Kidney International, Vol. 9 (1976) p. 323–332

Potassium secretion by the descending limb or pars recta of the juxtamedullary nephron *in vivo*

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Potassium secretion by the descending limb or pars recta of the juxtamedullary nephron *in vivo*. Potassium reabsorption by the juxtamedullary nephron up to the hairpin turn was studied by the micropuncture technique in the exposed renal papilla of rats. In 18 nondiuretic rats, the fraction of filtered potassium remaining at the end of the desdending limb averaged 113 ± 9%, indicating either that potassium is not reabsorbed by the juxtamedullary proximal tubule and descending limb or that potassium is reabsorbed and secreted in those segments. Furosemide, a drug which inhibits NaCl reabsorption in the ascending limb downstream from the descending limb, significantly decreased the potassium remaining at the end of the descending limb from 106 ± 12 to 72 ± 11% in seven rats. Benzolamide, a drug which inhibits reabsorption of $NaHCO_3$ and water in the proximal tubule upstream from the descending limb, significantly increased the potassium remaining from 103 ± 13 to 177 ± 32% in eight rats. These findings support the hypothesis that in the rat, potassium is normally reabsorbed by the proximal convoluted tubule and secreted in the pars recta or descending limb of the juxtamedullary nephron.

Secrétion de potassium *in vivo* par la branch descendante ou la pars recta du néphron juxta-médullaire. La réabsorption de potassium par le néphron juxta-médullaire jusqu'à la pointe de l'anse a été étudiée par microponction de la papille exposée du rein de rat. Chez 18 rats en antidiurèse, la fraction du potassium filtré qui est délivrée à la fin de la branche descendante est en moyenne de 113 ±9%, ce qui indique soit que le potassium n'est pas réabsorbé par le tube proximal et al branche descendante du néphron juxta-médullaire, soit que le potassium est, dans ces segments, réabsorbé et secrété. La furosémide, qui inhibe la réabsorption de NaCl dans la branche ascendante, en aval de la branche descendante, diminue significantivement la fraction de potassium délivré la fin de la branche descendante de 106 ± 12 à 72 ± 11% chez 7 rats, La benzolamide, qui inhibe la réabsorption de $NaHCO_3$ et d'eau dans le tube proximal en aval de la branche descendante, augmente significativement la fraction de potassium délivré de 103 ± 13 à 177 ± 32% chez 8 rats. Ces constatations étayent l'hypothèse que, chez le rat, le potassium est normalement réabsorbé par le tube contourné proximal et secrété dans la pars recta ou la branche descendante du néphron juxta-médullaire.

The potassium concentration in fluid at the bend of Henle's loop in the renal papilla is significantly higher than that of glomerular filtrate or proximal tubule fluid [1–7]. The average tubule fluid-to-plasma (TF/P) potassium ratio at the bend of the Henle's loop ranges from 2.4 in the Brattleboro rat in water diuresis [7] to 5 or greater in the salt-loaded *Psammomys* [6] or antidiuretic hamster [5] and is as high as 9 in the antidiuretic rat [3].

In a study of fragments of isolated perfused descending limbs of rabbit nephrons *in vitro*, Rocha and Kokko found the potassium permeability to be low, 2.5×10^{-5} cm sec^{-1} [8]. They proposed that the high concentration of potassium observed in fluid from Henle's loop *in vivo* is due to water abstraction from the descending limb. The purpose of the present experiments was to test that hypothesis *in vivo*.

Methods

Forty-five rats of either sex, and of Wistar and Sprague Dawley strains weighing 50 to 151 g, were studied. Prior to experiment, all rats were fed Berkeley rat chow containing 23 mEq/kg of potassium and were permitted free access to water. The rats were divided into three groups.

Group 1. Rats were prepared for micropuncture of the left renal papilla as previously described [3]. Urine from the right kidney was collected through a bladder catheter. The blood pressure was measured and samples of arterial blood were obtained through a femoral arterial catheter. During surgery and throughout the experiment, 0.9% saline solution was infused at 0.03 ml/min. In 18 rats inulin was added to the infusate to achieve plasma concentrations of 80 to 100 mg/100 ml. After an hour for equilibration, loops were punctured near the hairpin turn and fluid was collected at the intratubular flow rate using micropuncture techniques previously described [3]. Samples of urine and plasma were also obtained. In

Received for publication July 21, 1975;
and in revised form October 2, 1975.

five additional rats, inulin was not added to the infusate, and plasma was collected from vasa recta as previously described [9].

Group 2. Ten animals were used to study the effect of furosemide. Seven animals were prepared for micropuncture of the papilla. Samples of loop of Henle fluid, plasma and urine were obtained before and after the intravenous administration of furosemide, 5 mg/kg of body wt prime and 5 mg/kg of body wt/hr of infusion, a dose of furosemide lower than that employed in most micropuncture experiments (see [11] for references) in an attempt to confine the site of action to the ascending limb. Urinary and tubule fluid collections were begun 5 to 30 min after the injection of furosemide and continued thereafter for approximately 90 min. After the first pair of micropuncture and urinary specimens were obtained following furosemide, isotonic saline was infused at a rate adjusted to replace urinary losses of sodium and water [10], estimated as twice the urinary flow rate from the right kidney (urinary sodium concentration approximated that of plasma [11]).

