Role of urotensin II in health and disease

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Submitted 28 October 2009; accepted in final form 21 February 2010

Ross B, McKendy K, Giaid A. Role of urotensin II in health and disease. Am J Physiol Regul Integr Comp Physiol 298: R1156–R1172, 2010. First published February 24, 2010; doi:10.1152/ajpregu.00706.2009.—Urotensin II (UII) is an 11 amino acid cyclic peptide originally isolated from the goby fish. The amino acid sequence of UII is exceptionally conserved across most vertebrate taxa, sharing structural similarity to somatostatin. UII binds to a class of G protein-coupled receptor known as GPR14 or the urotensin receptor (UT). UII and its receptor, UT, are widely expressed throughout the cardiovascular, pulmonary, central nervous, renal, and metabolic systems. UII is generally agreed to be the most potent endogenous vasoconstrictor discovered to date. Its physiological mechanisms are similar in some ways to other potent mediators, such as endothelin-1. For example, both compounds elicit a strong vascular smooth muscle-dependent vasoconstriction via Ca^{2+} release. UII also exerts a wide range of actions in other systems, such as proliferation of vascular smooth muscle cells, fibroblasts, and cancer cells. It also 1) enhances foam cell formation, chemotaxis of inflammatory cells, and inotropic and hypertrophic effects on heart muscle; 2) inhibits insulin release, modulates glomerular filtration, and release of catecholamines; and 3) may help regulate food intake and the sleep cycle. Elevated plasma levels of UII and increased levels of UII and UT expression have been demonstrated in numerous diseased conditions, including hypertension, atherosclerosis, heart failure, pulmonary hypertension, diabetes, renal failure, and the metabolic syndrome. Indeed, some of these reports suggest that UII is a marker of disease activity. As such, the UT receptor is emerging as a promising target for therapeutic intervention. Here, a concise review is given on the vast physiologic and pathologic roles of UII.

UT receptor; somatostatin; human; experimental animals; autocrine/paracrine
molecule by cleavage at the dibasic residues (Lys-Arg). Cloning of the human and mouse orthologs of prepro-URP cDNA yielded a URP protein of identical sequence to that of rat URP (117). In an attempt to understand the relationship between UII and URP, the precursor molecules of each were compared, revealing only 18.8% sequence homology (116). As UII is found in the human genome at position 1p36 and URP is found at 3q29, they were thought to be derived from different ancestors (117).

The functional nature of URP was demonstrated by calcium mobilization in CHO cells expressing human or rat UII receptors (UT). URP demonstrated both high potency (EC₅₀ = 4.8 and 0.55 nM for human and rat GPR14/UT receptor-expressing cells) and high affinity of binding (Kᵋ = 170 pM), with similar results to those obtained using hUII (116). Intravenous administration of URP in anesthetized rats led to long-lasting hypotension. Cumulatively, these results point toward URP as an endogenous and functional ligand for the UT receptor (116).

The prepro-URP gene is largely expressed in lower levels than the prepro-UII gene in rats, whereas in humans, prepro-UII and prepro-URP were expressed in similar amounts in most tissues (117).
Evolutionary and Functional Relationships between UII and Somatostatin

Somatostatin was originally discovered nearly 40 years ago as an inhibitor of growth hormone release from the pituitary gland (19, 134) and was named growth hormone-inhibiting hormone for this reason. It also plays an important regulatory role over the release of insulin and a variety of other hormones and enzymes (103). In addition to its expression in nervous, neuroendocrine, and gastrointestinal cells, somatostatin is also expressed in cancer cells (103). With such important roles related to growth, metabolism, and digestion in the body, an indication of homology and conservation of somatostatin and UII is of great importance, particularly in the context of the metabolic syndrome and in diabetes, and therefore in cardiovascular disease.

