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Mechanism by which enhanced ammonia production reduces urinary potassium excretion

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Mechanism by which enhanced ammonia production reduces urinary potassium excretion. To determine the mechanism whereby an increase in ammonia production decreases urinary potassium excretion, we perfused isolated rat kidneys at a pH of either 7.0 or 7.4. After 45 min of perfusion at either pH, glutamine or ammonium chloride was added to the perfusate to result in concentrations of 5 and 0.8 mM, respectively and observations were continued for 50 min. Control kidneys were perfused at both pH's without further additions to the perfusate. At pH 7.0, glutamine increased ammonia production and increased urinary ammonium excretion strikingly; whereas the addition of ammonium chloride did not change ammonia production but increased urinary ammonium excretion to a comparable degree. Both maneuvers resulted in a reciprocal fall in urinary potassium excretion in comparison with control perfusions. The decrease in potassium excretion could not be accounted for by differences in perfusate or urinary acid-base parameters, or by changes in urinary sodium, water, or chloride excretion. At pH 7.4, glutamine also significantly increased ammonia production and perfusate ammonia concentration. In contrast to the studies at pH 7.0 in which the urine pH was acid (5.9), the urine remained alkaline (pH 7.2), and both urinary ammonium excretion and urinary potassium excretion were unaltered. Thus, potassium sparing is not a nonspecific effect of glutamine, its metabolism to ammonia, or perfusate ammonia concentration but is directly related to an increase in urinary ammonium excretion.

Mécanisme par lequel une augmentation de la production d'ammoniaque réduit l'excrétion urinaire de potassium. Afin de déterminer le mécanisme par lequel une augmentation de la production d'ammoniaque diminue l'excrétion urinaire de potassium, des reins de rats isolés ont été perfusés soit à pH 7,0, soit à pH 7,4. Après 45 min de perfusion à l'un ou l'autre pH, de la glutamine ou du chlorure d'ammonium ont été ajoutés au perfusat afin d'obtenir des concentrations respectivement de 5.0 et 0,8 mM, puis la préparation a encore été étudiée pendant 50 min. Des reins témoins ont été perfusés aux deux pH sans autres additions au perfusat. A pH 7,0 la glutamine augmente la production d'ammoniaque et augmente considérablement l'excrétion d'ammoniaque alors que l'addition de chorure d'ammonium ne modifie pas la production d'ammoniaque mais augmente de façon comparable l'excrétion urinaire. Les deux manoeuvres ont pour conséquence une diminution de l'excrétion urinaire de potassium par comparaison avec les perfusions contrôles. La diminution de l'excrétion de potassium ne peut pas être expliquée par des différences d'état acido-basique du perfusat ou de l'urine ou par des modifications de l'excrétion urinaire de sodium, d'eau ou de chlore. A pH 7,4 la glutamine augmente aussi la production d'ammoniaque et la concentration d'ammoniaque dans le perfusat. Cependant, à la différence des études à pH 7,0 au cours desquelles le pH de l'urine était acide (5,9), l'urine est restée alcaline (pH 7,2) et l'excrétion urinaire d'ammoniaque de même que l'excrétion de potassium n'ont pas été modifiées. Ainsi l'économie de potassium n'est pas un effet non spécifique de la glutamine, de son métabolisme en ammoniaque ou de la concentration en ammoniaque du perfusat, mais est directement liéeà l'augmentation de l'excrétion urinaire de potassium.

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The status of body potassium appears to influence renal ammonia production. Definitive evidence exists in whole animals and in vitro that potassium depletion stimulates ammoniagenesis [1–6], and there is suggestive evidence that a potassium surfeit diminishes renal ammonia production [6–9]. The reciprocal relationship between urinary potassium excretion and renal ammonia production led to the hypothesis that ammonia production, which is regulated by potassium status, might in turn alter urinary potassium excretion [10].

Previous experiments from this laboratory, which used glutamine as a tool for increasing renal ammonia production independent of changes in systemic acid-base or potassium status, suggested that enhanced ammonia production and urinary excretion could indeed modify potassium excretion [11]. Normal men, under normal acid-base conditions or acute metabolic acidosis, responded to glutamine ingestion with a decrease in potassium excretion. By contrast, when glutamine was administered following several days of alkali ingestion, its effect on renal ammoniagenesis was presumably diminished, urinary ammonium excretion changed minimally and the effect on potassium excretion was markedly dampened. These data provided strong support for the hypothesis that ammonia production and excretion could modify potassium handling.

But there were a number of unresolved issues. Although it was thought to be unlikely, the possibility that glutamine acted by modifying systemic hormones such as insulin, glucagon, or aldosterone could not be absolutely excluded. The possibility that alterations in renal sodium handling accounted for the effects was not definitively resolved. Finally, it was not clear whether the metabolism of glutamine itself or whether alterations in ammonium excretion resulting from its metabolism accounted for the changes in potassium excretion.

