Potassium-sparing effect of enhanced renal ammonia production

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The present studies were undertaken to determine whether a primary alteration in renal ammonia production can, in fact, affect potassium excretion. Since in most physiologic settings changes in ammonia production result from changes in acid-base metabolism, which in turn might independently alter potassium excretion, such models would not appear to be suitable. To circumvent this problem, ingestion of glutamine, the major precursor of ammonia, was utilized to increase ammonia production independent of concomitant acid-base manipulations. The results suggest that potassium excretion responds as predicted by the hypothesis, providing further evidence in support of the potential existence of a renal potassium-ammonia production homeostatic mechanism.

Methods

Fifteen normal male volunteers, who gave informed consent, were subjects for three experimental protocols. All protocols consisted of a water and a glutamine study performed in random order with a 6- to 18-day interval between studies. A low-electrolyte food powder (70005, kindly supplied by Mead Johnson and Company, Evansville, Ind.) supplemented with magnesium, sodium, and potassium chloride was ingested for 3 days preceding both phases of all protocols (22). It provided daily: 35 cal, 30 ml of water, 0.1 mmol of magnesium, 2 mmol of sodium, and 1.5 mmol of potassium per kilogram of body weight.

On the study day, after collection of four 30-min urine specimens, either glutamine (4.3 mmol/kg in 1000 ml of water) or water alone (1,000 ml) was ingested at exactly the same time of day over a 1-h period and six hourly urine collections were obtained. Heparinized venous blood samples were obtained prior to and at frequent intervals after ingestion of glutamine. Subjects were fasted with the exception of water intake for the duration of the acute study and maintained the same posture during both the water and glutamine studies.

Three modifications of this basic protocol were employed:

Protocol 1. Normal (4 studies); performed exactly as described above with the exception of one subject who received oral KCl 5 mmol/h during the water and glutamine studies.

Protocol 2. NH₄Cl (7 studies); during both the water and glutamine studies NH₄Cl in gelatin capsules (2 mmol/kg body wt) was ingested over a 2-h period, the 2nd h coinciding with the hour of glutamine ingestion.

Protocol 3. NaHCO₃ (7 studies); NaHCO₃ (1.35–2.0 mmol/kg per day) was ingested for the 3 days preceding both the water and glutamine studies.

Chemical Determinations

Urinary calcium determinations were performed on acidified urine by atomic absorption spectrophotometry with the use of lanthanum to eliminate phosphate inter-
ference. The other analytical procedures utilized in this laboratory have been described previously (23).

Calculations

Bicarbonate concentration and $\text{Pco}_2$ were calculated from the Henderson-Hasselbalch equation, assuming a $pK_a$ of 6.10 and a solubility coefficient of 0.0301 for blood and 0.0309 for urine. Titratable acidity was calculated from urine pH, blood pH and urinary phosphate and creatinine content using a $pK_a$ of 6.8 and a $pK_a'$ of creatinine of 4.92. Net acid excretion was calculated as ammonium plus titratable acid minus bicarbonate, but the bicarbonate contribution was not considered if the urine pH was less than 5.5. Paired data were analyzed statistically by the Wilcoxon matched-pairs signed-ranks test and nonpaired plus titratable acid minus bicarbonate, but the bicarbonate content using a $pK_a'$ of 6.8 and a $pK_a'$ of creatinine of 4.92. Net acid excretion was calculated as ammonium plus titratable acid minus bicarbonate, but the bicarbonate contribution was not considered if the urine pH was less than 5.5. Paired data were analyzed statistically by the Wilcoxon matched-pairs signed-ranks test and nonpaired data with the Mann-Whitney $U$ test (20).

RESULTS

$\text{NH}_4\text{Cl}$ and Normal Studies

The effect of glutamine lasted approximately 3 h from the onset of ingestion, with peak urinary changes during the 2nd h coinciding with peak plasma levels of glutamine as demonstrated by others (5, 24, 25). Therefore, the data from the first 3 h were considered together as the experimental period, and the next 3 h were referred to as the recovery period.