The remarkable conservation of somatostatin-like peptides underscores the vital role of UII as a basic physiological mediator. The somatostatin gene SS1 is particularly well-conserved and is present in virtually all vertebrate species (126). Likewise, UII exists in all vertebrate classes (126). Since the comparison of their amino acid sequences, UII was suspected to be a somatostatin analog (97). Originally, sequence alignments of both frog and human prepro-UII with the precursors of two somatostatin variants indicated that the two substances may not derive from the same ancestor (28). However, recent evidence has substantiated the notion that UII and URP belong to the same gene superfamily as somatostatin and cortistatin (see Fig. 1). The SS1 and URP genes and the SS2 (cortistatin) and UII genes, respectively, are closely linked on the same chromosomes (125). Thus, the emerging concept is that the UII gene and other somatostatin-related genes share a common ancestral gene. This gene may have undergone two rounds of gene duplication, the first round in tandem and the second round segmental, to create UII, SS1, URP, and SS2 (125, 126).

The UII and somatostatin families have been shown to carry out similar functions. Indeed, there is evidence of receptor cross-activation by UII and URP on somatostatin receptor subtypes and vice versa (82). UII and somatostatin may also elicit similar effects on the same system via different mechanisms. For example, UII and somatostatin both play important inhibitory effects on β-cell insulin secretion in the rat pancreas (86). Through pharmacological antagonism, the UII and somatostatin pathways were demonstrated to work independently of one another.

Discovery and Structure of the UII Receptor

The UT receptor was initially discovered in rats in an experiment looking for novel genes encoding peptide-binding receptors. Using PCR and genomic DNA library screening, an orphan G protein-coupled receptor was discovered and termed GPR14. GPR14 showed a 27% overall sequence homology with the somatostatin receptor SSTR4, with a 41% homology in the transmembrane domains (85). Subsequently, the functional role of the orphan GPR14 was discovered. Rat GPR14 was used to probe the human genomic library in an attempt to identify novel human GPCRs (5). A human 389 amino acid GPCR with 75% identity to rat GPR14 was identified. To determine the role of this receptor, the GPR14 gene was transfected into HEK-293 cells, and a calcium mobilization assay was conducted using multiple peptide ligands. The assay proved the human GPR14 receptor to be highly selective for gUII (5). Subsequent experiments showed that GPR14 was also highly selective for hUII. This led to the identification of GPR14 as the UT receptor (74, 90).

UT belongs to the class A, rhodopsin-like GPCR family. Some structural characteristics of the receptor include two potential N-glycosylation sites in the NH2-terminal domain (Asn29 and Asn33) and two cysteine residues in the first and second extracellular loops, which may be involved in disulfide bonding. The Glu/Asp-Arg-Tyr motif conserved in GPCRs and the phosphorylation sites on the tail can both be found on the intracellular portion of the receptor (15).

UII-UT Interaction

Several structure-activity relationship studies have been carried out between UII and the UT receptor. The nature of the ligand-receptor interaction is important in understanding the pharmacophoric requirements for UT activation. The importance of the cyclic hexapeptide sequence in UII has already been suggested from its conservation across species and further confirmed in elegant experiments. For example, the octapeptide hUII (4-11), which contains the cyclic hexapeptide sequence, has been demonstrated as the minimum active fragment necessary for high-affinity binding to the UT receptor (67). In fact, the potency of UII (4-11) (EC50 = 2.7) was shown to be four times greater than that of the complete molecule (EC50 = 11.9), indicating that the COOH-terminal region is largely responsible for biological activity (67). Each amino acid in the UII molecule was sequentially replaced by an L-alanine or a D-isomer, and the potency of each resulting compound was tested in deendothelialized rat aortic rings. Although replacement of the NH2- and COOH-terminal residues of the cyclic region had a minimal effect, the replacement of residues within the cyclic region itself significantly decreased contractile activity (67). Calcium mobilization studies in GPR14-transfected CHO cells suggest that the only amino acids essential for receptor recognition and activation are Trp-7, Lys-8, and Tyr-9 (38).

