UT-A Urea Transporter Protein in Heart
Increased Abundance During Uremia, Hypertension, and Heart Failure

Rafael Duchesne, Janet D. Klein, Jeffrey B. Velotta, John J. Doran, Patricia Rouillard, Brian R. Roberts, Alicia A. McDonough, Jeff M. Sands

Abstract—Urea transporters have been cloned from kidney medulla (UT-A) and erythrocytes (UT-B). We determined whether UT-A proteins could be detected in heart and whether their abundance was altered by uremia or hypertension or in human heart failure. In normal rat heart, bands were detected at 56, 51, and 39 kDa. In uremic rats, the abundance of the 56-kDa protein increased 1.9-fold compared with pair-fed, sham-operated rats, whereas the 51- and 39-kDa proteins were unchanged. We also detected UT-A2 mRNA in hearts from control and uremic rats. Because uremia is accompanied by hypertension, the effects of hypertension per se were studied in uninephrectomized deoxycorticosterone acetate salt–treated rats, where the abundance of the 56-kDa protein increased 2-fold versus controls, and in angiotensin II–infused rats, where the abundance of the 56 kDa protein increased 1.8-fold versus controls. The 51- and 39-kDa proteins were unchanged in both hypertensive models. In human left ventricle myocardium, UT-A proteins were detected at 97, 56, and 51 kDa. In failing left ventricle (taken at transplant, New York Heart Association class IV), the abundance of the 56-kDa protein increased 1.4-fold, and the 51-kDa protein increased 4.3-fold versus nonfailing left ventricle (donor hearts). We conclude that (1) multiple UT-A proteins are detected in rat and human heart; (2) the 56-kDa protein is upregulated in rat heart in uremia or models of hypertension; and (3) the rat results can be extended to human heart, where 56- and 51-kDa proteins are increased during heart failure. (Circ Res. 2001;89:139-145.)

Key Words: urea • cardiac hypertrophy • polyamine • human heart failure • rat models

Urea is a small but highly polar molecule that has a low permeability across lipid bilayers.1 Urea transport occurs by facilitated (or carrier-mediated) pathways in kidney inner medullary collecting ducts and in erythrocytes.2 Facilitated urea transporter cDNAs have been cloned from kidney (UT-A)3–9 and erythrocytes (UT-B).10–12 Five different UT-A isoforms have been identified: UT-A1, UT-A2, UT-A3, UT-A4, and UT-A5. These isoforms are thought to originate from the same gene (UT-A) by alternative splicing.8,14,15 Although UT-A protein expression was originally thought to occur only in kidney, we showed that liver expresses 51- and 39-kDa UT-A proteins and that the abundance of the 51-kDa protein is significantly increased in liver from rats made uremic by 5/6 nephrectomy.16 We previously detected a 3.1-kb UT-A mRNA in rat heart by Northern analysis,8 suggesting that UT-A protein may be expressed in heart. The rationale for a cardiac urea transporter would be the same as that in liver, to dispose of urea produced in the cell. Urea production is likely increased during cardiac hypertrophy, because an increase in polyamine synthesis from ornithine is associated with cardiac hypertrophy and urea is a byproduct of the production of ornithine from arginine.17–25 Therefore, the goals of this study were to determine whether UT-A proteins are expressed in rat and human heart and to determine whether their abundance is altered in a variety of models: 5/6 nephrectomy rats with uremia, hypertension, and cardiac hypertrophy; deoxycorticosterone acetate (DOCA) salt–treated rats with hypertension and cardiac hypertrophy; short-term angiotensin II–infused rats with hypertension before cardiac hypertrophy; and terminal human heart failure diagnosed as dilated cardiomyopathy.