The effect of furosemide on the accessible superficial proximal tubule was studied in three rats using the re-collection micropuncture technique, as previously described [11, 12]. Since the papilla was not exposed, urine was collected from the left kidney in these animals. Urinary and proximal tubule fluid collections were begun 5 min after the injection of furosemide and continued thereafter for approximately 90 min. After the first pair of micropuncture and urinary specimens were collected following furosemide administration, isotonic saline was infused at a rate to replace urinary losses of sodium and water.

Group 3. Twelve animals were used to study the effect of benzolamide. Benzolamide was added to a 300 mM $NaHCO_3$ solution and a single i.v. injection was given (2 mg/kg of body wt) as described by Kunau [13]. Total volume of the injectate was 0.2 ml. A maintenance dose of benzolamide was not administered. Eight rats were prepared for micropuncture of the renal papilla. Samples of loop of Henle fluid, plasma and urine were obtained before and after benzolamide administration as in the furosemide experiments. Since the changes in urinary excretion were small, the infusion rate (0.03 ml/min) of isotonic saline was not adjusted in the second period.

In four animals, the effect of benzolamide on the accessible superficial proximal tubule was studied using the re-collection micropuncture technique. The protocol of the experiments was the same as that for the study of the effect of benzolamide on the loop of Henle except that urine was collected from the left kidney.

Analytic procedures. Tubule fluid and urine, vasa recta and systemic plasma were analyzed for osmolality, sodium, potassium and inulin by methods previously described [2, 9]. To check the microanalytic method for measuring potassium, artificial solutions were prepared containing NaCl, KCl, urea and inulin in concentrations usually found in loop fluid samples in hydropenic rats [14]. Samples (volume, 5 nl) of the artificial solution were handled in the same manner as the micropuncture specimens. In 15 consecutive samples, the ratio of measured potassium to calculated potassium was 1.04 ± 0.04, SE.

Data are presented as the mean ± SEM. Differences were tested for significance using Student's *t* test [15].

Results

Nondiuretic rats (group 1). The body and kidney weights, blood pressure, packed cell volume and urinary data for all three groups are summarized in Table 1.

The results of analyses of loop fluid samples obtained from 18 antidiuretic rats are presented in Table 2. Note that the mean tubular fluid to plasma (TF/P) potassium ratio, 7.3 ± 0.58, is essentially the same as the mean TF/P inulin ratio, 7.1± 0.67. The percentage of filtered potassium remaining, [(TF/P K)/(TF/P inulin)] × 100, was 113 ± 9.3%.

In another group of five nondiuretic rats, the concentration of potassium in vasa recta plasma averaged 44 ± mEq/liter.

Furosemide-treated rats (group 2). The effect of furosemide on urinary excretion is summarized in Table 1 and on loop of Henle fluid in Table 2. Figure 1 illustrates one experiment. As we have previously observed [11], the effect of furosemide on the composition of loop fluid was striking. Within ten minutes after the diuretic was given (Fig. 1), TF/P inulin decreased from 8.5 to 2.4, TF/P osmolality from 5.2 to 1.4, TF/P sodium from 3.5 to 1.2 and TF/P potassium from 8.4 to near unity. Urinary excretion of solute, water, sodium and potassium (shown in the lower panel of Fig. 1) also increased concomitantly with the changes in loop fluid. When the first micropuncture and urinary collections were completed, isotonic saline was infused at a rate adjusted to replace urinary sodium and water losses. Thereafter, the changes in composition of tubule fluid and urine were less striking.

Data obtained for each variable determined after furosemide administration were averaged and treated as one value in each rat (Table 2). Although a striking reduction in TF/P ratios of inulin, total solute, sodium and potassium was noted after furosemide,

Table 1. Whole animal and kidney function data for control, furosemide-treated and benzolamide-treated rats[a]

	Group				
	Control	Furosemide		Benzolamide	
		First period	Second period	First period	Second period
		Whole animal profile			
Body wt, *g*	66 ± 2	77 ± 2		103 ± 7	
Kidney wt, *g*					
Left	0.37 ± 0.01	0.44 ± 0.03		0.47 ± 0.03	
Right	0.39 ± 0.01	0.42 ± 0.03		0.47 ± 0.03	
Blood pressure					
mm Hg	85 ± 3	98 ± 5	92 ± 5	98 ± 2.5	93 ± 3.1[d]
Hematocrit					
%	39 ± 1	39 ± 1	40 ± 0.9	42 ± 0.9	40 ± 1[d]
Sodium					
mEq/liter	150 ± 0.9	145 ± 0.9	144 ± 1	148 ± 2	150 ± 2
Potassium					
mEq/liter	5.4 ± 0.2	4.5 ± 0.2	4.3 ± 0.2	4.5 ± 0.1	4.5 ± 0.2
N (rats)	18	10		12	
		Kidney function			
Urine flow					
$\mu l\ min^{-1}\ g\ kw^{-1}$	6.2 ± 0.4	4.5 ± 0.4	123 ± 17[b]	4.4 ± 0.5	13 ± 1.3[b]
GFR					
$\mu l\ min^{-1}\ g\ kw^{-1}$	1107 ± 82	901 ± 92	880 ± 82	1092 ± 60	1091 ± 96
Osmolality					
$mOsm/kg\ H_2O$	1592 ± 61	1828 ± 82	337 ± 10[b]	2000[e] ± 131	1163[e] ± 68[b]
U/P inulin	186 ± 13	204 ± 14	8.0 ± 0.8[b]	276 ± 29	95 ± 12[b]
FE[f] sodium %	0.5 ± 0.11	0.3 ± 0.07	13.9 ± 1.3[b]	0.1 ± 0.04	2.2 ± 0.32[b]
FE[f] potassium %	36 ± 2.3	43 ± 5	73 ± 3.1[c]	24.1 ± 4.1	42.5 ± 3.1[c]
N (rats)	17	10		12	