To test these possibilities under conditions where the key parameters could either be rigorously controlled or carefully monitored, we carried out studies using the isolated perfused rat kidney.

Methods

Kidneys from male Sprague-Dawley rats were used. Each rat weighed between 250 and 330 g and was fed on standard

laboratory rat chow (Teklad 4% Mouse and Rat Diet, Winfield, Iowa). After the rats had been anesthetized with pentobarbital (50 mg/kg, i.p.), pulsatile perfusion of the isolated kidneys were carried out as described previously with 7.5% bovine serum albumin fraction V in Krebs-Henseleit saline equilibrated with 95% oxygen and 5% carbon dioxide [12, 13]. D-Glucose (5 mM) was added as substrate in all experiments. The mean perfusate pressure at the tip of cannula was kept between 90 and 100 mm Hg throughout the experiment. To maintain a constant pressure, we adjusted the flow of the perfusate with a needle valve and monitored it by a floating ball flowmeter. It was relatively stable throughout each experiment and averaged 37.1 ± 1.7 ml/min.

The perfusions were carried out for 95 min. After 15 min of equilibration, 10- to 15-min urine collections were started, with a perfusate sample taken at the beginning and at the end of each period. Two groups of studies were performed.

Group 1: Perfusate pH of 7.4. (a) Control study (N = 5). Kidneys were perfused for the 95 min without the addition of glutamine. *(b) Glutamine study (N = 5).* After the first 45 min of perfusion, ℓ-glutamine was added to achieve a concentration of 5 mM. *(c) Ammonium chloride study (N = 8).* After the first 45 min of perfusion, ammonium chloride was added to achieve a concentration of 0.8 mM. To achieve a control group with comparable baseline data, we performed a separate group of control studies for comparison with this ammonium chloride group.

Group 2: Perfusate pH of 7.0. These were similar to the first group except that the perfusions were carried out at a pH of approximately 7.0, rather than at 7.4, by isotonically substituting chloride for bicarbonate in the perfusate: *(a) control study (N = 6), (b) glutamine study (N = 6), (c) ammonium chloride study (N = 7).*

Analytical methods. Glomerular filtration rate was determined with ^{14}C-inulin. Sodium and potassium concentrations were measured by flame photometry. Perfusate and urine pH's were measured anaerobically at 37° C using a Corning pH meter with an Instrumentation Laboratory 329 electrode; and total carbon dioxide, with a Natelson microgasometer. Urine chloride was determined using a chloridometer. Ammonia concentration in the perfusate and urine from experiments in group 2, a and b, were measured with an Orion ammonia electrode [14, 15]. In the remaining experiments, concentration in perfusate was measured enzymatically [16]; and in the urine, colorimetrically [17]. These three methods have been shown to yield comparable results [15, 18]. The rate of ammonia production was calculated from the accumulation of ammonia in the perfusate plus the amount excreted in the urine. Significance was tested with Students *t* test, and the values given are the means ± SEM.

Results

Perfusate pH of 7.0. To take into account changes in kidney function over time, we analyzed the data by comparing the glutamine and ammonium chloride study at each perfusate pH with the respective control, during both the initial and experimental period. The experimental period was considered to begin 10 min after addition of glutamine or ammonium chloride to the perfusate, but analysis of the data including this initial 10-min period did not alter the conclusions.