The molecular structure and function of the UT receptor has also been investigated. Unfortunately, traditional methods of obtaining 3D structures, such as X-ray crystallography or NMR are not as applicable to GPCRs (13). Thus, information about the UT receptor has been gained by small increments. An experiment using mutational, as well as photo affinity labeling studies, demonstrated that radioactively labeled UII ([125I]-Bz-Phe6[UII]) clearly interacted with Met184 and Met185 located in the fourth transmembrane domain of rat UT, suggesting that transmembrane domain IV is involved in the formation of the ligand-receptor complex and contributes to ligand binding (15). A subsequent study, using hUT, found Met184 and Met185 to be located at the boundary of extracellular loop II (EC-II) and transmembrane helix IV. Through surface plasmon resonance technology, it was demonstrated that the ligands UII, URP, and the potent antagonist urantide all specifically bind to EC-II with high affinity, likely at the same site on the loop. In contrast, none of the compounds bind to EC-I, while only UII and URP bind to EC-III. EC-II may, therefore, be crucial in the signal transduction process (12). Another experiment indicated that one of the hUII binding sites is on one side of helix VII and...
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at the junction of EC-III/transmembrane domain VII, because of the alignment of six out of seven amino acids that face one side of the COOH-terminal helix (13). M288 was identified as a contact site in the rUT EC-III loop (13). This site is not conserved in the human receptor, although it may be in close proximity to an actual contact point between hUII and UT (48). Transmembrane domain VI and transmembrane domain VII are also both involved in the formation of the binding pocket, with the residue W278(6,54) serving as a focal point of interaction. In summary, the UT receptor appears to be quite similar to previously described class A GPCRs (49). The information that is known to date about the structure of UT is rather piecemeal. An understanding of how UII and UT interact could be of great clinical importance if this knowledge could be applied toward efficient pharmacotherapeutic intervention.

Circulating UII in the Plasma

In humans, the typical physiological plasma levels of UII range depending on the location of sampling and on the method of detection employed. In fact, a range of three or more orders of magnitude exists across human studies in the literature (123). Methods including radioimmunoassay, radioreceptor assays, enzyme immunoassays, and ELISA (20) are all used, and a standard method has yet to be determined.

Generally, plasma UII concentrations are found to be in the picomolar range in humans, sometimes even reported to be at the lower end of the detectable limit (87). Thus, UII is thought to act in an autocrine and paracrine fashion rather than as a hormone (145). The distribution of both UII and the UT receptor being widely scattered throughout the body and the concentration of UII in the plasma similar to other potent endothelium-derived autocrine/paracrine vasoconstrictors both lend support to this theory (80, 145).

Plasma UII levels are increased in a number of diseases. A few studies have indicated that there are no significant differences in plasma UII levels between males and females (10, 24, 65, 70, 129), whereas another study reported lower plasma levels of UII in women (89). The possibility of age or gender differences in UII expression, however, has yet to be rigorously explored. Table 1 compares the variations in plasma UII levels across different diseases (Table 1).

UII as a Biomarker

Altered plasma concentrations of UII in diseases, such as heart failure, essential hypertension, renal disease, diabetes, and liver cirrhosis (20) have raised the notion that UII may be a useful biomarker in detecting disease onset or progression (57). In heart failure, UII plasma concentrations are not only increased but also correlate with Big endothelin-1 (Big-ET-1) and brain natriuretic peptide levels as well as with New York Heart Association functional class (43, 93) and negatively correlate with left ventricular ejection fraction (43). Recent evidence suggests that UII is also a useful biomarker for diabetic retinopathy and carotid atherosclerosis in type 2 diabetes (119) and in rheumatic valve disease (93). Paradoxically, in some diseases, high plasma UII levels may actually be a marker for a decreased risk of fatal events (83).

UII and other Vasoactive Peptides

When compared with other vasoactive peptides, UII exhibits many functional similarities. UII and ET-1 both elicit strong vascular smooth muscle cell (VSMC)-dependent vasoconstriction and weak endothelium-dependent vasodilation (80). Like ET-1 and adrenomedullin, UII circulating concentrations are found to be very low and UT receptors are scattered throughout the body (145). Like the ET-1 and angiotensin II receptors, the UT receptor is coupled to a Gq11 subtype of heterotrimeric G proteins (145). Like angiotensin II, UII is thought to elicit RhoA membrane translocation and activation via receptor-Gq11 coupling (145).