[a]Values are means ± SEM.
[b]$P < 0.001$, compared to first period.
[c]$P < 0.005$, compared to first period.
[d]$P < 0.025$, compared to first period.
[e]$N = 8$.
[f]Fractional excretion.

there was no statistically significant change in the fraction of filtered sodium remaining. Fractional reabsorption of potassium, however, was significantly increased, as indicated by the decrease in filtered potassium remaining from 110 ± 13% to 74 ± 11% ($P < 0.025$).

These findings imply that furosemide inhibits potassium secretion in the descending limb (or pars

Table 2. Results of analyses of loop fluid samples in control, furosemide-treated and benzolamide-treated rats[a]

	Group				
	Control	Furosemide		Benzolamide	
		First period	Second period	First period	Second period
		Micropuncture of end-descending limb			
TF/P inulin	7.1 ± 0.67	7.2 ± 0.66	2.4 ± 0.15[b]	7.2 ± 0.81	5.0 ± 0.45[d]
TF/P sodium	2.8 ± 0.14	3.5 ± 0.34	1.2 ± 0.02[b]	2.8 ± 0.38	2.4 ± 0.26
TF/P potassium	7.3 ± 0.58	7.7 ± 1.10	1.7 ± 0.25[b]	7.2 ± 0.84	8.0 ± 0.83
TF/P osm	3.8 ± 0.24	4.9 ± 0.41	1.3 ± 0.02[b]	3.5 ± 0.55	2.7 ± 0.27
		Fraction of filtered load remaining at end-descending limb (%)			
Water	17 ± 1.4	15 ± 1.6	43 ± 2.6[b]	15 ± 1.5	21 ± 1.9[c]
Sodium	44 ± 2.8	52 ± 6.6	51 ± 3.5	39 ± 3.0	49 ± 3.5[d]
Potassium	113 ± 9.3	110 ± 13	74 ± 11[e]	103 ± 13	177 ± 32[d]
Total solute	60 ± 3.2	70 ± 6.4	57 ± 3.2	47 ± 3.7	55 ± 3.6[f]

[a]Values are means ± SEM. *N* of rats: control, 18; furosemide, 7; benzolamide, 8.
[b]$P < 0.001$, compared to first period.
[c]$P < 0.005$, compared to first period.
[d]$P < 0.01$, compared to first period.
[e]$P < 0.025$, compared to first period.
[f]$P < 0.05$, compared to first period.

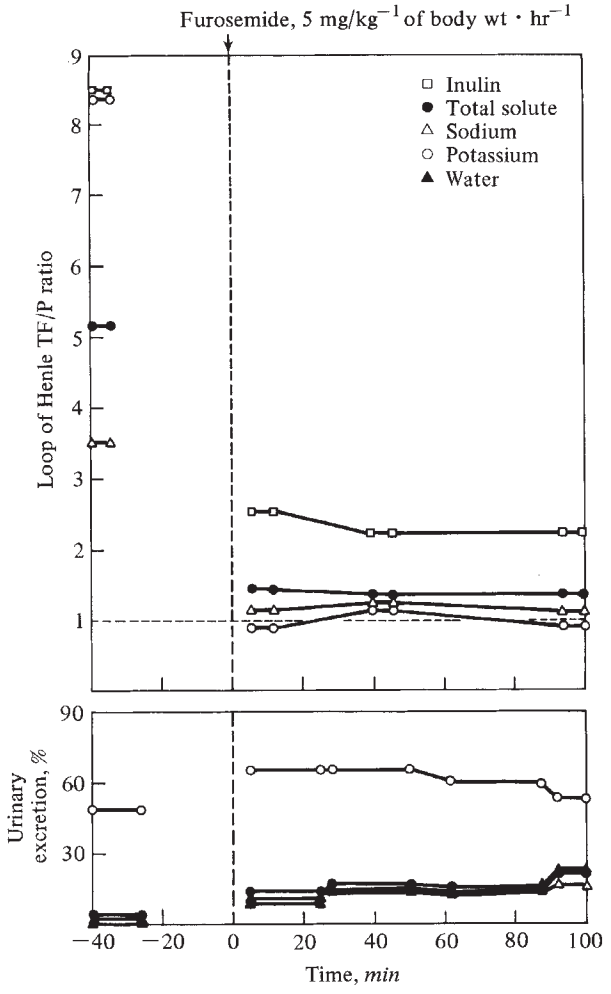

Fig. 1. *Effect of furosemide in one rat.* The ordinate in the *upper panel* represents the TF/P ratio for loop of Henle samples and the ordinate in the *lower panel* represents urinary excretion in percent. Abscissa is time in minutes. Length of *horizontal solid line* between symbols indicates duration of collection for tubule fluid and urinary samples. *Horizontal dashed line* in *upper panel* marks isotonicity with systemic plasma. At zero time indicated by *vertical dashed lines,* furosemide, 5 mg/kg of body wt, was injected as a priming dose and infused thereafter at 5 mg/kg of body wt/hr. Within five minutes all TF/P ratios fell dramatically; concomitantly, urinary excretion of water and all solutes increased substantially. For further description, see text.