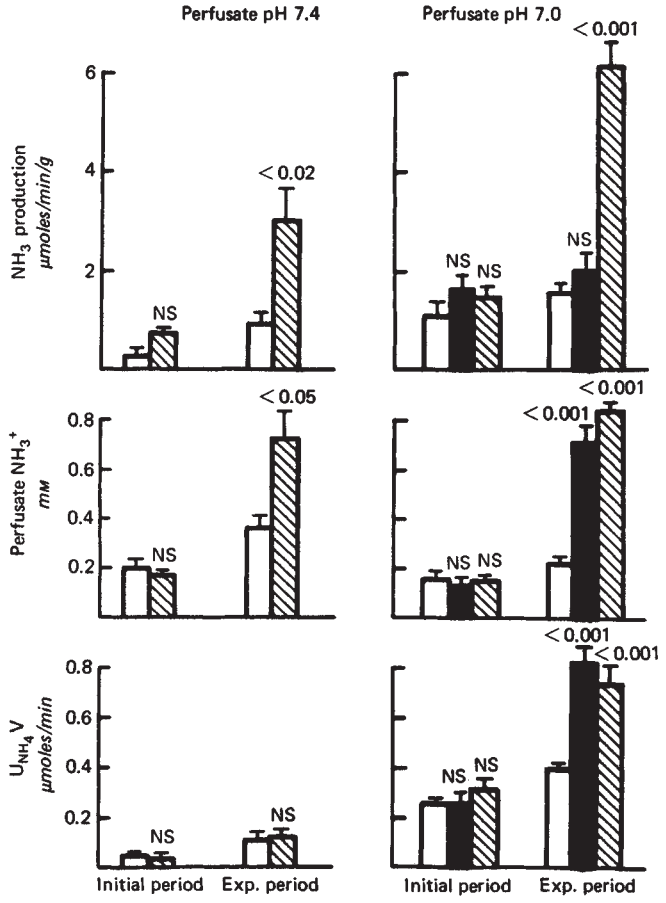

Fig. 1. *Effect of glutamine and ammonium chloride on renal ammonia production, perfusate ammonia level, and urinary ammonium excretion.* Glutamine significantly increased ammonia production and perfusate ammonia level at both perfusate pH's. Addition of ammonium chloride did not alter ammonia production but raised perfusate ammonia concentration to the same levels seen in the glutamine studies. Urinary ammonium excretion increased significantly in both the glutamine and ammonium chloride perfusions at pH 7.0. By contrast ammonium excretion did not change in the glutamine studies at pH 7.4 despite a significant increase in ammonia production and similar perfusate ammonia concentration. All *P* values indicate a comparison between each experimental group and the respective control. Control studies are represented by *open bars*, glutamine studies by *hatched bars*, and ammonium chloride studies by *dark bars*.

(a) Glutamine studies. During the initial period, perfusate and urinary acid-base and potassium parameters were similar in the control and glutamine studies. Ammonia production averaged 1.09 ± 0.29 and 1.50 ± 0.19 µmoles/min/g dry kidney wt, respectively (Fig. 1). Similarly ammonium excretion, urine pH, perfusate pH, perfusate total carbon dioxide, perfusate potassium, and both absolute and fractional potassium excretion did not differ between the two groups (Table 1, Figs. 1 and 2).

When glutamine was added to the perfusate during the experimental period, ammonia production increased strikingly in comparison with the control kidneys (6.18 ± 0.48 vs. 1.59 ± 0.16 µmoles/min/g) (Fig. 1), and this was accompanied by a significant increase in urinary ammonium excretion (Fig. 1). Urine pH remained acid in both the control and the glutamine studies, averaging 5.94 and 5.96, respectively, during the experimental period.

Table 1. Control, glutamine, and ammonium chloride experiments at perfusate pH 7.0

	Control study (N = 6)			Glutamine study (N = 6)			NH_4Cl study (N = 7)		
	Initial period	Experimental period	P[a]	Initial period	Experimental period	P	Initial period	Experimental period	P
GFR, *ml/min*	0.57 ± 0.08	0.39 ± 0.04	<0.01	0.47 ± 0.04	0.39 ± 0.03	NS	0.64 ± 0.05	0.41 ± 0.03	<0.001
$U_{Na}V$, *μmoles/min*	4.33 ± 0.99	4.82 ± 0.61	NS	5.02 ± 0.49	3.57 ± 0.70	<0.05	6.44 ± 1.16	6.08 ± 0.93	NS
FE_{Na}, *%*	5.68 ± 1.43	8.61 ± 0.91	NS	7.43 ± 0.50	6.28 ± 0.87	NS	7.36 ± 1.39	10.34 ± 2.02	<0.05
$U_{Cl}V$, *μmoles/min*	5.50 ± 1.07	7.14 ± 0.61	NS	6.27 ± 0.67[c]	5.50 ± 0.83[c]	NS	8.94 ± 1.18	7.99 ± 0.88	NS
Urine flow, *ml/min*	0.04 ± 0.01	0.06 ± 0.01	<0.05	0.04 ± 0.003	0.05 ± 0.004	NS	0.08 ± 0.01	0.11 ± 0.01[b]	<0.05
V/GFR	0.07 ± 0.02	0.17 ± 0.03	<0.01	0.09 ± 0.01	0.13 ± 0.01	<0.02	0.13 ± 0.02	0.28 ± 0.03[b]	<0.001
Kidney dry wt, *g*	0.25 ± 0.01	—		0.25 ± 0.01	—		0.24 ± 0.01	—	
$U_{NH_4}V$, *μmoles/min*	0.26 ± 0.02	0.40 ± 0.02	<0.01	0.32 ± 0.03	0.76 ± 0.07[b]	<0.001	0.26 ± 0.04	0.83 ± 0.06[b]	<0.001
U_KV, *μmoles/min*	0.93 ± 0.11	1.11 ± 0.13	NS	0.92 ± 0.12	0.66 ± 0.11[b]	<0.001	1.69 ± 0.17[b]	0.80 ± 0.07[b]	<0.01
FE_K	0.35 ± 0.04	0.67 ± 0.05	<0.01	0.42 ± 0.06	0.39 ± 0.06[b]	NS	0.53 ± 0.04[b]	0.44 ± 0.03[b]	NS
Urine pH	6.21 ± 0.10[d]	5.94 ± 0.09	<0.05	6.09 ± 0.05[e]	5.96 ± 0.10	NS	6.20 ± 0.11	6.23 ± 0.09[b]	NS
Perfusate pH	6.94 ± 0.03	6.97 ± 0.04	NS	6.91 ± 0.06	6.95 ± 0.06	<0.01	7.01 ± 0.01	7.06 ± 0.01[b]	<0.001
Perfusate total CO_2, *mM*	10.3 ± 0.43	10.8 ± 0.29	NS	9.2 ± 0.62	9.5 ± 0.67	NS	11.8 ± 0.17[b]	12.3 ± 0.24[b]	<0.01
Perfusate $[K^+]$, *mM*	4.9 ± 0.05	4.5 ± 0.06	<0.001	4.9 ± 0.03	4.5 ± 0.06	<0.001	5.0 ± 0.04	4.4 ± 0.12	<0.001
Perfusate $[NH_3^+]$, *mM*	0.16 ± 0.03	0.22 ± 0.03	<0.01	0.15 ± 0.01	0.84 ± 0.03[b]	<0.001	0.13 ± 0.03	0.71 ± 0.07[b]	<0.001
NH_3 production, *μmoles/min/g dry wt*	1.09 ± 0.29	1.59 ± 0.16	NS	1.50 ± 0.19	6.18 ± 0.48[b]	<0.001	1.65 ± 0.28	2.04 ± 0.34	NS