Materials and Methods

Animal Models
Animal protocols were approved by the Emory Institutional Animal Care and Use Committee. Sprague-Dawley rats (National Cancer Institute, Frederick, Md) were anesthetized using intraperitoneal ketamine (Fort Dodge Laboratories) and xylazine (Miles Agricultural Division). For the uremia model, rats underwent a 5/6 nephrectomy, were fed 40% protein, and drank 1/4 normal saline.16,26–28 Control rats underwent sham operation and were pair-fed. For the
DOCA model, rats underwent a right nephrectomy and a 100-mg slow-release DOCA pellet was inserted subcutaneously through a midscapular incision. Water was replaced by 1% saline. Control rats were uninephrectomized, implanted with a sham pellet, and given tap water to drink ad libitum. For the angiotensin model, angiotensin II (500 ng/min per kg) was administered by 3-day osmotic minipump. Control animals were pair-fed, and blood pressure was measured by tail cuff.

**Rat Heart**

Hearts were homogenized in isolation buffer (10 mmol/L triethanolamine, 250 mmol/L sucrose, 1 μg/mL leupeptin, and 0.1 mg/mL PMSF, pH 7.6, 0.025 to 0.1 g tissue per mL isolation buffer). Concentrated SDS was added to 1%, samples were sheared by passage through a 28-gauge needle and centrifuged for 15 minutes at 14000g, and protein was determined (DC protein assay kit, BioRad).

**Human Myocardium**

Left ventricular myocardia were obtained from 10 nonfailing and 10 terminally failing (dilated cardiomyopathy) human hearts, which overlap with the samples investigated previously. The failed and nonfailed hearts were from male and female patients who ranged in age from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported. Left ventricle samples from terminally failing hearts were obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis was dilated cardiomyopathy in all patients (New York Heart Association class IV). The patients’ age ranged from 33 to 59 years. Samples from left ventricle were dissected and frozen at −80°C. Nonfailing hearts were obtained from organ donors with brain death caused by traumatic injury. These hearts could not be used for transplantation for reasons previously reported.

**Northern Analysis**

Rat UT-A4 cDNA was cloned into the vector pcdNA3, which has a flanking Sp6 promoter site. Templates for GAPDH (loading control) and the molecular-weight marker probes were linearized pTRIPLEscript plasmids: pTRI-GAPDH rat and pTRI-MMP (Ambion). RNA probes were synthesized by in vitro transcription incorporating α-32P-UTP (6000 Ci/mmol) and purified through P-30 polyclaraylamide gel Micro Bio-Spin columns (Bio-Rad). The UT-A RNA probe includes the 410 nucleotides of the 3’-end of the coding region common to UT-A1, UT-A2, and UT-A4. Therefore, the probe detects UT-A1 (4.1-kb), UT-A2 (3.1-kb), and UT-A4 (2.7-kb) but not UT-A3 (2.1-kb) or UT-A5 (1.4-kb).

**Reverse Transcription and Polymerase Chain Reaction**

Representative RNA samples (3 μg) from uremic and control hearts and kidney outer medulla (control) were reverse-transcribed to cDNA (Omniscript, Qiagen). DNA was amplified using polymerase chain reaction (PCR) (Advantage 2 polymerase, Clontech) for 3 cycles (30 seconds at 95°C and 2 minutes at 70°C) and then for 32 cycles (30 seconds at 95°C and 2 minutes at 68°C). Three pairs of PCR primers were designed (Primer Designer v4.10, Science and Educational Software). Pair 1 amplifies a 968-bp region of UT-A2 including nucleotides 1147 through 2114 (forward, 5′-CCTTCCAGTGCCATCCTCAT-3′, and reverse, 5′-ACGTGTA-GGCTCTGACTT-3′) but does not amplify UT-A4. Pair 2 amplifies a 923-bp region of UT-A4 including nucleotides 557 through 1480 (forward, 5′-CGATCGGTAGGACAGCA-3′, and reverse, 5′-CATGCCACCAATACGATA-3′). Pair 3 amplifies a 1289-bp region of UT-A4 including nucleotides 482 through 3777 (forward, 5′-CCTACCTGGCTTCACACT-3′, and reverse, 5′-GACGTCTAGGGGCTGT-3′). Neither pair nor 3 nor amplifies UT-A2. PCR mixtures were size-separated by electrophoresis on 1% agarose gels in glyoxal buffer, blotted to nylon membranes, and crosslinked with UV light. Membranes were hybridized for 2 hours at 68°C (10 cpm/mL) with UT-A probe, GAPDH probe, and then the size-marker probe. The membrane was exposed to x-ray film after each hybridization.
then sequenced using an ABI Prism 310 genetic analyzer (Applied Biosystems).