recta) of the juxtamedullary nephron, assuming that furosemide did not increase potassium reabsorption by the juxtamedullary proximal tubule. While it is not possible to test that assumption directly because the juxtamedullary proximal tubule is inaccessible to micropuncture, it was assessed indirectly by determining fractional reabsorption near the end of the accessible superficial proximal tubule in three rats. Urinary excretion data in these rats did not differ from those of the other seven rats in group 2 and were therefore combined (Table 1). Micropuncture results are summarized in Table 3. In nine re-collections from three rats, fractional reabsorption of water, sodium and potassium did not change significantly. Thus, furosemide in the dose employed did not significantly affect overall reabsorption by the proximal tubule, in agreement with our previous findings [11].

Benzolamide-treated rats (group 3). The effect of benzolamide on urinary excretion is summarized in Table 1, and on loop of Henle fluid in Table 2. Figure 2 illustrates one experiment. Within ten minutes after the benzolamide injection (Fig. 2), TF/P inulin decreased from 4.8 to 4.3 and thereafter remained unchanged. TF/P potassium increased from 5.2 to 8.7 and then decreased to 4.9. TF/P osmolality rose slightly; TF/P sodium remained essentially unchanged. Urinary excretion of potassium increased

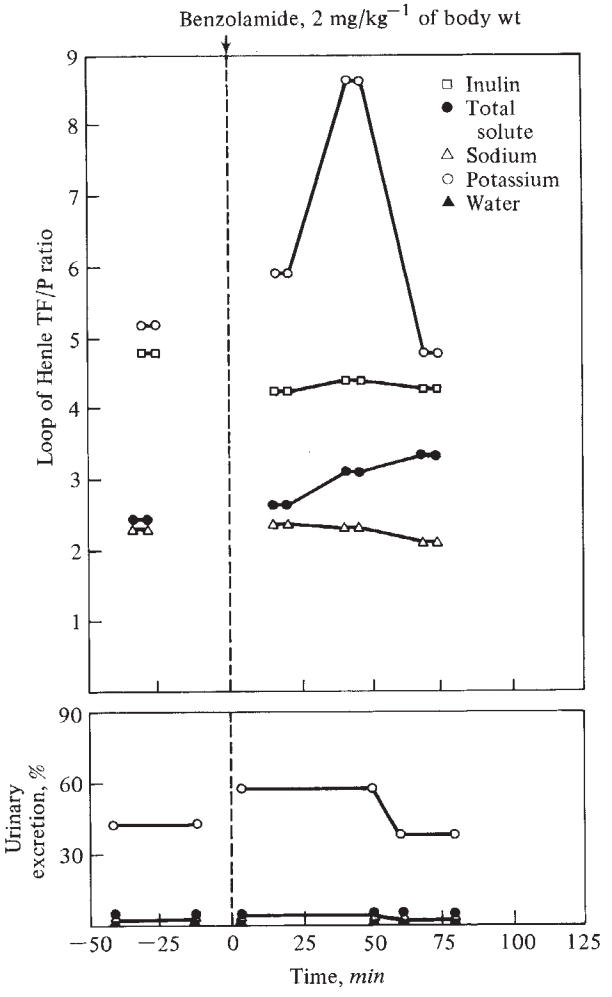

Fig. 2. *Effect of benzolamide in one rat.* Please see legend to Fig. 1. Urinary excretion of total solute, sodium and water is depicted as one line since values for each were similarly low. At zero time, indicated by *vertical dashed line,* benzolamide, 2 mg/kg of body wt, was injected. Note the changes in TF/P potassium and TF/P inulin. For further description, see text.

Table 3. Summary of micropuncture results (end-accessible proximal tubule) in control, furosemide-treated and benzolamide-treated rats[a]

	Group			
	Furosemide		Benzolamide	
	First period	Second period	First period	Second period
	Micropuncture of end-accessible proximal tubule			
TF/P inulin	2.37 ± 0.04	2.30 ± 0.07	2.70 ± 0.13	1.36 ± 0.09[b]
TF/P sodium	1.05 ± 0.00	1.06 ± 0.06	0.99 ± 0.04[c]	0.95 ± 0.02
TF/P potassium	1.12 ± 0.05	1.07 ± 0.03	1.12 ± 0.03[c]	0.96 ± 0.02
	Fraction of filtered load remaining at end-accessible proximal tubule (%)			
Water	43 ± 0.7	44 ± 1.6	37 ± 1.8	76 ± 4.7[b]
Sodium	45 ± 2.7	47 ± 1.4	37 ± 2.3[c]	74 ± 6.3[b]
Potassium	49 ± 2.2	47 ± 1.3	43 ± 3.9[c]	76 ± 4.2[b]

[a] Values are means ± SEM. N of rats: furosemide, 3; benzolamide, 4.
[b] $P < 0.001$, compared to first period.
[c] $N = 3$.

from 43 to 58%. The magnitude of the increase in urinary excretion of water, total solute, and sodium was much smaller than that seen following furosemide.

