Influence of chronic acidosis on plasma glutamine and urea production in the nephrectomized rat

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Nitrogen partition between glutamine and urea production in the nephrectomized rat. Am J Physiol 224(4): 796-802. 1973.—Nitrogen excretion in normal and chronic acidosis was investigated in bilaterally nephrectomized rats. Metabolic acidosis was induced by chronic administration of NH₄Cl in one group and by administration of acetazolamide, as a control for exogenous nitrogen contained in NH₄Cl loads, in a second group. Plasma glutamine and urea concentration were measured before and serially over 600 min after bilateral nephrectomy. Following nephrectomy, glutamine levels fell in both normal and chronically acidic groups. On the other hand, plasma urea rose linearly over the 600-min observation period. The rate of rise was significantly greater in acidotic rats than in normal rats. Urea production by the chronically acidic nephrectomized rats was greater than that of normal nephrectomized rats; furthermore, postnephrectomy urea production exceeded prenephrectomy rates only in chronically acidotic rats. These findings indicate accelerated urea production by chronically acidic nephrectomized rats, which, in part, may result from a shift of nitrogen from glutamine to urea production.

In chronic metabolic acidosis, ammonia excretion increases while urea excretion decreases (11, 10). This reciprocal decrease in urea excretion was originally attributed to urea serving as the ammoniagenic precursor (15, 18). However, it was subsequently shown that urea extraction was not augmented in acidosis (22), whereas amino acids (23), glutamine (31) in particular, had increased extraction rates. It is now firmly established that glutamine nitrogen accounts for essentially all of the endogenous ammonia production by the kidney of dog (23), man (20), and rat (14). Therefore, the original observation reflects the extrarenal partition of nitrogen between urea and glutamine production (24). Accordingly, chronic acidosis must exert some influence by which glutamine production is accelerated at the expense of urea production. It was our purpose to gain some insight into this mechanism by observing the effect of bilateral nephrectomy on plasma glutamine and urea production. We reasoned that if this mechanism was irreversible, glutamine should accumulate in the plasma following nephrectomy. If not, nitrogen destined for excretion as ammonia should be channeled into other end products and possibly urea.

Chronic metabolic acidosis was induced by administering ammonium chloride (NH₄Cl) in one group and by administering acetazolamide (Diamox) in another group. The effects of two acidifying agents were studied for the following reason: NH₄Cl is the most commonly used acidifying agent; the extra nitrogen supplied with it might affect the production of urea and glutamine independent of the acidifying influence. To discern this effect from that of chronic acidosis, we gave Diamox, which does not add an exogenous nitrogen load (8). Measurements of ammonia and urea excretion as well as renal artery-renal vein glutamine difference were performed prior to nephrectomy, whereas plasma glutamine level and urea production were measured following nephrectomy. Based on prenephrectomy data, we expected plasma glutamine to rise following nephrectomy. Surprisingly it did not. On the other hand, the rate of rise of plasma urea was significantly increased in chronically acidic as compared to control rats. The calculated production rates were increased 140% in Diamox and 175% in NH₄Cl acidosis, suggesting accelerated urea production occurring at the expense of glutamine production.

Materials and Methods

Studies were performed on large, approximately 400 g, male Sprague-Dawley rats maintained on water and rat chow fed ad libitum prior to experimentation. Twenty-four-hour studies were performed in metabolic cages. Food and water consumption, as well as urine production, were monitored. Urine samples were collected under mineral oil and preserved with a chloroform-thymol mixture.

Chronic metabolic acidosis was induced by substituting 1 5% NH₄Cl for drinking water over a 1-week period or by intraperitoneal injections of acetazolamide (Diamox), 150 mg/kg, administered in divided daily doses for 7 days. The degree of acidosis was judged by both plasma total CO₂ and 24-hr urinary ammonium levels.