Statistics
All data are presented as mean±SD, and n indicates the number of rats. An unpaired Student’s t test was used to test for statistical significance, except for protocols in which rats were pair-fed, where a paired Student’s t test was used.

Results
Normal Rats
Western analysis of rat heart tissue lysate revealed 3 bands with molecular masses of 56, 51, and 39 kDa (Figure 2A). The 51- and 39-kDa bands are also present in liver. The 97- and 117-kDa UT-A bands present in kidney inner medulla were not observed in rat heart. To verify that the heart urea transporter proteins recognized by the antibody or antibody that was preadsorbed with the immunizing peptide (+) or antibody that was preabsorbed with the immunizing peptide (−), all 3 UT-A bands are competed away by preabsorbed antibody. This result is representative of 3 similar experiments. C, Heart lysate probed before (Control) or after PNGase F (+PNGase F). This result is representative of 4 experiments.

Figure 2. UT-A proteins. A, Western blot of lysates from rat heart, liver, and kidney inner medulla. Heart shows bands at 56, 51, and 39 kDa. Liver shows bands at 51 and 39 kDa. Inner medulla shows bands at 97 and 117 kDa. B, Comparison of identical heart lysates probed with anti–UT-A antibody (−) or antibody that was preadsorbed with the immunizing peptide (+). All 3 UT-A bands are competed away by preabsorbed antibody. This result is representative of 3 similar experiments. C, Heart lysate probed before (Control) or after PNGase F (+PNGase F). C, Densitometric summary. There was a 1.9-fold increase in the abundance of the 56-kDa band in hearts from uremic rats compared with controls. There was no significant change in the abundance of the 51- or 39-kDa bands. Data are mean±SD, n=8 pairs of rats. *P<0.01 by paired Student’s t test.

Next, heart tissue lysate was treated with PNGase F. The 56-kDa band was not present after PNGase F treatment, and a new 47-kDa band appeared (Figure 2C). The 51- and 39-kDa bands were present both before and after PNGase F treatment. The disappearance of the 56-kDa band indicates that it is N-glycosylated.

Uremic Rats
We tested whether uremia altered the abundance of any UT-A protein in heart. Rats undergoing 5/6 nephrectomy had significantly higher blood urea nitrogen levels (uremic, 111±49; control, 29±11 mg/dL; P<0.05), did not gain weight, had significantly greater left ventricle weight, especially when compared with body weight, and had significantly increased systolic blood pressure (Table). The abundance of the 56-kDa UT-A protein was increased 1.9-fold in hearts from uremic rats compared with the pair-fed control rats (n=8, P<0.01, Figure 3). There was no significant difference in the abundance of the 51- or 39-kDa bands.

Next, hearts from uremic rats were treated with PNGase F. The 56-kDa band was not present after PNGase F treatment, and a new 47-kDa band appeared (Figure 3B), similar to the results observed in untreated rat hearts (Figure 2C). The 51- and 39-kDa bands were present both before and after PNGase F treatment.