As in the furosemide group, the data for each variable following benzolamide administration were averaged for each rat and considered as a single value (Table 2). Several differences between the effects of benzolamide and those of furosemide (Table 2) are apparent. Although there was a significant decrease in fractional reabsorption of water, as indicated by the decrease of TF/P inulin, the TF/P ratios for total solute, sodium and potassium did not change significantly. The fraction of filtered sodium remaining at the end of the descending limb increased significantly by 10% from 39 to 49%, consistent with inhibition by benzolamide of sodium reabsorption upsteam in the juxtamedullary proximal tubule. Especially noteworthy is the finding that the percentage of filtered potassium remainimg significantly increased to 177% of the filtered load, a value which is statistically greater than 100% ($P < 0.05$)—unequivocal evidence for net potassium secretion by the juxtamedullary nephron up to the hairpin turn.

As a check that benzolamide in the dose used inhibited fractional reabsorption in the proximal tubule of the superficial nephron, re-collection micropuncture experiments were performed in four rats. There was a substantial suppression of fractional reabsorption of water, sodium and potassium approximately from 65 to 25% (Table 3). Thus benzolamide, in contrast to furosemide, significantly reduced overall reabsorption by the accessible proximal tubule of the superficial nephron.

Discussion

The proposal that the high concentration of potassium found in loop fluid is due to water extraction from the descending limb [8] fails to account for the present results. Instead, the combined data, in our view, provide strong support for the hypothesis that potassium is normally secreted by the descending limb (or pars recta) of the juxtamedullary nephron *in vivo*[1].

Nondiuretic rats (group 1). The mean percentage of filtered potassium remaining at the end of the descending limb equalled the filtered load of potassium. If the rise in potassium concentration in fluid at the end of the descending limb is to be ascribed exclusively to water abstraction, that also requires that no net potassium reabsorption occurs in the juxtamedullary proximal tubule, which seems very unlikely.

Furosemide-treated animals (group 2). The decrease in potassium remaining at the end of the descending limb can be accounted for by *1)* increased potassium reabsorption or decreased potassium secretion in the juxtamedullary proximal tubule or *2)* increased potassium reabsorption or decreased potassium secretion in the abbreviated pars recta [17] or descending limb of the juxtamedullary nephron.

1) First alternative (increased potassium reabsorption or decreased potassium secretion in the juxtamedullary proximal tubule). According to the current view, furosemide directly inhibits active chloride reabsorption in the thick ascending limb but has no direct effect upstream in the proximal tubule [12, 18, 19]. One circumstance in which furosemide might enhance potassium reabsorption in the juxtamedullary proximal tubule is if the diuretic caused significant extracellular volume depletion, resulting in increased reabsorption of sodium, potassium and water (assuming reabsorption in the juxtamedullary proximal tubule, like that in the superficial proximal tubule, is normally isotonic). To avoid volume depletion, urinary losses of sodium and water

[1] The definition of secretion used in this paper is the transport of a substance from the peritubular fluid to tubule lumen. The mechanism involved may either be active or passive [16].

were replaced. No evidence of increased reabsorption by the superficial proximal tubule after furosemide (Table 3), as might be expected with significant volume depletion, was observed. Moreover, if furosemide enhanced reabsorption in the juxtamedullary proximal tubule, the fraction of filtered sodium remaining at the end of the descending limb should have decreased, like that of potassium, rather than remain unchanged, unless at the same time there was also an equivalent increase in sodium secretion into the descending limb. As yet, however, there is no clear evidence for sodium secretion by the descending limb in the rat [7, 11, 14, 20]. Thus, it seems unlikely that furosemide increased potassium reabsorption in the juxtamedullary proximal tubule. (If furosemide partially inhibited potassium reabsorption in the juxtamedullary proximal tubule, the interpretation that potassium is normally secreted beyond the proximal tubule is, of course strengthened, because the amount of potassium reaching the end of the descending limb was reduced, not increased after furosemide). Similar considerations make it unlikely that furosemide reduced potassium secretion by the juxtamedullary proximal tubule. Although le Grimellec has found that the concentration of potassium in proximal tubule fluid actually rises slightly above that in the glomerular filtrate [21], to our knowledge no evidence for potassium secretion in the convoluted proximal tubule or inhibition of a potassium secretory process therein by furosemide has been reported.

2) Second alternative (increased potassium reabsorption or decreased potassium secretion in the juxtamedullary descending limb or pars recta). No evidence for potassium reabsorption by the descending limb, to our knowledge, has been reported [8]. If potassium is normally reabsorbed in the descending limb, the finding that the potassium remaining at the end of the descending limb equals the filtered load automatically requires net potassium secretion in the proximal tubule of the juxtamedullary nephron. That possibility has already been discounted above as unlikely. By exclusion we are left with the interpretation that furosemide diminished net entry of potassium into the descending limb (or short pars recta—see following).