Differences among these peptides also exist. Unlike angiotensin II and ET-1, UII appears to have a minimal effect on systemic blood pressure or heart rate (2). Also, unlike ET-1, UII appears to be relatively ineffective on venous blood vessels (80). Perhaps the most striking differences between these peptides are properties, such as potency and absolute response. This is demonstrated in the classical experiment in which the three compounds were tested on the isolated proximal descending thoracic aorta (32). The -logEC50, or pEC50, of hUII was 9.09, significantly higher than both human ET-1 (pEC50 = 7.9) and goby angiotensin II (pEC50 = 8.52) in the rat aorta. The maximal effect brought about by hUII was also higher (Emax = 143.2) compared with goby angiotensin II (Emax = 78.1). Table 2 summarizes the differences between UII and ET-1, in terms of pEC50 and in terms of Emax, in various tissues and organisms (Table 2).

Physiological Actions of UII in Various Organ Systems

Blood vessels. Following its discovery and isolation, UII was quickly revealed to be a very potent vasoconstrictor. Although earlier studies showed that UII elicited contractile effects on most smooth muscle tissues of fishes, it was not until the mid-1980s that these effects were demonstrated in mammalian tissue (55). Using helically cut strips from major arteries of male Sprague-Dawley rats, UII was shown to produce concentration-dependent contractions, with EC50 obtained at a concentration of 6.8 × 10−10 M. This effect was greatest in the thoracic aorta. The action of UII was found to be independent of endothelial cells and to work via mobilization of intracellular calcium as well as through stimulation of extracellular calcium influx (39).

Contrary to these findings, a later study showed that endothelial cells did, in fact, mediate some of the effect of UII on blood vessels (40). This study found that gUII would cause endothelial cell-mediated relaxation of rat aortic strips precontracted with noradrenaline (100 nM) at low concentrations of gUII (0.1 to 0.5 nM), while causing further contraction at higher concentrations (1 to 10 nM). The vasodilatory effect was obliterated when endothelial cells were absent. Overall, however, contraction was observed to be the predominant effect of gUII. This contraction displayed both tonic and phasic components, thought to be mediated by plasma membrane-bound extracellular calcium and free extracellular calcium, respectively (40).

The contractile effect of UII was demonstrated again in the isolated rat aorta, with both gUII and the newly sequenced hUII-inducing potent contraction. The potency of hUII (-logEC50 of 9.09) was found to be greater than that of endothelin-1 (-logEC50 of 7.9),...
Table 1. Summary of the reported plasma levels of urotensin II in health and disease

<table>
<thead>
<tr>
<th>Technique</th>
<th>Disease Studied</th>
<th>Sample Details</th>
<th>Control</th>
<th>Experimental</th>
<th>Control</th>
<th>Experimental</th>
<th>Reference No.</th>
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<td>RIA</td>
<td>Healthy State</td>
<td>VA 15 m, 0 f; 37 yr</td>
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<td>12 ± 3</td>
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<tr>
<td>RIA</td>
<td>Blood Pressure</td>
<td>VA 10 m, 0 f; 42 yr</td>
<td>0</td>
<td>16 ± 1</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>RIA</td>
<td>HT</td>
<td>VA 28 m, 34 f; 54 yr</td>
<td>32 m, 30 f; 57 yr</td>
<td>8.8 ± 0.9</td>
<td>16.3 ± 4.4</td>
<td>166.2 ± 49.5</td>
<td>102</td>
</tr>
<tr>
<td>RIA</td>
<td>CHF</td>
<td>AR n = 88; 21–76 yr</td>
<td>n = 74; 52–89 yr</td>
<td>19 ± 0.9</td>
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<td>101</td>
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<td>n = 20</td>
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<td>150</td>
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<tr>
<td>RIA</td>
<td>Angina</td>
<td>VA 40 age, sex matched</td>
<td>69 m, 25 f; 64 yr</td>
<td>1.35 ± 0.3</td>
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Table 2. *Summary of the pharmacological profiles of urotensin II and endothelin-1 in various tissues and organisms*