[a] Comparison between experimental and initial period
[b] Significant difference ($P < 0.05$) in comparison with the control study
[c] Data from 5 studies
[d] Data from 3 experiments
[e] Data from 5 experiments

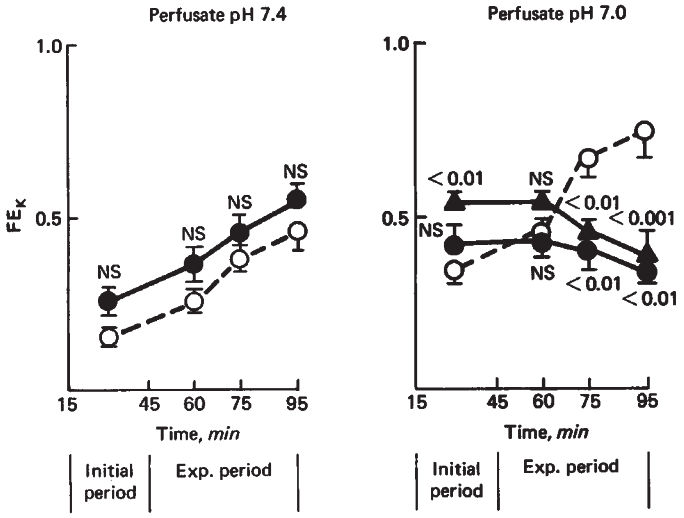

Fig. 2. *Effect of glutamine and ammonium chloride on fractional potassium excretion (FE_K).* At acidotic pH the addition of either glutamine or ammonium chloride decreased FE_K in comparison with the timed control. By contrast, at pH 7.4, no effect of glutamine on FE_K was apparent. Control studies are represented by *open circles*, glutamine studies by *closed circles*, and ammonium chloride studies by *triangles*.

Glutamine addition to the perfusate resulted in a significant decrease in both the absolute and fractional excretion (FE) rates of potassium in comparison with control studies (Fig. 2 and Table 1). Perfusate potassium concentration was identical in the glutamine and control studies.

As shown in Table 1, there were no significant differences in GFR, absolute or fractional sodium excretion, absolute or fractional urine flow rate, or chloride excretion between the control and glutamine studies. But, as shown in Table 2, when the difference between the experimental and initial period is considered, the changes in absolute and fractional sodium excretion, and fractional urine flow were all significantly lower in the glutamine studies in comparison with the controls, suggesting that glutamine may have enhanced both sodium and water reabsorption. As shown in Fig. 3, however, changes in FE_{Na}, V/GFR, and chloride excretion do not appear to account for the decrease in potassium excretion, because FE_K is significantly lower in the glutamine studies at a comparable FE_{Na}, FE_{H_2O}, and absolute chloride excretion.

(b) Ammonium choride studies. When ammonium chloride was added to an acidified perfusate, renal ammonia production was not altered, but the increases in the perfusate ammonia concentration and the urinary ammonium excretion were comparable to the glutamine studies (Table 1 and Fig. 1). This was accompanied by a decrease in absolute potassium excretion and a change in FE_K which paralleled the change seen with glutamine addition (Table 1 and Fig. 2). Although the absolute and fractional rates of potassium excretion were higher in the initial period of the ammonium chloride than the control studies, the values after ammonium chloride addition were similar to those in the glutamine study and significantly lower than in the control studies. Perfusate potassium concentration was similar to the controls.