Furosemide as a consequence of its inhibition of active chloride transport in the thick ascending limb reduced medullary interstitial sodium and total solute concentration to values approaching those in systemic plasma [11, 22]. It very likely reduces the concentration of potassium in the medullary interstitium too (ethacrynic acid, whose inhibitory action is the same as that of furosemide [12, 23], has been shown to reduce medullary interstitial potassium concentration [24]). If transtubular entry (secretion) of potassium into the descending limb, whatever its mechanism, normally occurs, then furosemide may diminish potassium entry into the descending limb by reducing the transtubular potassium gradient.

Benzolamide-treated animals (group 3). The purpose of giving benzolamide was opposite to that of administering furosemide: to inhibit reabsorption selectively in the juxtamedullary proximal tubule upstream to the descending limb. Benzolamide inhibits carbonic anhydrase and thereby reduces isotonic reabsorption in the proximal convoluted tubule [13]. Not only did the amount of filtered potassium remaining at the end of the descending limb increase significantly after benzolamide (Table 2) but it reached 177%, a value significantly greater than 100%, unequivocally indicating net potassium secretion. Presumably, this is due to benzolamide inhibition of reabsorption in the juxtamedullary proximal tubule. The validity of that assumption is supported by two independent observations following the administration of benzolamide: the suppression of isotonic reabsorption of water, sodium and potassium in the accessible portion of the superficial proximal tubule (Table 3), and the increase in sodium and water remaining at the end of the juxtamedullary descending limb (Table 2). The potassium flow at the end of the descending limb following benzolamide administration would be increased by the increased delivery of potassium to the beginning of the descending limb. In addition, benzolamide might have increased potassium secretion by the descending limb. If net transtubular potassium entry is a function of the difference in potassium concentration across the descending limb, the greater the volume flow of fluid entering the descending limb, the greater the transtubular potassium influx. This might account for the fact that the increment in percent of filtered potassium remaining at the end of the descending limb exceeded that of sodium.

Site of potassium secretion. An interpretation consistent with the combined results of the three experimental groups is that potassium is normally reabsorbed in the juxtamedullary proximal convoluted tubule and secreted either in the short pars recta or the descending limb[2]. Grantham, Qualizza and Irwin

[2] We assumed that in the juxtamedullary proximal convoluted tubule, benzolamide inhibited isotonic reabsortion and that furosemide did not enhance it, effects qualitatively similar to those of the two diuretics, respectively, in the superficial proximal convoluted tubule (Table 3). These assumptions were indirectly confirmed by the increase (benzolamide) or lack of significant change (furosemide), respectively, in the filtered sodium remaining at the end of the descending limb (Table 2). If either assumption is incorrect, our results require either that potassium is not reabsorbed at all or that it is actively secreted in the proximal convoluted tubule of the juxtamedullary nephron.

[25] have demonstrated potassium secretion by the pars recta of the rabbit nephron *in vitro.* They inserted a pipet into one end of a pars recta, occluded the other end and immersed the segment in a bath of rabbit serum. Normally, the lumen remained collapsed. In the presence of para-amino hippuric acid (PAH) in the bath, however, the lumen was opened by continuous transtubular fluid secretion into it. The sodium concentration of the secreted fluid and that of the bath were the same, but the potassium concentration of the secreted fluid, 6.9 mEq/liter, was significantly higher than that of the bath, 4.9 mEq/liter. The findings imply that potassium is secreted by the pars recta *in vitro,* at least in the presence of PAH. The mechanism of potassium secretion, while not understood, may be a form of cation cotransport coupled to organic anion secretion, since with PAH in the bath, the intraluminal negativity of the pars recta was increased by approximately 1 mv [25].

If such a phenomenon exists *in vivo,* it must be dependent on the secretion of naturally occurring organic acids. The stimulating effect of benzolamide on potassium secretion may be explained, at least in part, on this basis, since the drug is likely secreted by the same mechanism as PAH [26]. It seems unlikely, however, that potassium secretion is confined to the pars recta of the juxtamedullary nephron, since that fails to explain the reduced potassium flow at the end of the descending limb observed after furosemide administration. Furosemide is transported by the same secretory mechanism as PAH [27]. Thus, one might expect furosemide, as an organic anion, to stimulate potassium secretion by the pars recta, analogous to the observed effect of PAH in the pars recta *in vitro* and to the postulated stimulating action of benzolamide previously discussed. This would result in an increase in potassium remaining at the end of the descending limb, in direct contrast to what in fact was observed. It is therefore concluded that potassium is secreted across the juxtamedullary descending limb.

Mechanisms of potassium secretion in the descending limb. Since no active transport process has yet been discovered in the descending limb [8, 20, 27], a theoretical model was constructed to determine if the amount of potassium normally remaining in fluid at the end of the descending limb could be accounted for by passive driving forces across the descending limb (see Appendix). The assumptions concerning exterior conditions (Fig. 3) and membrane characteristics of the descending limb are stated in the Appendix. The resulting computation, illustrated in Fig. 4, indicates that the sharpest decrease in volume flow rate from 9 to 4.5 nl/min occurs in the first

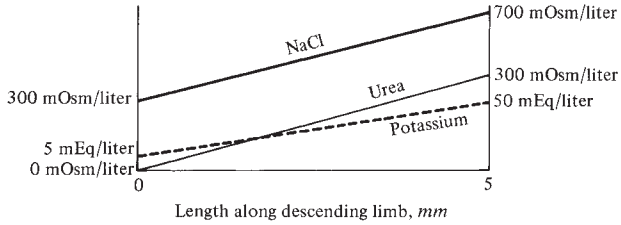

Fig. 3. *Exterior concentrations of NaCl, urea and potassium in the medullary interstitium from the beginning of the descending limb to the renal papillary tip which were used in computer simulation.* For further description, see Appendix.