<table>
<thead>
<tr>
<th>Heart and Body Weight and Systolic Blood Pressure</th>
<th>Uremia (n=8 to 9)</th>
<th>DOCA (n=7)</th>
<th>Angiotensin II (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>(−) 140±7</td>
<td>(+) 134±14</td>
<td>(−) 314±14</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>181±27</td>
<td>138±29*</td>
<td>373±7</td>
</tr>
<tr>
<td>Heart weight, total, g</td>
<td>0.8±0.1</td>
<td>1.0±0.1*</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Left ventricle weight, g</td>
<td>0.5±0.1</td>
<td>0.7±0.1*</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Left ventricle body weight, g/kg</td>
<td>2.8±0.2</td>
<td>4.9±0.7*</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>162±11</td>
<td>210±8*</td>
<td>126±2†</td>
</tr>
</tbody>
</table>

Data are mean±SD; n indicates number of rats per group. *Significantly different from control (−) value; P<0.05. †Values from Reference 29.

Figure 3. UT-A in hearts from uremic or pair-fed control rats. A, Western blot of a representative pair of heart samples from control (Ctrl) and uremic (Uremic) rats. Each lane represents a sample from a separate rat. B, Heart lysate from uremic rats before (Control) or after PNGase F (+PNGase F). C, Densitometric summary. There was a 1.9-fold increase in the abundance of the 56-kDa band in hearts from uremic rats compared with controls. There was no significant change in the abundance of the 51- or 39-kDa bands. Data are mean±SD, n=8 pairs of rats. *P<0.01 by paired Student’s t test.
UT-A mRNA
Northern analysis revealed a single 2.7-kb band in both uremic and pair-fed control rat hearts (Figure 4). The relative abundance of this band was unchanged by uremia. Using reverse transcriptase (RT)-PCR, the UT-A2–specific primer pair demonstrated the expected 968-bp product, whereas two UT-A4–specific primer pairs did not yield products (data not shown). All three primer pairs yielded the expected sized products from kidney outer medulla (control). Sequencing of both DNA strands of representative PCR products from primer pair 1 matched UT-A2 (data not shown).

Hypertensive Rats
Because uremia is frequently associated with hypertension, we studied the regulation of UT-A protein abundance in hearts in other rat models associated with hypertension. Uninephrectomized rats were treated with DOCA and given saline to drink for 21 days.29 The absolute heart and left ventricle weights were not different between rats receiving angiotensin II and control rats (Figure 5). There was no significant change in the abundance of the 51- or 39-kDa bands. Data are mean±SD; n=7 pairs of rats.

Human Heart Failure
To assess whether the changes that we observed in UT-A protein expression in the experimental rat models could be extended to pathological changes in human heart, we determined whether UT-A proteins were present in human heart and whether their abundance changed in heart failure. Western analysis of nonfailing human heart tissue lysate revealed immunoreactive bands with apparent molecular masses of 97, 56, and 51 and a fainter band corresponding to the mobility of the rat 39-kDa protein (Figure 7A). Only the 97-, 56-, and 51-kDa bands were detected in every sample. The 97-kDa band is analogous to the smaller UT-A1 protein detected in rat kidney inner medulla (Figure 2A); the 56- and 51-kDa bands are analogous to the UT-A proteins detected in rat heart.

Subcellular fractionation showed that the immunoreactive UT-A bands were present in the pellet (Figure 7B), indicating that they were in the membrane fraction. No bands were detected in the supernatant (data not shown), indicating that the UT-A bands were not soluble proteins.

In the terminally failed human hearts, the abundance of the 56-kDa UT-A1 protein increased 1.4-fold (P<0.05), and the 51-kDa UT-A protein increased 4.3-fold (P<0.005) compared with nonfailed human hearts (Figures 7C and 7D). The

Figure 4. UT-A2 mRNA in rat heart. A, Northern blot of a representative pair of hearts from control or uremic rats probed with radiolabeled RNA probes. Each lane represents a sample from a separate rat. UT-A riboprobe detects a 2.7-kb band (top), and GAPDH probe detects a 1.4-kb band (bottom). B, Densitometric summary of UT-A/GAPDH mRNA. There was no significant difference in mRNA abundance (relative to GAPDH) in hearts from uremic versus pair-fed controls. Data are mean±SD; n=8 pairs of rats.