Eighteen rats were divided into three groups: nonacidotic, in acidosis induced by NH₄Cl, and in acidosis induced by Diamox. They were bilaterally nephrectomized after a 12-hr postsorbptive period, as follows. Anesthesia, sodium pentobarbital 30 mg/kg, was given intraperitoneally and a tracheal cannula was inserted. A PE-10 cannula was placed in the femoral artery and a control prenephrectomy blood sample was obtained. Then bilateral nephrectomies were performed through a lumbar approach. Following nephrec-
PLASMA GLUTAMINE AND UREA PRODUCTION

TABLE 1. Influence of ammonium chloride- or Diamox-induced acidosis on relevant blood and urine parameters in the intact rat

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>Intake</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H2O ml/day, NH4Cl, μmoles/ day</td>
<td>Total CO2, μmoles/ml</td>
<td>Vol, ml/day, NH4Cl, μmoles/ml</td>
</tr>
<tr>
<td>Nonacidotic (6)</td>
<td>388 ± 14</td>
<td>26 ± 8</td>
<td>25.8 ± 0.9</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>NH4Cl acidotic (6)</td>
<td>402 ± 16</td>
<td>39 ± 9</td>
<td>6.500</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>Diamox acidotic (6)</td>
<td>391 ± 11</td>
<td>44* ± 8</td>
<td>18.8* ± 1.2</td>
<td>31* ± 5</td>
</tr>
</tbody>
</table>

Values are means ± st. Numbers in parentheses are numbers of rats. * Significantly different from nonacidotic (P < 0.05).

An evaluation of the linearity of the rate of rise of blood urca allows an assessment of nephrectomy, as the signal event, upon urea production. With a single sample, differences between experimental groups may be attributed to variable hourly production rate and not necessarily related to the experimental treatment. This modification becomes critical when calculating production rates over postnephrectomy periods of greater than 20 hr, after which time a linear rate of urea rise is no longer observed (7). With this modification, the increment in total body urea content (TBU) was obtained from the linear rise in plasma urea concentration, (urea), times the total body water (TBW). TBW was taken to be 70% of body weight as an average based on previously published values (6, 7). Providing a similar degree of hydration in the experimental groups, the accuracy of this estimate is not critical. Thus:

\[
\text{urea}_p \times \text{TBW} = \text{TBU/linear time course} = \frac{\text{urea production} \times \text{μmoles/ml} \times \text{ml}}{\text{μmole/600 min} = \text{μmole/min}}
\]

From preliminary experiments, we established that a difference in urea production rate as small as 1.1 μmoles/min could be readily detected\(^1\) (29).

Pre-nephrectomy urea production rates were estimated from the 24-hr excretion rate immediately preceding bilateral nephrectomy. In comparing pre- and postnephrectomy production, the accuracy of the postnephrectomy rate depends on the TBW estimate. The finding that both pre- and postnephrectomy rates were similar in control nonacidotic rats indicates that 70% for total body water is an acceptable estimate.

Comparisons between nonacidotic and NH4Cl acidotic or Diamox acidotic rats were made using the Student t test. Differences were considered significant at the 5% level.

RESULTS

Pre-nephrectomy data. Table 1 depicts the change in urinary nitrogen partition with the development of chronic metabolic acidosis. After 7 days on the acidifying regimes, ammonia nitrogen excretion had increased from 7 to 26

\(^1\) (x - m)² = (t²n) / n

where t = 2.6, n = 6, the number of rats, s = 1.9 μmoles/min, s, and (x - m) = the difference between the mean of control and test group.
and 29% of urea plus ammonia nitrogen excreted in Diamox and NH₄Cl acidosis, respectively. In both cases of acidosis, this change reflects an absolute increase in ammonia excretion and, in Diamox acidosis, a significant decline in urea excretion. In NH₄Cl acidosis, urea excretion remained unchanged, suggesting the extra nitrogen intake as NH₄Cl spares urea production. This interpretation is consistent with the similar amounts of daily ammonia nitrogen excreted and consumed as well as with the observation that glutamine concentration is significantly lower in Diamox acidosis. Consequently, the nitrogen source of the ammoniagenic precursor glutamine appears to depend on the nature of the acidifying stimulus employed, Diamox drawing on endogenous nitrogen sources while NH₄Cl supplies exogenous ammonium nitrogen.