In contrast to the studies with glutamine, there was no suggestion that addition of ammonium chloride to the perfusate enhanced either sodium or water excretion. Values for both absolute and fractional sodium, chloride, and water excretion during the experimental period were either not significantly different or greater than in the control studies (Table 1). In addition, when the difference between the initial and experimental periods were compared, there was no significant difference in sodium and chloride values, and fractional water excretion was actually higher in the ammonium chloride group (Table 2).

Table 2. Comparison of the change between the experimental and initial period for the control, glutamine, and ammonium chloride studies at perfusate pH 7.0[a]

	Glutamine study	*P*[b]	Control study	*P*[b]	NH_4Cl study
GFR, *ml/min*	−0.08 ± 0.03	NS	−0.19 ± 0.04	NS	−0.23 ± 0.03[c]
$U_{Na}V$, *μmoles/min*	−1.45 ± 0.43	<0.05	0.48 ± 0.61	NS	−0.35 ± 1.03
FE_{Na}, *%*	−1.15 ± 0.68	<0.02	2.93 ± 1.24	NS	2.98 ± 1.19[c]
$U_{Cl}V$, *μmoles/min*	−0.78 ± 0.56	<0.05	1.65 ± 0.66	NS	−0.95 ± 1.16
Urine flow, *ml/min*	0.01 ± 0.004	NS	0.02 ± 0.01	NS	0.03 ± 0.01
V/GFR	0.03 ± 0.01	<0.05	0.10 ± 0.02	<0.05	0.15 ± 0.02[c]
$U_{NH_4}V$, *μmoles/min*	0.44 ± 0.05	<0.001	0.14 ± 0.02	<0.001	0.57 ± 0.08
U_KV, *μmoles/min*	−0.26 ± 0.03	<0.02	0.18 ± 0.14	<0.001	−0.89 ± 0.15
FE_K	−0.03 ± 0.03	<0.01	0.32 ± 0.07	<0.001	−0.09 ± 0.04
$P_{[NH_3^+]}$, *mM*	0.69 ± 0.03	<0.001	0.07 ± 0.01	<0.001	0.58 ± 0.06
NH_3 production, *μmoles/min/g dry wt*	4.68 ± 0.44	<0.001	0.50 ± 0.35	NS	0.40 ± 0.49[c]

[a] Change is calculated from the experimental period value minus the initial period value.
[b] Comparison with the control study
[c] Significant difference ($P < 0.05$) in comparison with the glutamine study

Perfusate pH of 7.4: (a) Glutamine studies. As with perfusions at the pH of 7.0, there were no significant differences in ammonia production (0.40 ± 0.16 vs. 0.75 ± 0.12 μmoles/min/g) between the control and glutamine studies in the initial period (Fig. 1). In addition, ammonium excretion, perfusate pH, perfusate total carbon dioxide, perfusate potassium, and both absolute and fractional potassium excretion did not differ between the two groups (Figs. 1 and 2, Table 3).

When glutamine was added to the perfusate, ammonia production increased significantly in comparison with the control kidneys (3.03 ± 0.66 vs. 0.97 ± 0.21 μmoles/min/g) (Fig. 1). Ammonia concentration in the perfusate increased to the same extent as in the studies at an acid pH (0.73 ± 0.14 vs. 0.84 ± .05 mM, Fig. 1). In contrast to the studies at a low perfusate pH, the urine remained alkaline in this group (Table 3) and, as shown in Fig. 1, despite the increase in ammonia production, ammonium excretion was not altered by glutamine.

Furthermore, neither absolute nor fractional potassium excretion was altered by glutamine when perfusions were carried out at pH 7.4 (Fig. 2 and Table 3).

There were no significant differences in GFR, absolute or fractional sodium excretion, absolute or fractional urine flow, or chloride excretion between the control and glutamine studies (Table 3). This was also the case when the data were analyzed as the difference between the experimental and initial period.

(b) Ammonium chloride studies. These ammonium chloride studies were carried out at a different time than the glutamine studies, and for reasons which are unclear, potassium excretion during the initial period differed substantially from the earlier control studies. Therefore, these data are compared with a second set of control studies that were performed at the same time and had similar baseline values. Although the absolute values in this second control group differed from the earlier controls, the qualitative response of all the measured parameters to 95 min of perfusion under control conditions is similar. In both groups, all variables changed in similar fashion when the experimental (last 40 min) and the initial (first 45 min) periods are compared (Tables 3 and 4).

When ammonium chloride was added during the perfusion at pH 7.4, ammonia production was unchanged; but, the increase in perfusate ammonia concentration was accompanied by a small but significant increase in urinary ammonium excretion (Table 4). Absolute potassium excretion decreased modestly in contrast to the increase seen in control studies. Although there was an increase in fractional potassium excretion, the change was less than in the timed control (Table 4).