2 mm of the descending limb; the concomitant rise in potassium concentration from 5 to 12.5 mEq/liter in the same segment is accounted for primarily (but not completely) by water abstraction, as suggested by Rocha and Kokko on the basis of perfusing similarly short fragments (approximately 1 to 2 mm) of descending limb *in vitro* [8]. Thereafter, the diminution in volume flow is much less striking, but the rise in potassium flow rate, indicating potassium secretion, continues throughout the remainder of the descending limb. The tubule fluid potassium concentration continuously increases (Fig. 4), reaching a value at the hairpin turn similar to that found in normal rats (group 1).

Two points deserve emphasis. The first is that al-

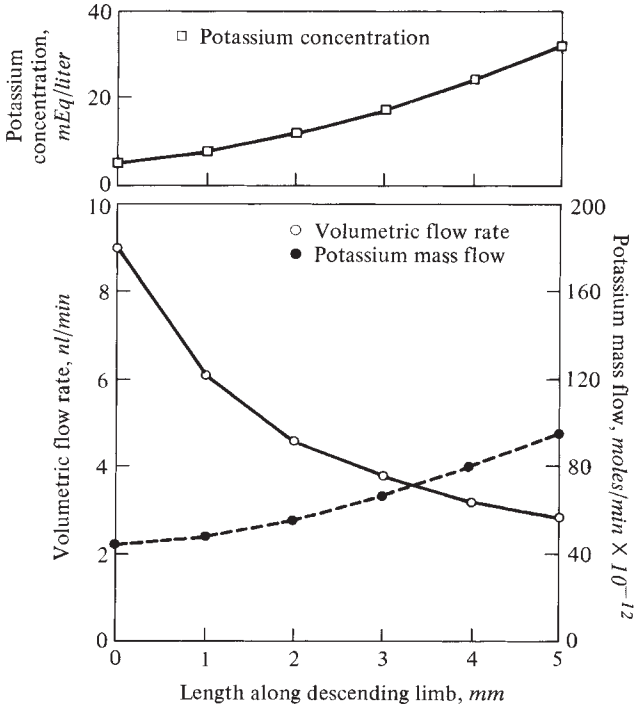

Fig. 4. *Results of computer simulation. Upper panel:* ordinate represents potassium concentration in fluid along the descending limb. *Lower panel:* ordinate on left (*open circles* and *solid line*), volumetric flow rate; ordinate on right (*closed circles* and *dashed line*), mass flow of potassium. For further description, see text and Appendix.

though our interpretation of these findings differs from Rocha and Kokko's interpretation of theirs, the disagreement reflects no fundamental conflict in experimental data. In fact, the present interpretation that potassium is normally secreted into the descending limb *in vivo* is based on a model incorporating membrane characteristics reported for the rabbit descending limb *in vitro*. The basis of the contrasting interpretations lies in the difference between *in vitro* and *in vivo* exterior conditions. The concentration of potassium in the *in vitro* bath was only 5 mEq/liter [8], while the concentration of potassium of vasa recta plasma, which presumably reflects that of papillary interstitial fluid [9, 28], is greater by an order of magnitude. Furthermore, it is impossible to duplicate *in vitro* the profile of the progressive rise in exterior total solute concentration along the descending limb *in vivo*.

The second point is that while an active potassium secretory process is not essential to explain these results, such a process is not excluded. The source of the high potassium concentration in the medullary interstitium is unclear. As countercurrent exchangers, vasa recta act to preserve a high interstitial potassium concentration, but cannot be its source [1, 28, 29]. Potassium reabsorption by the papillary collecting duct [3, 30, 31] or medullary collecting duct [31, 32] is frequently absent in nondiuretic rats and is regularly demonstrable only in potassium depletion [30] or in water diuresis [31]. Thus, the most likely source of potassium secreted into the outer medullary segment of the descending limb is the thick ascending limb, while the potassium secreted into the descending limb in the inner medulla comes from the thin ascending limb, as previously suggested[3][3–5].

Physiological significance of potassium secretion in the juxtamedullary pars recta or descending limb. The present experiments shed no light on the physiological significance of potassium secretion at this site in the nephron. It is conceivable that the high potassium concentration in the medullary interstitium exercises a regulatory influence on transtubular potassium transfer by the collecting duct. The fact that papillectomy significantly reduced the rate of potassium secretion in potassium-loaded rats, but does not impair sodium reabsorption in the same animals (according to a preliminary report [33]), is consistent with this speculation. Determination of the extent of potassium secretion by the juxtamedullary descending limb in potassium-loaded rats might prove enlightening in this regard.

[3] Cell potassium could not be the source in the steady state. We calculated that the amount of potassium removed by flow through loops of Henle and vasa recta would exhaust all intracellular potassium in the papilla within two minutes.