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<th>HUMAN ET-1</th>
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<td>Effect</td>
<td>pEC(_{50})</td>
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<tr>
<td><strong>Arterial Vessels</strong></td>
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<td>Left circumflex</td>
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<tr>
<td>Left anterior descending</td>
<td>Dog (beagle)</td>
<td>++</td>
</tr>
<tr>
<td>Common carotid</td>
<td>Dog (beagle)</td>
<td>/</td>
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<tr>
<td>Common carotid</td>
<td>Cynomolgus monkey</td>
<td>++</td>
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<tr>
<td>Coronary vessels</td>
<td>Dog (beagle)</td>
<td>/</td>
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<tr>
<td>Coronary vessels</td>
<td>Cynomolgus monkey</td>
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<tr>
<td>Renal</td>
<td>Dog (beagle)</td>
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<tr>
<td>Renal</td>
<td>Cynomolgus monkey</td>
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<td>Basilar</td>
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<tr>
<td>Basilar</td>
<td>Cynomolgus monkey</td>
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<td>Femoral</td>
<td>Dog (beagle)</td>
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<tr>
<td>Radial</td>
<td>Human</td>
<td>9.5</td>
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<tr>
<td>Mammary</td>
<td>Human</td>
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<tr>
<td>Internal mammary</td>
<td>Cynomolgus monkey</td>
<td>++</td>
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<tr>
<td>Dog (beagle)</td>
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<tr>
<td><strong>Venous Vessels</strong></td>
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<tr>
<td>Jugular</td>
<td>Dog (beagle)</td>
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<tr>
<td>Saphenous</td>
<td>Dog (beagle)</td>
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<tr>
<td>Saphenous</td>
<td>Cynomolgus monkey</td>
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<td>Saphenous</td>
<td>Cynomolgus monkey</td>
<td>/</td>
</tr>
<tr>
<td>Portal</td>
<td>Cynomolgus monkey</td>
<td>/</td>
</tr>
<tr>
<td>Umbilical</td>
<td>Cynomolgus monkey</td>
<td>9.3</td>
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- **E\(_{\text{max}}\)**: maximal effect brought about by human urotensin II (hUII); ET-1, endothelin-1; n, no vasoconstriction; (+), weakest/smallest vasoconstriction; +, weak/small vasoconstriction; ++, moderate vasoconstriction; ++++, strong vasoconstriction; /, consistently unresponsive.
- ET-1 compared with UII: *P < 0.05, †P < 0.01, §P < 0.005, ¥P < 0.001.

Contradictory information often arises in UII experimentation. Differences can result from using different types of UII, using different animal species models, and within each species, using different anatomical vessels. All of these factors alter the manner in which the UII system functions. For example, a differential contractile potency of fish UII has been shown to exist across different vessel segments in the rat, with binding in the thoracic aorta > abdominal aorta > mesenteric artery (56). In addition, UII action is variable in different vascular beds of mice, pigs, dogs, and monkeys (32). While almost no response was observed in the mouse aorta, the rat thoracic aorta and dog coronary circulation displayed pronounced constrictive responses. Likewise, large variability and variable reproducibility was observed in tissue from monkeys and pigs (32).
to activate the l-arginine/NO synthase/NO pathway, resulting in vasodilation (72). Somewhat surprisingly, hUII was shown to cause sustained vasodilation in the coronary arteries of perfused male Fischer rat hearts after causing a transient decrease in coronary flow. This was inhibited by the cyclooxygenase inhibitor diclofenac and attenuated by the NO synthase inhibitor N\textsuperscript{ω}-nitro-l-arginine (l-NNA). This vasodilatory effect was thus attributed to the UII-mediated release of prostacyclin and NO, respectively (60). The mechanism of coronary vasodilation was subsequently compared in rats at different ages (54). Once again, diclofenac attenuated hUII-induced coronary vasodilation in both young and aged rats, demonstrating the importance of prostacyclin in hUII-induced coronary vasodilation. Conversely, hUII-mediated NO production and the inhibitory effect of l-NNA were only observed in young rats. It was concluded that in aged rats, a damaged endothelium over time likely impairs endothelial NO production (54). In addition to hUII, rUII was also subsequently demonstrated to be a potent dose-dependent vasodilator of the coronary arteries in the perfused rat heart (98). In contrast to the previous studies, the use of nonnative UII on the perfused heart either attenuated or reversed the vasodilator effects of rUII. URP acted similarly to rUII, causing dose-dependent vasodilation of the coronary arteries. However, unlike the rUII-mediated vasodilation, the URP-mediated vasodilation was not maintained, demonstrating the inferior potency of URP to its rUII counterpart (98). In addition to the coronary circulation, UII-mediated NO-dependent vasodilation has also been observed in the pial arteries of newborn pigs (68), in rat renal arteries (147), and in human small muscular pulmonary and abdominal arteries (115).