Mean urinary data from the control, experimental, and recovery periods are given in Table 1. These data were analyzed statistically in a paired fashion by comparing each subject’s water and glutamine studies. In addition, each hourly value obtained during a subject’s glutamine study was compared with the value obtained during the same time hour of the water study. In addition, each hourly value obtained during a subject’s glutamine study was compared with the value obtained during the same time hour of the water study. In addition, each hourly value obtained during a subject’s glutamine study was compared with the value obtained during the same time hour of the water study. These comparisons are plotted in the figures. The studies were designed and analyzed in this fashion to obviate changes resulting from diurnal patterns of electrolyte excretion. Mean plasma data are given in Table 2. Blood values for the experimental period are the averages of samples obtained from 30 to 210 min after the onset of glutamine ingestion.

In the original design of the study, it was anticipated that changes in ammonium excretion and hence, potentially, potassium excretion would be accentuated by acute $\text{NH}_4\text{Cl}$ ingestion as compared to the nonacidotic state. During the course of the study, however, it became apparent that considerable overlap occurred in both parameters between the $\text{NH}_4\text{Cl}$ and normal studies and that the overall response to glutamine ingestion was similar in both instances. Therefore the data from both these protocols have been pooled and are considered together.

Control period. As shown in Tables 1 and 2 no differences were apparent in urinary or plasma parameters between the paired water and glutamine studies, indicating that 3 days of prefeeding had resulted in comparable base line states.

Experimental period (0-3 h). Ingestion of glutamine resulted in the anticipated increase in urinary ammonium excretion (Fig. 1), which was paralleled by an increase in net acid excretion and a rise in urine pH (Table 1). This effect on urinary ammonia metabolism was accompanied by a significant decrease in potassium excretion (Fig. 2), which reached maximal effect during the 2nd h concomitant with the peak in ammonium excretion. A significant difference in potassium excretion was also apparent ($P < .01$) if the increment in excretion from control values was compared for the water ($+3.0 \mu \text{mol/min}$) and glutamine ($-14.2 \mu \text{mol/min}$) studies. As shown in Fig. 3, there were no consistent changes in sodium excretion to account for the decrease in urinary potassium. Although during the experimental period mean changes in sodium and potassium are not correlated, when individual points are analyzed (as shown in Fig. 7) a significant direct correlation ($r = 0.36, P < 0.001$) is found between changes in sodium and potassium excretion. The diuretic effect of sodium may account for the lack of potassium sparing during the 3rd and 4th h when ammonium excretion is still increased, since at this time sodium excretion is also elevated (Figs. 1-3).

Changes were not detected in creatinine clearance, urine flow rate, or phosphate excretion (Table 1). Chloride and calcium excretion was increased (Table 1 and Fig. 4); however, the increase in calcium excretion was not significant statistically. When the $\text{NH}_4\text{Cl}$ studies were analyzed individually a statistically significant ($P < .02$) increase in calcium excretion from 3.7 to 5.2 $\mu \text{mol/min}$ was found.

No differences were detected in plasma sodium, potassium, bicarbonate, and $\text{Pco}_2$ concentration or in plasma pH between the water and glutamine studies.

Recovery period (4-6 h). During the recovery period urine pH, ammonium, and net acid excretion were comparable, while sodium excretion was strikingly higher and potassium excretion modestly increased in the glutamine compared with the water studies. In addition, urinary phosphate and chloride excretion were elevated, but no changes were noted in urine flow rate, creatinine clearance, or calcium excretion.

### Table 1. Urine parameters for $\text{NH}_4\text{Cl}$ and normal studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{K}^+$, $\mu\text{mol/min}$</td>
<td>92.2</td>
<td>92.1</td>
<td>NS</td>
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<tr>
<td>$\text{Na}^+$, $\mu\text{mol/min}$</td>
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<td>61.7</td>
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<tr>
<td>$\text{NH}_4^+$, $\mu\text{mol/min}$</td>
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<td>41.0</td>
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<tr>
<td>Net acid, $\mu\text{mol/min}$</td>
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<td>99.2</td>
<td>NS</td>
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<tr>
<td>$\text{pH}$</td>
<td>5.94</td>
<td>5.87</td>
<td>NS</td>
</tr>
<tr>
<td>$\text{Cl}^-$, $\mu\text{mol/min}$</td>
<td>130</td>
<td>162</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate, $\mu\text{mol/min}$</td>
<td>7.5</td>
<td>7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium, $\mu\text{mol/min}$</td>
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<td>5.8</td>
<td>NS</td>
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<tr>
<td>Creatinine clearance, $\mu\text{g/m}^2\text{min}$</td>
<td>120</td>
<td>118</td>
<td>NS</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
<td>4.6</td>
<td>4.3</td>
<td>NS</td>
</tr>
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</table>

