Acidosis Mediates the Upregulation of UT-A Protein in Livers from Uremic Rats

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Abstract. Liver expresses a 49-kD UT-A protein whose abundance is increased by uremia. Chronic renal failure causes acidosis; therefore, the role of acidosis in increasing UT-A abundance was tested. Rats underwent 5/6 nephrectomy, and half were given bicarbonate mixed in their food. Bicarbonate administration significantly increased blood pH. Compared with sham-operated rats, UT-A protein abundance was significantly increased by 50% in livers from uremic, acidotic rats; bicarbonate administration prevented the increase in UT-A protein. To determine whether acidosis alone would increase UT-A protein in liver, rats were made acidotic, but not uremic, by feeding them HCl. HCl-feeding significantly lowered blood pH, increased urea excretion, and increased the abundance of the 49-kD liver UT-A protein by 36% compared with pair-fed nonacidotic rats. HCl-feeding significantly increased the abundance of the 117-kD UT-A1 protein in kidney inner medulla but did not change aquaporin-2 protein. Next, rats were fed urea to determine whether elevated blood urea would increase UT-A protein. However, urea feeding had no effect on UT-A in liver or kidney inner medulla. It was, therefore, concluded that acidosis, either directly or through a change in ammonium concentration, rather than other dietary components, stimulates the upregulation of UT-A protein in liver and kidney inner medulla.

Urea is a highly polar, small molecule that has a low permeability across artificial lipid bilayers (1). Urea is transported by facilitated (or carrier-mediated) transport pathways in kidney inner medullary collecting ducts (IMCD), erythrocytes, and liver (2–4). Four urea transporter cDNAs have been cloned from rat kidney (UT-A1, UT-A2, UT-A3, and UT-A4) that originate from a single gene by alternative splicing (5–11). Although UT-A protein expression was initially thought to be limited to the kidney, we showed that liver expresses 49- and 36-kD UT-A proteins (3), and Northern analysis shows that liver expresses a 2.7-kb UT-A mRNA (12), which is consistent with the size of UT-A2b (8).

In liver, the abundance of the 49-kD UT-A protein is significantly increased in rats made uremic by 5/6 nephrectomy (3). We proposed (3) that upregulation of this UT-A protein may allow hepatocytes to increase urea production (13,14) to reduce the accumulation of ammonium (15–17). In searching for signals that could upregulate UT-A, we have studied metabolic acidosis because renal failure frequently causes metabolic acidosis (18). Acidosis, in turn, enhances protein degradation, both in rats and patients with renal failure, and bicarbonate administration has been shown to improve nitrogen balance in patients (19). The goal of our study was to determine whether metabolic acidosis causes an increased abundance of the 49-kD UT-A protein in liver, both in uremic and nonuremic rats.

Materials and Methods

Tissue Preparation

Male Sprague-Dawley rats (National Cancer Institute, Frederick, MD) were anesthetized with intraperitoneal pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL), an aortic blood sample was obtained for blood gas and biochemical analysis, and the liver and kidneys were removed. The kidney inner medulla was dissected into base and tip portions, as described previously (20). Tissue samples were homogenized in isolation buffer (10 mM triethanolamine, 250 mM sucrose, 1.46 g/ml leupeptin, 0.1 mg/ml phenylmethylsulfonyl fluoride, pH 7.6; 0.025 to 0.1 g tissue per ml isolation buffer) (3,20,21). Concentrated sodium dodecyl sulfate (SDS) was added to achieve a final concentration of 1%, and samples were sheared by passage through a 28-gauge needle and centrifuged for 15 min at 14,000 × g. Protein was determined by using the BioRad DC protein assay kit (BioRad, Richmond, CA).

Animal Models

Plasma and urine chemistries were measured by the Emory University Veterinary Services Laboratory. Arterial blood gas was measured by using a blood gas analyzer (Opti 1; AVL Scientific, Roswell, GA). Urine osmolality was measured by using a vapor pressure osmometer (model 5500; Wescor, Logan, UT).

Chronic Renal Failure. To induce uremia, rats underwent 5/6 nephrectomy, were fed 40% protein, and drank 0.225 mg/dl NaCl; control rats underwent sham-operation and were pair-fed the same food and drink (22,23). To test for an effect of acidosis, a second group of rats underwent 5/6 nephrectomy but were given bicarbonate.
for 8 d by adding 1.7 g NaHCO₃/100 g chow to their food and 0.125 mg/dl NaHCO₃ to their drinking fluid; these rats were pair-fed to the 5/6 nephrectomy rats that were not given bicarbonate (23).

**Acidosis.** To induce acidosis in nonuremic rats, normal rats were fed a 50:50 mixture of normal chow and chow with 0.8 M HCl (wt/vol) for 4 to 11 d and given water ad libitum; control rats were pair-fed a 50:50 mixture of normal chow and chow with water (24).