The in vivo vasoactive effects of UII in humans have been assessed in a variety of experiments on healthy human subjects. Experiments relied largely on measuring forearm blood flow to assess the effects of hUII infusion. In one experiment, even with hUII concentrations of 300 pmol/min for 20 min that increased plasma concentrations of UII 30-fold, no UII-mediated effect on forearm blood flow was observed (143). This is in contrast to other vasoactive peptides, such as ET-1 and angiotensin II, which have been shown to produce effects at much lower concentrations. Inhibition of NO and prostanoid production also failed to reveal any vasoactive effect of UII, and no changes in systemic hemodynamics or EKG alterations were observed (143). Another study found very similar results, where, despite a 100-fold increase from baseline in circulating UII concentrations, intravenous UII infusions did not affect hemodynamics or arterial stiffness (2). However, one study did report a significant decrease in forearm blood flow following UII administration and concluded that this was as a result of potent UII-mediated vasoconstriction (11).

Other human studies have focused on comparing healthy and diseased subjects. The effects of UII administration were compared between patients with essential hypertension and healthy subjects by assessing changes in the skin microvasculature (113). Interestingly, in healthy volunteers, a vasodilatory effect was observed, whereas a vasoconstrictive effect was observed in subjects with essential hypertension following UII administration. This same phenomenon was observed when the skin microvasculature tone was compared between healthy subjects and subjects with congestive heart failure: upon UII administration normal subjects displayed UII-mediated vasodilation, whereas patients with CHF displayed UII-mediated vasoconstriction (71). The reported variations in UII activity in diseased vs. healthy humans provides an important example of the difference between physiological and pathophysiological states and how the role of UII can drastically change in these distinct settings.

In summary, UII induces vasoconstriction, migration, and proliferation of vascular smooth muscle cells. UII also induces vasodilation in an endothelial-dependent manner (see Fig. 2). The vasoconstrictive effects are thought to be mediated via a number of pathways upon binding the UT receptor. Experimental evidence points to Ca\textsuperscript{2+} release via PLC/dependent IP\textsubscript{3} and calmodulin-dependent myosin light-chain kinase pathways, with ERK and RhoA/Rho kinase pathways also partially involved. The latter two pathways are also important in VSMC proliferation and migration (138). The endothelium-dependent vasodilatory effects of UII occur through the release of NO, prostaglandin E\textsubscript{2}, and endothelium-derived hyperpolarizing factor (94, 138) (Fig. 2).