* $n = 11$ ($\text{NH}_4\text{Cl} = 7$; normal = 4). $^*$ $\text{H}_2\text{O}$ = studies with water ingestion only. $^+$ Glu = glutamine studies. $^\dagger$ Statistics were performed on paired data by the Wilcoxon matched-pairs signed-ranks test; NS = not significant.
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TABLE 2. Blood parameters

<table>
<thead>
<tr>
<th></th>
<th>NH₄Cl and Normal Studies*</th>
<th>NaHCO₃ Studies†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental‡</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>Gln</td>
</tr>
<tr>
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<td>134</td>
</tr>
<tr>
<td>K⁺, mM</td>
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<tr>
<td>pH</td>
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<td>7.36</td>
</tr>
<tr>
<td>HCO₃⁻, mM</td>
<td>23.6</td>
<td>23.9</td>
</tr>
<tr>
<td>Pco₂, mmHg</td>
<td>44.7</td>
<td>45.0</td>
</tr>
</tbody>
</table>

* n = 11 (normal studies = 4; NH₄Cl studies = 7). † n = 7. ‡ H₂O and Pco₂ determinations for normal and NaHCO₃ studies are from recovery period. These parameters were virtually identical during control and recovery periods in these studies. § NS = not significant.

NaHCO₃ Studies

In an attempt to determine whether the effect of glutamine on urinary potassium excretion is directly related to its effect on renal ammonia production, studies were performed after 3 days of NaHCO₃ ingestion, a maneuver that suppresses the effect of glutamine on renal ammonia production (24). Mean urinary values for these studies are given in Table 3 and plasma values in Table 2.

Control period. As in the previous studies, no significant differences in urinary parameters were apparent in the base-line values. In comparison with the NH₄Cl and normal protocols, urinary ammonia and net acid excretion were considerably lower and urine pH was higher, while the rate of potassium excretion was similar (Tables 1 and 3).

Experimental period. A significant increase in ammonium excretion was not apparent in these studies, with the mean increase averaging 3.4 µmol/min (Fig. 5 and Table 3). On the other hand, net acid excretion did not parallel ammonium excretion, and as shown in Fig 5 a small but statistically significant increase averaging 8.8 µmol/min was noted. This resulted largely from a combined, although not individually significant, increase in ammonium and decrease in bicarbonate excretion (mean = -4.5 µmol/min). No changes in urine pH were detected.

In association with the diminished effect on ammonium and net acid excretion the effect on potassium excretion was blunted, with an average decrement of 10.8 µmol/min that was not statistically significant (Fig. 6). These studies include one subject who during glutamine administration manifested a vasovagal reaction with a striking antidiuresis and marked decrease in potassium excretion (39 µmol/min). Excluding this subject the mean decrease in potassium averaged 6 µmol/min. This small decrement in potassium excretion was accompanied by an average decrease in sodium excretion of 20 µmol/min, which also was not statistically significant. No significant alterations in creatinine clearance, urine flow rate, and chloride or phosphate excretion were detected (Table 3); however, calcium excretion increased significantly. No differences were noted in plasma acid-base or electrolyte parameters (Table 2).

When the changes in urinary potassium during the NaHCO₃ protocol are compared to those of the NH₄Cl and normal protocol in a nonpaired fashion by the Mann-Whitney U test, a significant difference is not apparent. On the other hand, a more detailed comparison of these two experimental groups that takes into account the effects of urinary sodium on potassium excretion suggests that a real difference does exist.
Although mean rates of sodium and potassium excretion are not correlated in this study, when the individual points obtained during the experimental period are analyzed, as shown in Fig. 7, a significant correlation is found between the changes in sodium and potassium excretion. This relationship is apparent when the NH₄Cl and normal protocol is considered alone, the NaHCO₃ study alone (r = 0.62, p < 0.01), or all protocols are combined (r = 0.50, P < 0.001), and analysis of the recovery period yields similar results. Furthermore, it is apparent from Fig. 7 that the most striking decreases in potassium excretion in the NaHCO₃ protocol are usually accompanied by large decreases in sodium excretion, whereas significant depression of potassium excretion occurs frequently in the NH₄Cl and normal protocol even in the presence of an increase in sodium excretion. When the NaHCO₃ studies are compared to the NH₄Cl and normal studies under conditions of comparable sodium effect, i.e., when the change in sodium excretion does not exceed ±40 μmol/min (as outlined by the