The similar daily amount of ammonium ingested and excreted by NH₄Cl acidotic rats is consistent with incorporation of ingested ammonium into liver glutamine (5), subsequent renal extraction, and hydrolysis resulting in increased ammonia excretion. Alternatively, ingested ammonium may elevate blood levels and the filtered ammonium load (26). Thus, it is necessary to determine whether the intact kidney exhibits a net glutamine uptake or release; furthermore, this information will allow a prediction concerning the influence of nephrectomy on plasma glutamine level. Arteriovenous (A-V) glutamine differences across the kidney and ammonia excretion rates are presented in Table 2. A-V glutamine was positive in both nonacidotic and NH₄Cl acidotic rats. Thus, the kidney of the rat exhibits net glutamine uptake. An approximate value for the magnitude of glutamine extraction can be obtained by multiplying A-V glutamine by estimated renal plasma flow (30). The extraction rates obtained in this manner, 0.06 and 0.51 μmole/min, reflect the increased ammonium excretion rate in NH₄Cl acidosis. This supports the contention that excreted ammonia nitrogen is derived from glutamine nitrogen.

**Postnephrectomy data.** Based on the prenephrectomy glutamine uptake, plasma glutamine should increase in all three groups following nephrectomy. Furthermore, based on the greater extraction rate in acidosis, the rate of glutamine rise should be faster in the chronic acidotic rats. Surprisingly, plasma glutamine (Fig. 1) did not increase in any of the groups, and in fact actually decreased from 0.61 ± 0.02 to 0.43 ± 0.08 μmole/ml (P < 0.02) in NH₄Cl acidotic and from 0.59 ± 0.04 to 0.45 ± 0.03 μmole/ml (P < 0.05) in nonacidotic rats over the first 150 min. On the other hand, Diamox-treated rats demonstrated only a small, but statistically insignificant (P > 0.1) fall in glutamine level over the 600-min postnephrectomy period. Interestingly, the glutamine levels reached in all three groups after the first 150 min did not decrease significantly thereafter. Since renal A-V glutamine difference was consistently positive, the possible role of the kidney as a glutamine-contributing organ appears unlikely and thus cannot explain the decrease in plasma glutamine. Neither does the fall in glutamine appear related to the acid-base state as both nonacidotic and NH₄Cl acidotic rats exhibited a similar decline. Rather, the fall in glutamine appeared related to the prenephrectomy level, since Diamox-treated rats had a lower prenephrectomy glutamine level, which was unaffected by nephrectomy; while the decline in glutamine following nephrectomy plateaued at this level in the other two groups. This suggests the kidney of nonacidotic and NH₄Cl acidotic rats plays an indirect role in maintaining "normal" glutamine levels.

Equally surprising, the rate of plasma urea rise was significantly greater in either NH₄Cl or Diamox acidosis than in nonacidotic rats (Fig. 2). Plasma urea concentration in both acidic groups, 9.82 ± 0.050 μmole/ml in NH₄Cl and 9.60 ± 0.06 μmole/ml in Diamox acidosis, was significantly higher than the control 7.68 ± 0.47 μmole/ml after only 150 min. The degree of hydration would not appear to be a factor in these observations, since body weights and water balances, urine volume minus water intake, were similar in all three groups (Table 1). Both Diamox and NH₄Cl do have some initial diuretic action, which is lost upon chronic administration or; more

<table>
<thead>
<tr>
<th>Blood Glutamine</th>
<th>Arterial</th>
<th>Venaous, μmole/ml</th>
<th>A-V</th>
<th>Renal Plasma Flow, ml/min</th>
<th>Glutamine Uptake, μmole/min</th>
<th>Urine Flow, ml/min</th>
<th>Ammonia Excretion, μmole/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonacidotic (4)</td>
<td>0.55 ± 0.05</td>
<td>0.51 ± 0.07</td>
<td>0.04 ± 0.05</td>
<td>2.2</td>
<td>0.06 ± 0.003</td>
<td>0.25 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>NH₄Cl acidotic (3)</td>
<td>0.55 ± 0.04</td>
<td>0.32 ± 0.09</td>
<td>0.23 ± 0.12</td>
<td>2.2</td>
<td>0.51 ± 0.0036</td>
<td>0.99 ± 0.15</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se. Numbers in parentheses are numbers of rats. *Handbook of Biological Data (30).
PLASMA GLUTAMINE AND UREA PRODUCTION

150. $A! i = 13.0 a!z 11.0$

Mean and SE

Non-Acidotic

NH₄Cl Acidotic

Diamox Acidotic

Minutes post-nephrectomy

FIG. 2. Plasma urea concentration before and after bilateral nephrectomy in nonacidotic and chronically acidotic rats.