To control for the increase in water intake of the HCl-fed rats, a second group of HCl-fed rats were water-restricted by limiting their water intake to that of the pair-fed control rats. The water-restricted, HCl-fed rats were pair-fed to the HCl-fed rats receiving water ad libitum.

**Urea Feeding.** To increase blood urea nitrogen (BUN), rats were fed standard chow supplemented with 30% urea for 7 d; control rats were pair-fed with standard chow (25).

**Western Blot Analyses**

Proteins (10.46 g/lane) were separated on 10% SDS-polyacrylamide gels and then transferred to a polyvinylidene difluoride membrane. Membranes were probed with our affinity-purified polyclonal antibody (3.8 mg/ml used at 1:5000 for Western blot) to UT-A (3,20). This antibody was prepared against the C-terminal portion of UT-A1 antibody (3.8 mg/ml used at 1:5000 for Western blot) to UT-A (3,20). Autoradiograms were scanned using the Bio-Rad Gel Doc 1000 digital imaging densitometer (Bio-Rad Laboratories, Hercules, CA). Scanned bands were quantified by using the system’s Multi-Analyst version 1.0.1 software. Results are expressed as arbitrary units/0.46 g protein loaded.

**Statistical Analyses**

All data are presented as mean ± SEM, and n = number of rats. The t test was used to test for statistical significance between two groups. An ANOVA was used to test for statistical significance between three groups, followed by Tukey’s protected t test (26) to determine which groups were significantly different. The criterion for statistical significance was P < 0.05.

**Results**

**Uremic Rats**

The rats that underwent a 5/6 nephrectomy had significantly increased serum creatinine and BUN and decreased pH and serum bicarbonate and albumin compared with sham-operated rats (Table 1). Feeding bicarbonate to uremic rats significantly increased pH and serum bicarbonate and decreased serum chloride (Table 1) compared with uremic rats that were not given bicarbonate. Compared with sham-operated rats, the 49-kD UT-A protein abundance was significantly increased by 50% in liver from uremic, acidotic rats, which is consistent with our previous study (3); administering bicarbonate to uremic rats reversed the increase in the 49-kD UT-A protein (Figure 1).

**HCl-Fed Rats**

To determine whether acidosis alone can increase UT-A protein abundance, normal rats were fed HCl. Arterial blood pH and bicarbonate were reduced after 4, 6, 7, and 11 d of HCl feeding (Table 2). The 49-kD UT-A protein in liver was increased at each time point (Figure 2).

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**Table 1. Blood chemistries in uremic rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham Operation</th>
<th>Uremia</th>
<th>Uremia + Bicarbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>3.3 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>4.9 ± 0.1</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>137 ± 2</td>
<td>131 ± 3</td>
<td>135 ± 3</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>100 ± 1</td>
<td>105 ± 2</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>26 ± 3</td>
<td>191 ± 7</td>
<td>159 ± 13</td>
</tr>
<tr>
<td>Arterial blood pH</td>
<td>7.41 ± 0.02</td>
<td>7.02 ± 0.05</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>Arterial blood pCO₂</td>
<td>37.9 ± 1.9</td>
<td>32.2 ± 2.7</td>
<td>35.1 ± 2.5</td>
</tr>
<tr>
<td>Serum HCO₃ (mEq/L)</td>
<td>24.9 ± 0.7</td>
<td>7.7 ± 1.1</td>
<td>25.3 ± 0.9</td>
</tr>
</tbody>
</table>

* Data are mean ± SE; n = 6 rats/group; BUN, blood urea nitrogen.

* P < 0.01 versus sham operation.

* P < 0.05 versus CRF.

* P < 0.01 versus CRF + Bicarbonate.

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**Figure 1.** Urea transporter protein abundance in livers from rats made uremic by 5/6 nephrectomy. (Left panel) representative Western blot showing UT-A protein bands in liver from a sham-operated rat (Sham), a uremic rat (Uremic), and a uremic rat given bicarbonate (Uremic + Bicarb). Each lane represents a sample from a separate rat. (Right panel) densitometric summary of the 49-kD band on the gels. There was a significant increase in the density of the 49-kD band in liver from uremic rats compared with control rats. There was no change in the abundance of the 36-kD band (data not shown). Data are mean ± SE; n = 6 rats/group; *P < 0.05.
Additional rats were fed HCl for 7 d, and compared with pair-fed control rats, the rats fed HCl had significantly decreased arterial blood pH, serum bicarbonate, and urine pH and significantly increased arterial blood pCO₂ and urine urea excretion (Table 3). The abundance of the 49-kD liver UT-A protein was significantly increased by 36% (Figure 3).