### Table 3. Urine parameters for NaHCO₃ studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺, μmol/min</td>
<td>102</td>
<td>104</td>
<td>NS</td>
</tr>
<tr>
<td>Na⁺, μmol/min</td>
<td>120</td>
<td>101</td>
<td>NS</td>
</tr>
<tr>
<td>NH₄⁺, μmol/min</td>
<td>22.0</td>
<td>19.0</td>
<td>NS</td>
</tr>
<tr>
<td>Net acid, amol/min</td>
<td>4.4</td>
<td>2.0</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>0.81</td>
<td>0.87</td>
<td>NS</td>
</tr>
<tr>
<td>Cl⁻, amol/min</td>
<td>162</td>
<td>144</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate, pmol/min</td>
<td>5.1</td>
<td>6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium, amol/min</td>
<td>4.4</td>
<td>3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>115</td>
<td>117</td>
<td>NS</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
<td>4.9</td>
<td>4.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Effect of glutamine on sodium excretion. No alterations in sodium excretion are apparent in response to glutamine ingestion during experimental period.

**Fig. 4.** Effect of glutamine on calcium excretion. NH₄Cl and normal studies are represented by ● and NaHCO₃ studies by ○. No differences in magnitude of calciuresis were apparent between NH₄Cl and normal studies and NaHCO₃ studies.

**Fig. 5.** Effect of glutamine on net acid and ammonium excretion. NaHCO₃ markedly blunted the response to glutamine ingestion of both net acid and ammonium excretion.

**Fig. 6.** Effect of glutamine on potassium excretion. NaHCO₃ blunted the response to glutamine ingestion, with an average decrease of 11 μmol/min that was not statistically significant.
DISCUSSION

Recent studies of potassium depletion and loading suggested that a renal potassium-ammonia production homeostatic mechanism might exist, with an increase in ammonia production leading to potassium conservation and a decrease having the opposite effect (8, 9, 22, 23). To determine whether a primary alteration in ammonia production can affect potassium excretion, ingestion of glutamine was used to increase ammonia production independent of concomitant acid-base manipulations.

Paired studies were designed with a water and a glutamine study performed on separate days to obviate the influence of diurnal variations in potassium excretion. Each study was preceded by ingestion of a constant-formula diet for 3 days. This resulted in a comparable base-line state, as demonstrated by the similar plasma and urinary control values on both the water and glutamine study days.

Ingestion of glutamine either under conditions of normal acidification or concomitant with an acute acidifying stimulus resulted in the anticipated increase in urinary pH, ammonium, and net acid excretion. Both in vivo and in vitro experimental evidence suggest this is the result of an increase in renal ammonia production (16).

As shown in Fig. 2, this increase in renal ammonia production was accompanied by a concomitant decrease in potassium excretion, which averaged 17% when compared with the water studies. There were no changes detected in plasma potassium or acid-base status to account for the decrease in potassium excretion, nor were changes in creatinine clearance detected.

Furthermore, sodium excretion was not altered in any apparent fashion that could explain the decrease in potassium excretion, mean rates of excretion remaining comparable for the water and glutamine studies during the experimental period when potassium excretion was diminished. Since analysis of net sodium excretion cannot detect counterbalancing alterations in sodium reabsorption of different renal tubular sites, the possibility that proximal tubular reabsorption was enhanced and distal reabsorption diminished comparably cannot be excluded. Although no correlation was apparent when overall changes in sodium and potassium were considered, when individual collections from the experimental period were analyzed a significant positive correlation existed between potassium and sodium excretion. Presumably this reflects the well-proven influence of sodium on potassium excretion and represents experimental background noise independent of the glutamine effect, since consistent changes in response to glutamine ingestion were found only in the case of potassium ion. In support of this conclusion, a significant correlation between sodium and potassium excretion also was found when individual collections from the recovery period were analyzed.