Figure 5. UT-A abundance in hearts of DOCA-infused rats. A, Western blot of a representative pair of hearts from control (−) and DOCA-infused (+) rats. Each lane represents a sample from a separate rat. B, Densitometric summary. There was a 2-fold increase in the abundance of the 56-kDa band in the hearts of DOCA-infused rats compared with controls. There was no significant change in the abundance of the 51- or 39-kDa bands. Data are mean±SD; n=7 pairs of rats. *P<0.005 by unpaired Student’s t test.

Figure 6. UT-A abundance in hearts of angiotensin II–infused rats. A, Western blot of a representative pair of hearts from control (−) and angiotensin II–treated (+) rats. Each lane represents a sample from a separate rat. B, Densitometric summary of UT-A bands. UT-A abundance in hearts of DOCA-infused rats. A, Western blot of a representative pair of hearts from control (−) and DOCA-infused (+) rats. Each lane represents a sample from a separate rat. B, Densitometric summary. There was a 2-fold increase in the abundance of the 56-kDa band in the hearts of DOCA-infused rats compared with controls. There was no significant change in the abundance of the 51- or 39-kDa bands. Data are mean±SD; n=7 pairs of rats. *P<0.005 by unpaired Student’s t test.
Previously, we observed a 3.1-kb mRNA band in rat heart that corresponds to the size of UT-A2, whereas You et al did not detect any UT-A mRNA in rabbit heart. In this study, we detected a 2.7-kb transcript, and the DNA sequence of the RT-PCR product-matched UT-A2. A smaller UT-A2 transcript named UT-A2b (2.5 kb), which has the same coding region as UT-A2 but a shorter 3’ untranslated region, is expressed in kidney inner medulla. Because the PCR product that we sequenced represents almost the entire coding region of UT-A2 (968 of 1190 bp), the small difference in mRNA size on Northern analysis may indicate that the heart transcript results from differences in the untranslated regions of UT-A2. However, to prove this would require cloning the UT-A2 transcript from heart.

Although we cannot definitively assign a UT-A isoform number to each of the UT-A protein bands, the 97-kDa band observed in the human heart migrates at the same mobility as one of the UT-A1 glycoproteins in kidney inner medulla, and the 56-kDa heart band migrates at the same molecular mass as UT-A2 in rat kidney outer medulla. The 51- and 39-kDa heart bands comigrate with the UT-A bands identified in liver. On treatment with PNGase F, the 56-kDa band disappeared and a 47-kDa band appeared in hearts from either control or uremic rats. The 51- and 39-kDa bands do not seem to be changed by PNGase F treatment, similar to the result of PNGase F treatment in liver. These findings suggest that the 56-kDa protein is being deglycosylated to a 47-kDa protein. However, we cannot exclude the possibility that the 56- and 51-kDa proteins are being deglycosylated to 51- and 47-kDa proteins, respectively. All heart samples were kept on ice, and protease inhibitors were added in an attempt to avoid any proteolysis. Nevertheless, we cannot exclude the possibility that some of the heart bands are proteolytic products.

Possible Physiological Role for UT-A in Heart

Whereas the presence of UT-A urea transporter proteins in kidney and liver is logical considering the role of urea in the urine concentrating mechanism and the fact that liver is the principal site of ureagenesis, the function of a urea transporter in heart is not as obvious. In liver, urea production occurs primarily from arginine in the urea cycle. In extrahepatic tissue, urea can also be produced as a byproduct of ornithine synthesis from arginine via arginase in the first step of the polyamine synthesis pathway. Spermatogenesis is associated with an increase in urea production by the polyamine pathway, and urea transporter proteins are present in the Sertoli cells of rodent seminiferous tubules. The polyamine pathway is present in heart, and polyamine production via the rate-controlling enzyme, ornithine decarboxylase, increases in conditions associated with cardiac hypertrophy. The increase in polyamine synthesis may be a critical contributor to cardiac hypertrophy, because ornithine decarboxylase inhibitors protect the heart from becoming hypertrophic during aortic coarctation or during treatment with a β-adrenergic agonist in vivo or in cultured cardiomyocytes. The hypertrophic response is also dependent on autocrine stimulation with transforming growth factor-β. However, the dependence of cardiac hypertrophy on polyamine production has been questioned in transgenic mice that...
overexpress an inhibitor of ornithine decarboxylase, antizyme-1, because β-adrenergic agonist-induced cardiac hypertrophy occurs without an increase in polyamine synthesis.47

