractional excretion (FE) of Na+, FE of Ca++, or FE of K+ during the course

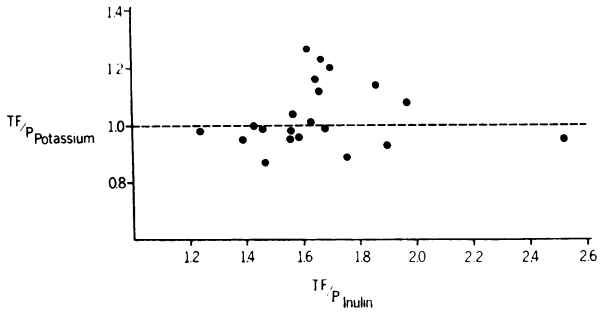

FIGURE 1 Mean values for $(TF/P)_K$ plotted against corresponding mean $(TF/P)_{In}$ in 21 intact dogs during control collections. Each point represents the mean of 4–7 tubules.

of the micropuncture study. On the other hand, the infusion of isotonic NaCl solution (equivalent to 5% body wt) to six dogs resulted in a significant increase in each of these parameters, In the eight dogs that received acetazolamide, C_{In} fell an average of 28%. Despite the fall in C_{In}, FE of Na+ increased and there was a marked kaliuresis. The FE of K+ after acetazolamide, 61.1%, was significantly greater than that achieved after NaCl, 24.5% ($P < 0.001$), despite equivalent natriuresis.

Micropuncture results. Fig. 1 shows the mean values for $(TF/P)_K$ plotted against $(TF/P)_{In}$ in each of 21 intact dogs during control collections. The mean $(TF/P)_K$ values varied aboout a value of 1.0, and the overall mean value was 1.04. There was no relationship between $(TF/P)_K$ and $(TF/P)_{In}$. In seven control dogs, $(TF/P)_{In}$, $(TF/P)_{Na+}$, $(TF/P)_{K+}$, and $(TF/UF)_{Ca++}$ did not change during control and recollection periods (Table I, right). Fractional reabsorption (FR) to the point of micropuncture during control and recollection, respectively, for Na+ was 0.39±0.02 and 0.40±0.01, for K+ was 0.35±0.03 and 0.36±0.03, and for Ca++ was 0.34±0.03 and 0.36±0.03. None of these changes

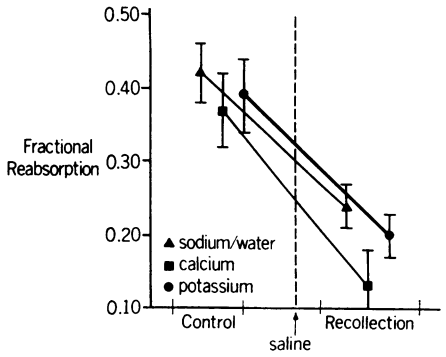

FIGURE 2 Effect of saline expansion upon FR of sodium, calcium, and potassium in proximal tubule of six intact dogs. Lines connect data points representing mean values (±SEM) during control and recollection periods.

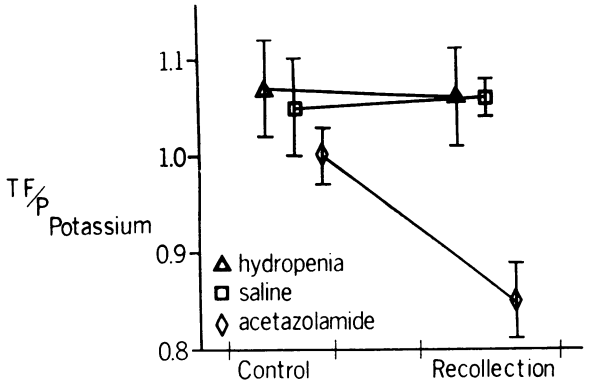

FIGURE 3 Mean±SEM $(TF/P)_K$ in three groups of intact dogs: hydropenic control ($n = 7$), saline expansion ($n = 6$), and acetazolamide ($n = 8$). Lines connect corresponding values during control and recollection periods.

was statistically significant. Saline infusion resulted in a significant fall in $(TF/P)_{In}$ but no change in $(TF/P)_{K+}$, $(TF/P)_{Na+}$, or $(TF/UF)_{Ca++}$ (see Table I). Fractional reabsorption of Na+, Ca++, and K+ was therefore inhibited in parallel (Fig. 2); FR of Na+ fell from 0.42±0.04 to 0.24±0.03 ($P < 0.001$), FR of Ca++ from 0.37±0.05 to 0.13±0.05 ($P < 0.001$), and FR of K+ from 0.39±0.05 to 0.20±0.03 ($P < 0.005$) as a result of the NaCl infusion.

Acetazolamide also caused a significant fall in $(TF/P)_{In}$ (Table I). Since neither $(TF/P)_{Na}$ nor $(TF/UF)_{Ca}$ changed, there was a parallel inhibition of FR of Na+ 0.34±0.03 to 0.26±0.04 ($P < 0.005$), and of Ca++ 0.26±0.04 to 0.20±0.04 ($P < 0.01$). On the other hand, $(TF/P)_K$ fell significantly after acetazolamide from 1.00±0.03 to 0.85±0.04 (Fig. 3; Table I), so that FR of K+ remained unchanged (0.31±0.04 to 0.34±0.03; NS), as shown in Fig. 4.