The HCl-fed rats also had a significant increase in urine volume (Table 3). Therefore, we measured UT-A1 and aquaporin-2 (AQP2) protein abundances in the renal inner medulla (IM). In the IM tip, the abundance of the 117-kD UT-A1 protein was significantly increased by 129% and the abundance of the 97-kD UT-A1 protein was unchanged (Figure 4). In the IM base, the 117-kD UT-A1 protein was nearly undetectable in control rats but clearly present in HCl-fed rats; the abundance of the 97-kD UT-A1 protein was unchanged (Figure 5). AQP2 protein abundance was unchanged in the IM tip of control versus HCl-fed rats (data not shown).

To determine whether the increase in UT-A1 protein in the kidney of HCl-fed rats was due to the increase in urine volume, HCl-fed rats were water-restricted by limiting their water intake to the amount drunk by the control rats (Table 4). In the IM tip, the abundance of the 117-kD UT-A1 protein was significantly increased and the abundance of the 97-kD UT-A1 protein was unchanged, which is similar to the results obtained in the HCl-fed rats drinking water ad libitum (Figure 6).

Urea-Fed Rats

To determine whether an increase in BUN alone can increase UT-A in liver, rats were fed a standard protein diet to which urea was added. The urea-fed rats had a significantly higher BUN and urine urea excretion than pair-fed control rats (Table 4). There was no significant change in the abundance of the 49-kD UT-A protein in liver, the 117- or 97-kD UT-A1 proteins in the IM tip, or in AQP2 protein abundance in the IM tip of control versus urea-fed rats (data not shown).

Discussion

Liver

We previously showed that the liver expresses two UT-A proteins: a 49-kD protein that is present in the membrane and a 36-kD protein that is present in the cytoplasm (3). We also found that our model of uremia is associated with an increase in the abundance of the 49-kD, but not the 36-kD, UT-A protein in rat liver (3). The 49-kD UT-A protein is most likely UT-A2b and is expressed in hepatocyte membranes (3,8,12). The goal of this study was to identify a mediator of the in vivo increase in UT-A. Because uremia results in so many pathophysiologic changes, we chose to study metabolic acidosis because it can be manipulated and has been shown to be a mediator of increased protein and amino acid catabolism and increased gene transcription (23). Our major result was that giving bicarbonate to uremic rats normalized blood pH and blocked the increase in the abundance of the 49-kD UT-A protein in their livers. This response occurred despite no difference in dietary constituents (other than bicarbonate). Moreover, the major endproduct of dietary protein is urea; therefore, our finding that BUN values between uremic acidotic rats and uremic nonacidotic rats were not statistically different (Table 1) indicates that the acidosis, either directly or through a change in ammonium concentration, rather than the accumulation of urea (or other nitrogenous products), was the principal cause of the increase in UT-A protein expression. This was confirmed when we studied HCl-fed, nonuremic rats; the abundance of the 49-kD UT-A protein was increased there as well.

<table>
<thead>
<tr>
<th>Days HCl Feeding</th>
<th>Pair-Fed Control Rats</th>
<th>HCl-Fed Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>pCO₂</td>
</tr>
<tr>
<td>Day 4</td>
<td>7.29</td>
<td>44.6</td>
</tr>
<tr>
<td>Day 6</td>
<td>7.34</td>
<td>34.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>7.34</td>
<td>47.8</td>
</tr>
<tr>
<td>Day 11</td>
<td>7.45</td>
<td>37.3</td>
</tr>
</tbody>
</table>

* Values shown are average of 2 rats/time point.

Figure 2. Western blot showing UT-A protein bands in liver from rats fed HCl (HCl) or pair-fed control (C) rats. Rats were fed HCl for the number of days indicated. The abundance of the 49-kD band is increased in the HCl-fed rat at each time point. Each lane represents a sample from a separate rat; two HCl-fed and control rats were studied at each time point.
The percent increase (50%) in the 49-kD UT-A protein in uremic rat liver was somewhat greater than the percent increase (36%) in HCl-fed, nonuremic rat liver. This difference could be due to the larger change in pH in the uremic rats or to assorted consequences of acidosis and/or uremia. In short, our results strongly suggest that acidosis, either directly or through a change in ammonium concentration, is the principal cause of the upregulation of UT-A protein expression in rat liver.

We also tested whether an increase in BUN would increase UT-A protein in liver by feeding rats large quantities of urea. The BUN of the urea-fed rats was significantly higher than in the pair-fed control rats (25) but not as high as in the 5/6 nephrectomized rats. We found that UT-A protein abundance was unchanged in liver, which suggests that an elevated BUN alone was not responsible for the increase in UT-A protein. However, we cannot exclude the possibility that a larger increase in BUN would have an effect on UT-A protein.