Heart. The heart is one of the major sites of UII release (21, 22). The many hemodynamic and cardiac effects of UII have been assessed in a variety of animal and human experiments. Low doses (<30 pmol·kg\textsuperscript{-1}·10\textsuperscript{-7}) of hUII in rats were shown to slightly increase cardiac output, decrease regional vascular resistance, and leave systemic blood pressure unchanged. At higher doses (≥10 pmol·kg\textsuperscript{-1}·10\textsuperscript{-5}), however, hUII caused a dose-dependent decrease in cardiac output and severely impaired stroke volume, myocardial contractility, and systemic vasoconstriction (5). Another study also found dose-dependent differences in rats. At low concentrations (3 pmol/kg), neither rUII nor hUII administration seemed to have any effect. At a 30 pmol/kg dose, however, rUII was shown to cause transient mesenteric vasodilation, while at 300 and 3,000 pmol/kg, dose-dependent tachycardia and mesenteric and hindquarter hyperemic vasodilation was noted (39).

A strong, dose-dependent vasoconstrictive response to hUII was also noted when administered to anesthetized monkeys with a dose of 300 pmol/kg (5). This response was marked by a 300% increase in total peripheral resistance and resulted in myocardial depression and fatal circulatory collapse. Notably, when substantially larger doses of other vasoconstricting peptides, such as angiotensin-II and ET-1 (1 nmol/kg iv) were administered, a simple sustained increase in arterial pressure was observed (5). The mechanism of circulatory collapse that occurred in anesthetized cynomolgus monkeys with bolus systemic administration of hUII was further investigated (153). Negative chronotropic and inotropic effects of UII on the heart. Arterial vasodilation leading to decreased afterload, and pulmonary hypertension were found to be the factors that ultimately contributed to cardiovascular breakdown and death (153).

Intravenous UII administration in ewes caused a sustained tachycardia with no significant effect on cardiac output, contractility, or total peripheral conductance (140). In a recent human forearm blood flow experiment, administration of UII yielded no effect on forearm blood flow in both normal subjects and subjects with cardiovascular disease (23). However, systolic and mean pressures increased significantly in both groups. In addition, an increased heart rate was observed in the subjects with cardiovascular disease (23).
Lungs. There appear to be regional differences in UII action along the pulmonary vasculature. Although there is a potent vasoconstrictive response in main rat pulmonary arteries, small rat pulmonary arteries were not constricted by hUII (78). The same phenomenon is observed in small human pulmonary arteries (47). hUII caused potent vasodilation in human small muscular pulmonary arteries precontracted by ET-1. This vasodilation was equivalent to the effects of adrenomedullin and greater than those of sodium nitroprusside and acetylcholine (115).

Administration of hUII to the pulmonary circulation of monkeys results in a very potent vasoconstrictive response (5, 32). Indeed, the potency of UII (-log[EC₅₀] = 9.29) was reported to be 28 times greater than that of ET-1 (-log[EC₅₀] = 7.84) (5). However, whereas administration of ET-1 in humans, isolated perfused pulmonary arterial and lung specimens elicited marked vasoconstriction, UII infusion was found to yield no vasoconstrictive effect (9).

Kidneys. UII is thought to play a role in maintaining renal vascular tone and tubular function. hUII was found to produce endothelial NO-mediated vasodilation in rat renal arteries (147). A continuous infusion of hUII resulted in a dose-dependent increase in renal blood flow, as well as increased urinary water and sodium excretion. The administration of L-NAME completely abolished all of these effects, indicating the involvement of NO. These findings were similar in isolated, phenylephrine, precontracted rat renal arteries. When the arteries were denuded of their endothelium, no effect was seen.
Using 4,5-diaminofluorescein diacetate-based fluorescence imaging analysis, hUII was shown to double the amount of NO in the endothelium of dissected renal arteries, an effect that was inhibited by L-NAME (147). A later study in rats where rUII was used found contradictory results (114). Injection of rUII led to reductions in GFR, urine flow, and sodium excretion, whereas the UT antagonist urantide caused these variables to increase (114). This discrepancy regarding the action of UII in glomerular filtration and natriuresis likely derives from the differing types of UII used and from the method of UII administration (20).