TPTX dogs

Clearance results (Table I). Clearance values from the three groups of TPTX dogs were remarkably similar to their intact counterparts. Initial FE of Ca++

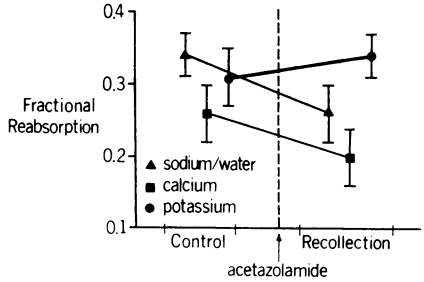

FIGURE 4 Effect of acetazolamide upon FR of sodium, calcium, and potassium in proximal tubule of eight intact dogs. Lines connect data points representing mean values (±SEM) during control and recollection periods.

tended to be higher in TPTX than in intact dogs, but this difference was not statistically significant. As in intact, the FE of K+ after acetazolamide, 47.5±3.2%, was greater than that after NaCl, 24.8±1.0% ($P < 0.001$), despite equivalent natriuresis.

Micropuncture results. The changes in proximal concentration ratios during the three experimental procedures in TPTX dogs were qualitatively similar to those in intact dogs (Table I). Saline infusion resulted in parallel falls in FR of Na+ from 0.45±0.01 to 0.26±0.03 ($P < 0.005$), of Ca++ from 0.43±0.03 to 0.22±0.04 ($P < 0.005$), and of K+ from 0.45±0.02 to 0.30±0.03 ($P < 0.01$), with no change in TF/P ratios of all three ions. Acetazolamide caused similar decreases in FR of Na+, 0.39±0.02 to 0.23±0.06 ($P < 0.05$), and of Ca++ 0.32±0.03 to 0.17±0.06 ($P < 0.005$). However, FR of K+ did not change significantly (0.36±0.03 to 0.34±0.05) in association with a significant decrease in $(TF/P)_{K+}$ from 1.03±0.03 to 0.89±0.04.

DISCUSSION

Several prior micropuncture studies in rats and dogs, summarized by Giebisch (2), have indicated that the proximal $(TF/P)_{K+}$ generally is close to unity. The present data confirm that during hydropenia and saline infusion, no major chemical gradient exists for K+ between tubular fluid and plasma, so that K+ transport parallels net fluid reabsorption in both situations. Since $(TF/P)_{Na+}$ under these conditions is also 1.0, it is not possible to determine if K+ reabsorption is independent of Na+ transport. In fact, Bennett, Clapp, and Berliner, on the basis of finding a $(TF/P)_{K+}$ close to 1.0 in the proximal tubule of the dog during varied experimental conditions, proposed that proximal tubule K+ transport might be entirely passive in nature (8). A recent study by Khuri, Agulian, and Wise (9), in which K+ activity was measured by a K+-selective electrode, reported that the TF/P activity ratio for K+ was less than unity, suggesting a K+ transport pathway independent of Na+. However, since intratubular Na+ concentration (or activity) was not measured, such an interpretation is speculative.

In an attempt to dissociate the movement of Na+ from K+ we used two different maneuvers: saline, which inhibits proximal sodium reabsorption in large measure by affecting the passive back-diffusion component of net reabsorption (10), and acetazolamide, a diuretic that has been shown to inhibit Na+ reabsorption in the proximal tubule (6) and, by its capacity to inhibit hydrogen ion secretion, might be expected to inhibit the efflux component of net reabsorption. Proximal Ca++ transport was also evaluated, since its movement is closely associated with that of Na+ in the proximal tubule (11). After acetazolamide, proximal Na+ and Ca++ reabsorption were inhibited in parallel, whereas FR of K+ remained unchanged (Fig. 4). As a result of this dissociation, $(TF/P)_{K+}$ fell significantly to less than 1.0, uncovering an uphill K+ transport system in the proximal tubule functioning independently of Na+ transport. A prior study by Watson (12) in the dog had suggested that acetazolamide caused a fall in proximal $(TF/P)_{K+}$, although not to values less than 1.0, whereas Meng was unable to demonstrate any effect of acetazolamide on $(TF/P)_{K+}$ in the proximal tubule of the rat (13).

Our data suggest the following interpretation of proximal tubular K+ transport. Under normal circumstances, Na+ and K+ transport systems work in parallel in such a manner that both TF/P ratios remain at unity. Similarly, under conditions in which changes in peritubular capillary uptake of fluid might affect passive back-leak of interstitial fluid (10), probably via intercellular channels (saline loading and glomerulotubular balance), TF K+ and Na+ concentrations remain close to that of extracellular fluid; hence parallel changes in net reabsorption of both ions occur. Acetazolamide apparently inhibits the efflux component of Na+ reabsorption, and under this circumstance K+ reabsorption continues at its original rate. The concomitant inhibition of fluid reabsorption produces a fall in TF K+ concentration. In fact, in this view, it seems likely that acetazolamide would, if anything, decrease the passive back-leak and allow the capacity of the independent K+ pathway to be expressed more fully. The similarity of results between intact and TPTX dogs suggests that PTH, which may affect Na+ and fluid reabsorption in the proximal tubule (4), plays no important role in the regulation of proximal K+ transport.

ACKNOWLEDGMENTS

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