What is the physiologic advantage for an increase in hepatic UT-A protein expression since urea synthesis consumes two ammonium and two bicarbonate molecules and should not alter acid-base balance? One proposed function for a hepatic urea transporter is to facilitate the rapid transport of urea out of hepatocytes. Consistent with this hypothesis, we found that urine urea excretion was significantly increased in the HCl-fed rats (Table 3). However, previous studies of HCl-fed rats did not find an increase in urine urea excretion (24,34,35). Although the protocols for HCl-feeding differ, we do not have an explanation for this difference.

**Kidney**

UT-A1 exists as two glycoproteins, which run at 117 and 97 kD, in rat inner medulla (36). Deglycosylation of rat inner medullary proteins results in a single 88-kD UT-A1 band (36). Both glycoproteins are expressed in the IMCD apical membrane, and the reason for the two forms is not known. We found that acidosis increased the abundance of the 117-kD, but
not the 97-kD, UT-A1 protein in the kidney inner medullary tip. We previously showed that UT-A1 protein abundance is increased in five conditions associated with increased urine volume: water diuresis, furosemide diuresis, low-protein diet, hypercalcemia, and adrenalectomy (reviewed in references 37 and 38). The HCl-fed rats also had an increased urine volume compared with pair-fed control rats, which is consistent with earlier studies (24). However, the increase in the 117-kD UT-A1 protein in the inner medullary tip of HCl-fed rats is not due simply to polyuria, because it was also increased in HCl-fed rats whose water intake was limited to the amount drunk by control rats.

There are interesting differences between HCl-feeding and other polyuric conditions. One is that AQP2 protein is generally reduced in polyuric conditions (39), but in HCl-fed rats, we did not find a reduction in AQP2 protein in the inner medullary tip. Another difference is that the 117-kD UT-A1 protein was clearly expressed in the inner medullary base of HCl-fed rats; the inner medullary base typically expresses only the 97-kD UT-A1 protein (reviewed in reference 37). The abundance of the 117-kD, but not the 97-kD, UT-A1 protein was also increased in both the inner medullary base and tip regions in diabetic rats that were studied two weeks after streptozotocin injection (40). These rats were polyuric, but their pH was not measured.

Lastly, we tested whether an increase in BUN would alter UT-A1 or AQP2 proteins in kidney inner medulla by feeding rats large quantities of urea (25). Despite significant changes in urine volume and urea excretion, both UT-A1 and AQP2 protein abundances were unchanged.

In conclusion, we have identified that acidosis stimulates the expression of UT-A protein in the liver and kidney. It is tempting to conclude that the increased expression represents transcriptional stimulation as acidosis in uremia increases the transcription of genes involved in the ubiquitin-proteasome pathway, at least in muscle (23,32,33,41). Regardless, the responses of the liver and kidney, coupled with those in muscle

Table 4. Water-restricted HCl-fed rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HCl-Fed</th>
<th>Water-Restricted, HCl-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>167 ± 5</td>
<td>168 ± 6</td>
<td>163 ± 5</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>169 ± 6</td>
<td>168 ± 13</td>
<td>156 ± 4</td>
</tr>
<tr>
<td>Water intake (ml/d)</td>
<td>22.7 ± 1.2</td>
<td>49.7 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.3 ± 3.2</td>
</tr>
<tr>
<td>Urine volume (ml/d)</td>
<td>7.7 ± 2.0</td>
<td>28.8 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0 ± 1.6</td>
</tr>
<tr>
<td>Arterial blood pH</td>
<td>7.31 ± 0.03</td>
<td>7.20 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.22 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arterial blood pCO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>50.8 ± 1.3</td>
<td>44.0 ± 1.6</td>
<td>45.5 ± 3.5</td>
</tr>
<tr>
<td>Urine pH</td>
<td>8.43 ± 0.64</td>
<td>7.77 ± 0.34</td>
<td>6.95 ± 0.57</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are mean ± SE; <i>n</i> = 3 rats/group.

<sup>b</sup> Significantly different from control rats at <i>P</i> < 0.05.
Table 5. Blood and urine chemistries in urea-fed rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pair-Fed Control Rats</th>
<th>Urea-Fed Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>19 ± 2</td>
<td>41 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine volume (ml/d)</td>
<td>8 ± 1</td>
<td>94 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine urea excretion (mmol/d)</td>
<td>6.1 ± 0.9</td>
<td>119.2 ± 8.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>2510 ± 170</td>
<td>1090 ± 30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are mean ± SE; n = 7 rats/group.
<sup>b</sup> P < 0.05 versus control rats.

(19), indicate that acidosis induces a coordinated set of physiologic responses in different organs to defend both acid-base and nitrogen homeostasis. Additional studies will be necessary to identify the mechanisms underlying these responses, such as whether acidosis activates stress or immediate early response genes and whether acidosis per se or a change in ammonium concentration is the signal.

Acknowledgments

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References