Acute metabolic acidosis develops following bilateral nephrectomy (Fig. 3). For both NH₄Cl- and Diamox-treated groups, this was superimposed on an already existing chronic acidosis. The decrement in plasma total CO₂ reflects the elimination of renal acid excretion with subsequent retention of acid within the body fluid. The larger decrement, 6.2 mM, observed in NH₄Cl-treated rats, as compared to only 4.0 mM in Diamox-treated rats, characterizes the two modes of inducing acidosis, since nephrectomy eliminates bicarbonate excretion in Diamox-treated rats while it eliminates ammonium excretion in NH₄Cl-treated rats.

Comparison of pre- and postnephrectomy data. Urea production rates, calculated as described in METHODS, before and after nephrectomy are shown in Table 3. As is clearly evident, postnephrectomy urea production rates were greater ($P < 0.02$) in both groups of chronically acidotic rats than in nonacidotic controls. In comparing each rat with its prenephrectomy rate, only chronically acidotic rats had increased production rates following nephrectomy. A comparison of prenephrectomy nitrogen excretion, expressed as the sum of ammonium plus urea nitrogen, with urea production following nephrectomy appears in Fig. 4. This figure presents a quantitative comparison of nitrogen partition before nephrectomy, at which time both urea and ammonia are produced, with urea production after nephrectomy. If glutamine production is shifted to urea production following removal of the kidney, then urea production should be equal to the sum of ura plus ammonia excretion. Accordingly, prior to nephrectomy, nonacidotic rats excreted 9.0 μmoles urea nitrogen per minute (Table 3) plus 0.6 μmole ammonia nitrogen per minute (Table 1) or 9.6 μmoles nitrogen compared to 9.6 μmoles nitrogen produced following nephrectomy. In NH₄Cl acidosis, 13.9 μmoles nitrogen were excreted per minute compared to 16.8 μmoles/min incorporated into urea. Therefore, these findings are consistent with a shift of nitrogen from glutamine to urea production. The

TABLE 3. Urea excretion before nephrectomy compared to urea production after nephrectomy by nonacidotic and chronically acidotic rats

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>Prenephrectomy Urea Excretion, μmoles/min per 400 g</th>
<th>Postnephrectomy Urea Production, μmoles/min per 400 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonacidotic (6)</td>
<td>388 ± 14</td>
<td>4.57 ± 0.28</td>
<td>4.80 ± 0.34</td>
</tr>
<tr>
<td>NH₄Cl acidotic (6)</td>
<td>402 ± 16</td>
<td>5.00 ± 0.17</td>
<td>8.36* ± 0.51</td>
</tr>
<tr>
<td>Diamox acidotic (6)</td>
<td>391 ± 11</td>
<td>3.37 ± 0.18</td>
<td>7.01* ± 0.47</td>
</tr>
</tbody>
</table>

Values are means ± se. Numbers in parentheses are numbers of rats. * Significantly different from nonacidotic ($P < 0.05$).
findings support the contention that glutamine is the major plasma precursor of ammonia produced by the rat kidney (Table 2). Similar findings have been reported by others (14). Therefore, there is little doubt that excreted ammonia nitrogen is derived from extracted glutamine nitrogen. Our findings suggest that the nitrogen source for glutamine synthesis varies with the acidifying stimulus employed. In the case of NH$_4^+$ acidosis, absorbed NH$_4^+$ is readily incorporated into liver glutamine (5). Consistent with this finding, Addae and Lotspeich (1) proposed that ammonia arising from the gastrointestinal system stimulates hepatic glutamine release. In our study, equal amounts of ammonia were ingested and excreted per day (Table 1) supporting the above interpretation. If glutamine synthesis is the predominant fate of administered ammonium as suggested (5), it is perhaps not too surprising that glutamine has not been implicated as a limitation of renal ammonia production when NH$_4$Cl is the acidifying agent. When nitrogen availability is reduced, for example, in protein deficient patients, there is a spontaneous acidosis and an inability to respond adequately to an acid load that may be related to a limitation imposed by glutamine production (13). Diamox-induced acidosis, unlike NH$_4$Cl acidosis, draws from endogenous nitrogen sources to produce glutamine, and these are sources common to urea synthesis since urea excretion decreases (Table 1). If these sources are amino acids derived from muscle catabolism, then increased muscle glutamine release might be expected (16). On the other hand, with NH$_4$Cl acidosis hepatic glutamine release is stimulated (1). Obviously in evaluating the relative contribution of various organ systems in regulating glutamine homeostasis, the nature of the acidifying agent is of considerable importance. Finally, some stimulus resulting directly or indirectly from acidosis influences nitrogen metabolism, since during the development of chronic acidosis, ammonia nitrogen increases from 7 to almost 30% of the urea plus ammonia nitrogen excretion (Fig. 4). Extrarenal glutamine production appears coordinated with renal extraction, since plasma concentrations are not altered (23). This raises the possibility of the kidney emitting a signal by which glutamine production is coupled to renal glutamine uptake. Since renal vein ammonia content increases in acidosis (1) and as ammonia stimulates both muscle (12) and liver (1) glutamine release, ammonia itself may function in some manner to attune total body glutamine metabolism to the needs of the kidney.