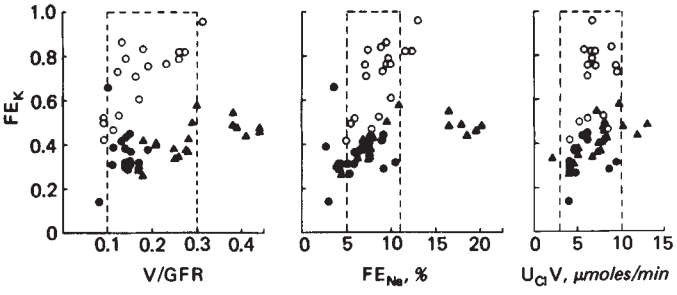

Fig. 3. *Relationship of fractional potassium excretion (FE_K) to V/GFR, FE_{Na} and $U_{Cl}V$ in studies conducted with acidotic perfusate.* All values from the last 35 to 40 min of the experimental period are plotted. When FE_K was analyzed at similar fractional urine flow rate, FE_{Na}, and $U_{Cl}V$ (outlined by *insert*), it was significantly lower in both the glutamine and ammonium chloride studies in all three instances ($P < 0.001$). Symbols are defined in Fig. 2.

Absolute and fractional rates of water excretion appeared to be increased in response to the addition of ammonium chloride to the perfusate, but there were no significant differences in other renal functional parameters in comparison with control studies.

Discussion

When kidneys were perfused with acidified perfusate, the addition of glutamine resulted in a substantial increase in both renal ammonia production and urinary ammonium excretion. As in the prior studies in humans, the alteration in ammonia metabolism was accompanied by a striking decrease in potassium excretion, analyzed in either absolute or fractional terms. When ammonium chloride was added instead of glutamine, there was no significant change in ammonia production, but the perfusate ammonia concentration and urinary ammonium excretion increased comparably. This was accompanied by changes in absolute and fractional potassium excretion, which were similar to those seen in the glutamine studies.

In contrast to the earlier studies in humans, alternative

Table 3. Control and glutamine experiments at perfusate pH 7.4

	Control study ($N = 5$)				Glutamine study ($N = 5$)			
	Initial period	Exp. period	Exp. − control	P	Initial period	Exp. period	Exp. − control	P
GFR, *ml/min*	0.66 ± 0.03	0.44 ± 0.05	−0.22 ± 0.05	<0.02	0.57 ± 0.06	0.40 ± 0.04	−0.17 ± 0.03	<0.01
$U_{Na}V$, *μmoles/min*	1.36 ± 0.35	2.54 ± 0.75	1.19 ± 0.45	NS	2.46 ± 0.56	2.52 ± 0.69	0.06 ± 0.23	NS
FE_{Na}, *%*	1.36 ± 0.35	3.62 ± 0.79	2.26 ± 0.49	<0.02	3.26 ± 1.03	4.69 ± 1.62	1.43 ± 0.63	NS
$U_{Cl}V$, *μmoles/min*	2.38 ± 0.56	3.85 ± 0.96	1.46 ± 0.73	NS	3.59 ± 0.66[b]	3.68 ± 0.59	0.32 ± 0.12[b]	NS
Urine flow, *ml/min*	0.02 ± 0.002	0.04 ± 0.01	0.02 ± 0.01	<0.05	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.003	<0.01
V/GFR	0.03 ± 0.002	0.08 ± 0.02	0.06 ± 0.02	<0.02	0.05 ± 0.02	0.11 ± 0.03	0.06 ± 0.01	<0.02
Kidney dry wt, *g*	0.27 ± 0.01	—	—		0.26 ± 0.01	—	—	
$U_{NH_4}V$, *μmoles/min*	0.05 ± 0.01	0.11 ± 0.03	0.06 ± 0.03	NS	0.04 ± 0.01	0.12 ± 0.03	0.08 ± 0.02	<0.05
U_KV, *μmoles/min*	0.52 ± 0.06	0.77 ± 0.12	0.26 ± 0.09	<0.05	0.65 ± 0.05	0.79 ± 0.04	0.14 ± 0.03	<0.01
FE_K	0.16 ± 0.02	0.40 ± 0.05	0.24 ± 0.04	<0.01	0.26 ± 0.04	0.48 ± 0.05	0.22 ± 0.02	<0.001
Urine pH[c]	—	7.21 ± 0.06	—	—	—	7.18 ± 0.02	—	—
Perfusate pH	7.38 ± 0.02	7.38 ± 0.01	0.004 ± 0.01	NS	7.39 ± 0.03	7.36 ± 0.02	−0.02 ± 0.02	NS
Perfusate total CO_2, *mM*	26.5 ± 0.30	26.9 ± 0.39	0.36 ± 0.37	NS	26.5 ± 0.27	27.1 ± 0.53	0.64 ± 0.32	NS
Perfusate $[K^+]$, *mM*	4.8 ± 0.08	4.5 ± 0.07	−0.24 ± 0.02	<0.001	4.8 ± 0.09	4.3 ± 0.11	−0.5 ± 0.03[a]	<0.001
Perfusate $[NH_3^+]$, *mM*	0.20 ± 0.04	0.37 ± 0.04	0.17 ± 0.02	<0.01	0.17 ± 0.02	0.73 ± 0.14[a]	0.56 ± 0.15[a]	<0.05
NH_3 production, *μmoles/min/g dry wt*	0.41 ± 0.15	0.97 ± 0.21	0.56 ± 0.34	NS	0.75 ± 0.11	3.03 ± 0.66[a]	2.28 ± 0.77[a]	<0.05