Appendix

The purpose of this appendix is to determine whether passive driving forces can account for the amount of potassium secreted and the level of potassium concentration in fluid at the end of the juxtamedullary descending limb (DLH) in nondiuretic rats. The results suggest that passive forces are indeed sufficient.

The model chosen is a semi-flow-through system in which exterior conditions in the medullary interstitium are forced to represent linear concentration profiles for NaCl, K plus univalent anion and urea in the medullary interstitium. For simplicity, transepithelial salt flux is assumed to be negligible [7, 14, 20].

The mass balance equations for water and solutes can be written as follows:

$$dQ/dx = -J_v \text{ for water} \quad (1)$$

$$d(C_kQ)/dx = +J_k \text{ for potassium} \quad (2)$$

$$d(C_{urea}Q)/dx = +J_u \text{ for urea} \quad (3)$$

$$C_{NaCl}Q = \text{constant for sodium chloride} \quad (4)$$

where Q = volumetric flow rate; J_v = flux of water per unit length out of descending limb; J_k = flux of potassium per unit length into descending limb; J_u = flux of urea per unit length into descending limb; and C_{NaCl}, C_k, C_{urea} = tubule fluid concentrations of NaCl, KCl and urea.

The transmural fluxes of water and solutes can be described by the following equations from linear nonequilibrium thermodynamics [34].

$$J_v = L_pRT\, S_p\, (\Sigma\sigma_i \cdot \Delta C_i) \quad (5)$$

$$= L_pRTS_p\, [\sigma_{NaCl}\, (\Delta C_{NaCl}) + \sigma_{KCl}\, (\Delta C_{KCl}) + \sigma_{urea}\, (\Delta C_{urea})] \quad (6)$$

$$J_k = P_k\, S_p\, (\Delta C_K) \quad (7)$$

$$J_u = P_u\, S_p\, (\Delta C_{urea}) \quad (8)$$

where L_p = hydraulic permeability of the membrane; R = universal gas constant; T = absolute temperature; P_k, P_u = permeability of membrane to potassium and urea, respectively; σ_{NaCl}, σ_{KCl}, σ_{urea} = reflection coefficients of membrane to NaCl, KCl and urea, respectively; S_p = surface area per unit length; ΔC_{NaCl}, ΔC_{KCl}, ΔC_{urea} = concentration gradients of NaCl, KCl and urea, respectively, interstitium to lumen. The solvent drag terms in equations [7] and [8]

were excluded because the high reflection coefficients would render them negligible. This was confirmed *a posteriori* from the solutions.

The solute mass balance equations:

$$d(C_sQ)/dx = J_s \cdot (\text{where "s" is any solute}) \quad (9)$$

can be simplified to

$$Q \times dC_s/dx + C_s \times dQ/dx = J_s \quad (10)$$

and the term dQ/dx obtained from the previous volume mass balance equation (1).

Therefore,

$$dC_s/dx = 1/Q\ (J_s + C_cJ_v) \quad (11)$$

$$dQ/dx = -J_v. \quad (12)$$

This is a set of nonlinear first order differential equations which may be solved using the Runge-Kutta-Merson scheme. The equations were solved step-wise, using a step size of 0.05 mm, over 5 mm of length, assuming the exterior concentrations varied linearly, as shown in Fig. 3. The external potassium concentration was assumed to vary linearly from 5 mEq/liter (systemic plasma value) at the beginning of the DLH to 50 mEq/liter at the tip of the papilla (based on the mean potassium concentration in vasa recta plasma).

In addition, the following assumptions were made: initial flow rate, 9 nl/min [7, 14]; initial tubule fluid composition, same as external initial conditions; length of descending limb, 5 mm [7, 14]; $L_p = 1.71 \times 10^{-4}$ cm^3/(cm^2·sec·atm) [20]; $P_u = 1.5 \times 10^{-5}$ cm/sec [27]; $P_k = 2.5 \times 10^{-5}$ cm/sec [8]; $S_p = 60 \times 10^{-4}$ cm (27); $\sigma_{urea} = 0.95$ [27]; and $\sigma_{NaCl} = \sigma_{KCl} = 0.96$ [8, 20]. To calculate the interior and exterior osmolality due to potassium and its attendant anion, the potassium concentration was multiplied by 2.

The results are depicted in Fig. 4. Water reabsorption occurs principally in the earlier portion of the descending limb, and accounts for most of the rise in potassium concentration in the first 2 mm. Potassium secretion occurs along the entire DLH, resulting in a potassium concentration in fluid at the end of the DLH similar to that observed in nondiuretic rats (group 1).

Acknowledgments

Preliminary reports of portions of this work have been presented to the American Federation for Clinical Research and have appeared in abstract form: *Clin Res* 22:533A, 1974, and 23:367A, 1975. This investigation was supported by Public Health Service research grants HL 14784 and 18077 from the National Institutes of Health and by an American Heart Association grant-in-aid (72821). Dr. Jamison was a recipient of Public Health Service Research Career Development Award 7-K04-HL42685 from the National Heart and Lung Institute and was a Markle Scholar in Academic Medicine. Professor Thomas Maren supplied the benzolamide used in this study and provided advice on how to use it. Mary Fisher and Steven Pottier provided technical assistance; Cassie Laffoon and Helen Ramey, secretarial assistance; and Roy Maffly and Channing Robertson, helpful discussions.

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