Another variable that can affect potassium excretion is the level of circulating mineralocorticoid and its effect on the distal nephron. Measurements of aldosterone were not performed and these studies were not carried out with exogenous mineralocorticoid administration. Nevertheless, it seems unlikely that alterations in aldosterone secretion or its effect on the renal tubules are responsible for the decrease in potassium excretion. Although the effect of aldosterone on sodium and potassium handling can be dissociated, in the physiologic setting a decrease in aldosterone effect would be expected to result in both a natriuresis and concomitant decrease in potassium excretion (19). Such a natriuretic effect was not apparent during the experimental period, and the natriuresis during the recovery period was accompanied by an increase in potassium excretion.

Finally, glutamine administration increases plasma glucose, glucagon, and insulin levels (18), all of which can alter electrolyte excretion. Glucagon is both natriuretic and kaliuretic (3, 12, 17, 21), and insulin is antinatriuretic and antikaliuretic (1, 14, 15). Glucose administration can decrease potassium excretion in the absence of changes in sodium excretion and in the presence of increased plasma potassium levels (3, 7, 10, 11, 13). Its mechanism of action is unexplained. These variables were not measured in the present study, and even if they had been their combined effects on electrolyte excretion would be difficult to predict. It should be emphasized, however, that ornithine, lysine, and arginine, which do not increase ammonia production but can affect glucose, glucagon, and insulin in a fashion similar to glutamine, are kaliuretic (2, 18).

On the other hand, an increase in renal ammonia production and a concomitant decrease in potassium excretion in response to glutamine ingestion did not indicate a causal linkage between these events. Therefore, studies were undertaken after chronic ingestion of NaHCO₃, a maneuver that depresses the influence of glutamine on urinary ammonium excretion and renal ammonia production (24). In this setting glutamine administration resulted in a minimal, but
not significant, increase in urinary ammonium excretion and a slight increase in net acid excretion. This suppressed effect of glutamine on ammonia production was accompanied by a diminished effect on urinary potassium, with a small mean decrease in excretion that was not significant statistically. Furthermore, when the NaHCO₃ studies were compared with the normal and NH₄Cl studies under conditions where differences in rates of sodium excretion had been accounted for, a significant difference in the response of potassium to glutamine ingestion was apparent. Therefore the potassium-sparing effect of glutamine does not appear to be related to its potential systemic effects on glucose, insulin, or glucagon but rather is tightly linked to its ability to increase ammonia production.

However, the intimate relationship between renal ammonia production and potassium excretion observed in these studies still does not constitute definitive proof that ammonia secretion can directly influence potassium excretion. When the effects of glutamine on ammonia production are suppressed by sodium bicarbonate administration, other renal metabolic events are altered and renal gluconeogenesis is suppressed. It is conceivable that glucose availability at this site, in which fashion similar to the effects of systemic glucose administration, may alter electrolyte handling. One consistent effect of glucose ingestion is the production of a calciretis. In these studies a calciretis of similar magnitude was found when the NH₄Cl and normal studies were compared with the NaHCO₃ studies. To the extent that calcium excretion reflects the effects of glucose on renal electrolyte handling, any potential influence of glucose would appear to be similar in these two settings, suggesting that differences in potassium handling result from some other mechanism.

In conclusion these studies indicate that an increase in renal ammonia production induced by glutamine ingestion can influence potassium excretion and that this effect most likely results from an increase in ammonia secretion. Although additional exploration is clearly required, these observations provide further evidence consistent with the hypothesis that a renal potassium-ammonia production homeostatic mechanism might exist.

We have suggested previously that an increase in ammonia production by producing a more favorable gradient for hydrogen ion secretion could, in net terms, enhance hydrogen ion excretion in exchange for sodium reabsorption and thereby diminish potassium excretion. Presumably, this mechanism would take place at distal nephron, potassium secretory sites, i.e., distal tubule and/or collecting duct, even though a significant portion of the ammonia is produced by proximal tubules. This explanation is plausible since ammonia is a freely diffusible gas that influences tubular fluid buffering capacity throughout the entire nephron, irrespective of its actual site of production. Based on current concepts the most likely explanations whereby an increase in hydrogen ion excretion secondary to enhanced ammonia genesis would diminish potassium excretion are either through an alteration in transtubular potential difference or an effect on potassium entry into renal tubular cells at the peritubular site. Studies designed specifically to address these issues will be required for a definitive answer.

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