[a] Significant difference ($P < 0.05$) in comparison with the control study.
[b] Data from 4 experiments
[c] Data from 2 control and 2 glutamine studies

Table 4. Control and ammonium chloride experiments at perfusate pH 7.4

	Control study ($N = 7$)				NH_4Cl study ($N = 8$)[a]			
	Initial period	Exp. period	Exp. − control	P	Initial period	Exp. period	Exp. − control	P
GFR, *ml/min*	0.74 ± 0.05	0.45 ± 0.02	−0.29 ± 0.03	<0.001	0.64 ± 0.07	0.40 ± 0.02	−0.24 ± 0.06	<0.01
$U_{Na}V$, *μmoles/min*	4.52 ± 1.09	5.83 ± 0.93	1.31 ± 0.31	<0.01	4.18 ± 0.98	7.22 ± 1.38	3.04 ± 0.83	<0.01
FE_{Na}, *%*	3.96 ± 0.92	8.43 ± 1.38	4.46 ± 0.55	<0.001	4.45 ± 0.79	12.04 ± 2.23	7.59 ± 1.60	<0.01
$U_{Cl}V$, *μmoles/min*	5.50 ± 1.08	6.44 ± 0.75	0.94 ± 0.45	NS	5.20 ± 0.98	6.76 ± 1.31	1.56 ± 0.64	<0.05
Urine flow, *ml/min*	0.05 ± 0.01	0.08 ± 0.01	0.04 ± 0.004	<0.001	0.04 ± 0.01	0.10 ± 0.01	0.06 ± 0.01[a]	<0.001
V/GFR	0.06 ± 0.01	0.18 ± 0.02	0.12 ± 0.01	<0.001	0.07 ± 0.01	0.26 ± 0.02[a]	0.19 ± 0.02[a]	<0.001
Kidney dry wt, *g*	0.25 ± 0.01	—	—		0.26 ± 0.01	—	—	
$U_{NH_4}V$, *μmoles/min*	0.07 ± 0.01	0.15 ± 0.02	0.08 ± 0.01	<0.01	0.06 ± 0.01	0.29 ± 0.03[a]	0.23 ± 0.03[a]	<0.001
U_KV, *μmoles/min*	1.41 ± 0.15	1.54 ± 0.15	0.13 ± 0.22	NS	1.20 ± 0.15	0.98 ± 0.08[a]	−0.21 ± 0.14	NS
FE_K	0.38 ± 0.03	0.86 ± 0.11	0.48 ± 0.11	<0.01	0.37 ± 0.03	0.56 ± 0.05[a]	0.19 ± 0.04[a]	<0.01
Urine pH	7.41 ± 0.04	7.35 ± 0.04	−0.06 ± 0.02	<0.05	7.26 ± 0.07	7.33 ± 0.03	0.07 ± 0.07	NS
Perfusate pH	7.41 ± 0.01	7.45 ± 0.01	0.03 ± 0.01	<0.01	7.39 ± 0.01[a]	7.43 ± 0.01[a]	0.04 ± 0.01	<0.001
Perfusate total CO_2, *mM*	24.1 ± 0.20	24.5 ± 0.28	0.39 ± 0.15	<0.05	25.2 ± 0.08[a]	25.3 ± 0.11[a]	0.19 ± 0.16	NS
Perfusate $[K^+]$, *mM*	4.9 ± 0.06	4.0 ± 0.11	−0.90 ± 0.12	<0.001	5.1 ± 0.06	4.6 ± 0.11[a]	−0.50 ± 0.07[a]	<0.001
Perfusate $[NH_3^+]$, *mM*	0.14 ± 0.03	0.37 ± 0.09	0.24 ± 0.07	<0.02	0.13 ± 0.02	0.84 ± 0.10[a]	0.71 ± 0.09[a]	<0.01
NH_3 production, *μmoles/min/g dry wt*	1.05 ± 0.25	1.15 ± 0.27	0.10 ± 0.12	NS	1.04 ± 0.08	1.21 ± 0.26	0.17 ± 0.25	NS