It was our hope that characteristics of this system and the possible role of the kidney would be more clearly discernible following nephrectomy. First, nephrectomy eliminates an important site of glutamine uptake. Some estimate of its importance can be realized from uptake rates of 1–3 μmoles/min (Table 2) when the apparent total body glutamine turnover rate in the rat approximates 7–8 μmoles/min (5). Consequently, elimination of renal glutamine uptake should lead to an accumulation of glutamine in the plasma. However, this did not occur. Second, based on nephrectomy rates we expected postnephrectomy urea production rates to be equal to or less in acidic rats than in the nonacidotic controls. However, postnephrectomy production rates were actually 140–175% greater in acidic

higher prenephrectomy nitrogen excretion rate by NH$_4$Cl-treated rats (13.9 μmoles/min) compared to that by controls (9.6 μmoles/min) reflects nitrogen ingested as NH$_4$Cl. In Diamox acidosis, the sum of urea plus ammonia nitrogen excretion was similar to nonacidotic controls. However, with nephrectomy, nitrogen incorporation into urea jumped to 14.0 μmoles/min compared to 8.6 μmoles urea plus ammonia nitrogen per minute prior to nephrectomy, indicating in Diamox-induced acidosis that accelerated urea production could not have been derived solely from shift of nitrogen from glutamine.
rats. Since glutamine did not accumulate, nor does the glutamine precursor, ammonia, accumulate following nephrectomy (18), it appeared that glutamine nitrogen was transferred to urea synthesis. Furthermore, the increment in urea production over control nonacidotic rats was 2.2 μmoles/min in Diamox acidosis and 3.5 μmoles/min in NH₄Cl acidosis, and these values are in the range expected from renal glutamine uptake rates of 1-3 μmoles/min in acidotic rats. Another way of calculating the possible contribution of prenephrectomy renal glutamine extraction to the increased urea production during the postnephrectomy period is to compare the urea plus ammonia nitrogen excretion rate with urea production rates following nephrectomy (Fig. 4). The close agreement for nonacidotic and NH₄Cl acidotic rats suggests a reversal of the prenephrectomy nitrogen partition. Since this difference between pre- and postnephrectomy nitrogen partition was observed as a linear function of time following nephrectomy, the removal of the kidneys may be the important factor in accelerating urea production. In this regard, there are numerous reports of a nitrogen partition. Since glutamine did not accumulate, nor does the glutamine precursor, ammonia, accumulate following nephrectomy (27). Finally, the acidosis associated with renal insufficiency reportedly accelerates the rate of urea rise measured 48 hr after ureter ligation, and this can be nullified by titrating the blood with alkali (28). Our findings are in agreement with an influence of the kidney on urea production and specifically with a role of glutamine in accelerating urea production in chronically acidotic nephrectomized rats. However, nephrectomy and the ensuing acute metabolic acidosis do not accelerate urea production in nephrectomized rats not previously made chronically acidotic.

Acute renal insufficiency in the nephrectomized rat bears some resemblance to clinical cases of chronic renal insufficiency. In the nephrectomized rat the renal venous blood ammonia contribution is eliminated, plasma glutamine is reduced, and urea production is accelerated. In chronic renal insufficiency, renal ammonia release is presumably reduced (32), plasma glutamine is lowered (32), and a negative nitrogen balance usually persists (9). Conceivably, such subjects resemble our acidic nephrectomized rats in lacking the renal influence on nitrogen partition between glutamine and urea. Consequently, endogenous nitrogen sources would appear in urea, rather than in the renal amminoniagenic precursor, glutamine, as in healthy subjects.

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REFERENCES