Whether or not the induction of polyamine synthesis is critical for cardiac hypertrophy, there is agreement that it is activated in association with cardiac hypertrophy. In the present study, we found a significant increase in the left ventricle/body weight in both uremic rats and the DOCA salt–treated rats (Table), which is evidence for the presence of left ventricular hypertrophy. Although the left ventricle/body weight was not increased in the angiotensin II–treated rats, these rats received angiotensin II for only 3 days, which may not have been long enough to detect significant cardiac hypertrophy. In an earlier study of angiotensin II–infused rats, increased polyamine synthesis in the heart was detected within hours, whereas measurable levels of cardiac hypertrophy were not detected until angiotensin II had been infused for several days.21

Relevant to the human heart, a study of patients with chronic heart failure established that the plasma urea level was an independent prognostic factor of mortality.48 Half of the patients with elevated plasma urea also had elevated creatinine, evidence of decreased renal function, but half did not, perhaps evidence of increased catabolism, a stimulus that might warrant increased urea transporter abundance in tissues such as heart.

In the human heart samples used in this study, we previously studied how heart failure affects the levels of sodium pump isoforms and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger protein levels in the left ventricle. Protein levels of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase α1, α2, and β1 (but not α2) were significantly reduced to between 60% and 70% of control, as were Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity and ouabain binding, whereas levels of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and calsequestrin remained unchanged.34 Thus, membrane transport proteins seem to display unique patterns of change in human heart failure, indicating that the changes are not secondary to changes in the cell membrane to volume relationship. We speculate that upregulation of the UT-A proteins in the hearts of uremic or hypertensive rats and in human dilated cardiomyopathy may be important for urea exit in conditions where urea production is increased.

Summary

This study shows the expression of UT-A proteins in rat and human heart lysate. In rat, the abundance of the 56-kDa UT-A protein is increased in conditions (uremia and hypertension) that predispose to left ventricular hypertrophy and suggest the hypothesis that upregulation of the 56-kDa UT-A protein in the heart of uremic rats may be related to hypertension. This observation was reexamined in a set of human hearts diagnosed as dilated cardiomyopathy, where it was found that the abundance of the 56- and 51-kDa UT-A proteins was significantly increased compared with their expression in nonfailing hearts.

Acknowledgments

This work was supported by National Institutes of Health grants R01-DK41707 and P01-DK50268 (to J.M.S.) and by a Grant-in-Aid from the American Heart Association Western States Affiliate (to A.A.M.). The authors thank Dr Robert H.G. Schwinger and his colleagues (Klinik III for Innere Medizen, University of Cologne, Germany) for providing myocardial tissue samples, Dr David G. Harrison (Emory University, Atlanta, Ga) for providing hearts from DOCA-treated rats, and Dr William E. Mitch (Emory University) for critically reading this manuscript.

References


UT-A Urea Transporter Protein in Heart: Increased Abundance During Uremia, Hypertension, and Heart Failure
Rafael Duchesne, Janet D. Klein, Jeffrey B. Velotta, John J. Doran, Patricia Rouillard, Brian R. Roberts, Alicia A. McDonough and Jeff M. Sands

Circ Res. 2001;89:139-145; originally published online July 5, 2001; doi: 10.1161/hh1401.093293

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/89/2/139

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/