[a] Significant difference ($P < 0.05$) in comparison with the control study

explanations for the ammonia-induced reduction in potassium excretion could be eliminated more definitely with this experimental model. Because the kidney was perfused isolated from the animal, changes in circulating, potentially potassium modifying hormones such as insulin, glucagon, and aldosterone cannot account for the findings [19]. Perfusate potassium concentration was virtually identical in all these groups, and perfusate acid-base status was also similar in the control and glutamine studies. Although perfusate pH was slightly higher in the ammonium chloride group (7.06 vs. 6.97), it is unlikely that this minimal difference exerted any effect on the pattern of potassium excretion, because each kidney also served as its own control.

The effect of glutamine administration on sodium and water handling by the renal tubule is not entirely clear. When the absolute or fractional rates of sodium and water excretion are compared between the glutamine and control studies, no significant differences are apparent. But, when the experimental period in each perfusion is compared with the initial 45 min of perfusion, glutamine appears to result in less sodium and water excretion (Table 2). This latter analysis may reflect enhanced sodium and water reabsorption induced by glutamine. It seems unlikely, however, that a decrease in the distal delivery of sodium or fluid accounts for the decrease in potassium excretion found in either the glutamine or ammonium chloride studies. As shown in Fig. 3, potassium excretion is significantly lower in both the glutamine and ammonium chloride studies than it is in the controls at similar rates of sodium and water excretion. In addition, in the ammonium chloride studies, comparison of the experimental and initial periods revealed no significant differences in absolute and fractional sodium excretion or absolute water excretion, and fractional water excretion was increased significantly in comparison with the time controls (Table 2).

Finally, the rate of urinary anion excretion can influence potassium excretion. In these studies performed with a hyperchloremic perfusate, the major anion excreted is chloride. The change in chloride excretion between the initial and experimental period was significantly less in the glutamine studies than in the timed controls (Table 2). But, absolute rates of chloride excretion did not differ significantly between the three protocols. Furthermore, as shown in Fig. 3, potassium excretion was lower in the ammonium chloride and glutamine studies than in the controls when compared at the same rate of chloride excretion. Taking all these observations into account it appears unlikely that altered rates of chloride excretion are responsible for the change in potassium excretion.

Thus, the change in potassium excretion appears to be related in some fashion to a modification in ammonia metabolism. Because potassium excretion changed similarly in both the glutamine and ammonium chloride studies, neither an increase renal ammonia production nor other events related to glutamine metabolism or its renal tubular reabsorption would appear to be responsible for the alteration in renal potassium handling. But, glutamine and ammonium chloride addition both increased perfusate ammonia concentration and urinary ammonium excretion. Therefore, the experiments at an acid pH do not delineate which of these two changes are responsible for the decrease in potassium excretion. Experiments carried out at the perfusate pH of 7.4 help resolve this issue.

Prior studies had demonstrated that the isolated perfused rat kidney excretes an alkaline urine under normal acid-base conditions, and that significant urinary acidification is only achieved during perfusion at a low bicarbonate concentration [13]. When glutamine was added to perfusion at pH 7.4, ammonia production increased threefold. Perfusate ammonia concentration rose to levels comparable to that seen in perfusions with glutamine and ammonium chloride under acidotic conditions, but urinary ammonia excretion did not increase because of the alkaline urine. In striking contrast to the studies carried out under acidotic conditions, urinary potassium excretion also was unaltered. Thus, an increase in urinary ammonium excretion appears to be the critical determinant for potassium sparing.

We anticipated that addition of ammonium chloride to the perfusate at pH 7.4 would result in a change similar to the experiments with glutamine; but, it appeared to result in a modest antikaliuresis. The mechanism accounting for the change is difficult to ascertain, because, despite the alkaline urine, ammonium excretion increased by a small but significant amount in these experiments. Thus, these experiments cannot be used to discriminate between the effects of perfusate concentration and excretion of ammonia, but they are consistent with the premise that increased ammonium excretion can account for a reduction in potassium excretion.

Our studies do not define the nephron site or precise mechanism whereby ammonium excretion modifies urinary potassium excretion. Recent experiments by Jaeger, Karlmark, and Giebisch in which glutamine was infused into intact rats suggest that ammonia alters potassium at some site beyond the distal convoluted tubule, presumably the collecting duct [20]. We have previously speculated that the mechanism might involve generation of a more favorable gradient for hydrogen ion secretion, which in some fashion possibly related to transepithelial PD would diminish potassium excretion [10], but additional experiments are required to clarify this question. Finally, a change in ammonium excretion altered potassium excretion by approximately 50% in our studies. This substantial effect lends support to the hypothesis that ammonia metabolism might be an important mechanism for potassium regulation.

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