A significant amount of UII-like immunoreactivity in human urine was found using radioimmunoassay technique (87). Abundant expression of mRNA transcripts for both UII and UT in the kidney was also observed. Because the clearance of hUII exceeded the glomerular filtration rate, it was determined that urinary hUII is of renal tubular origin (87). Immunostaining for UII in normal human kidneys demonstrated that the strongest presence of UII is in the epithelial cells of the renal tubules and ducts (106). Subsequent studies in normal human kidneys have confirmed the presence of UII in the renal epithelium (81). Saturation binding experiments also displayed diffuse presence of UT in the renal cortex in normal human kidneys (79). Thus, in the physiological state, UII and UT are both endogenously expressed in the kidney and are likely involved in normal renal function.

Adrenal glands. In addition to renal function, there is also structural and functional evidence that UII plays a role in regulating normal adrenal function. UII and UT receptor mRNA expression was found in cultured rat adrenocortical cells using RT-PCR (4). Furthermore, adrenal hormones are influenced by UII administration. For example, corticosterone secretion was decreased by UII in cultured rat adrenocortical cells, an effect abolished by the UII antagonist palosuran (4). Upon intracerebroventricular infusion of UII in conscious ewes, plasma levels of epinephrine and ACTH increased, remaining elevated for up to 4 h after infusion (140). Thus, UII appears to play an important regulatory role in adrenal steroidogenesis and in adrenal hormonal release.

Nervous system. Central UII regulation is not limited to the sympathoadrenal medullary and pituitary-adrenal cortex pathways. Although UII is typically thought of as a direct vasoactive mediator, it is also found in motor neurons of the spinal cord as well as in the brain stem (74). In rats, the UT receptor is widely expressed throughout the brain and spinal cord, in a variety of functional nuclei (74). Indeed, there is evidence for central and peripheral nervous modulation of UII over a number of physiological processes (58). For example, UII is becoming increasingly implicated in a variety of mood and sleep processes. UII plays a role in releasing norepinephrine in the cerebral cortex (62), influences rapid eye movement in the rat via direct cholinergic activation (53), and alters behavior in mice upon central administration (30). UII is also implicated in digestive behavior. UII increases mesenteric, iliac, and renal blood flow (140) and contracts strips of isolated ileum from the guinea pig via mesenteric neuron activation (52).

Contrasting hemodynamic effects exist when UII is microinjected into different regulatory nuclei in anesthetized rats (76). The effects of UII were assessed on the medulla, notably areas designated A1 and A2. These areas contain important noradrenergic neurons that play a role in central cardiovascular control. While microinjection of UII into the A1 brain area resulted in a dose-related long-lasting hypotensive and bradycardic response, microinjection of UII into the A2 area produced no significant effect on mean arterial blood pressure or heart rate. When injected into the paraventricular and arcuate nuclei, UII caused transient, significant, hypertensive and tachycardiac responses. This study demonstrates the central neural effects of UII as well as its variability of action on different areas of the brain (76).

Intracerebroventricular administration studies have also demonstrated the potential of UII-mediated central cardiac control. In rats, intracerebroventricular administration of UII yielded no response at a low dose (1 nmol), but at a higher dose (10 nmol) UII increased both arterial pressure and heart rate. These effects were proposed to be mediated by sympathetic activation, as the observed effects were significantly attenuated by preinfusion of the ganglionic-blocking agent pentolinium (73). Intracerebroventricular administration of UII to conscious ewes caused an increase in heart rate, contractility, and cardiac output (140). These effects were blocked upon administration of propranolol (30 mg bolus followed by 0.5 mg·kg\(^{-1}\)·h\(^{-1}\) iv) in a subsequent experiment in sheep, implicating the importance of β-adrenoceptor activity in central UII cardiac control (51).

Liver. The effect of UII on the liver is variable. In fact, while some studies have reported the presence of UII and the UT receptor in the liver, others have failed to observe their presence (64). Nevertheless, the liver is thought to be one of the main producers of circulating UII, as evidenced by the aforementioned studies in sheep (21) and in humans (22). Interestingly, the vasodilatory effects of UII may dominate over vasoconstriction in the portal circulation (45) confirmed by the local overproduction of NO (1). This vasodilatory dominance in the portal circulation is important in maintaining portal blood flow and therefore in regulating portal blood pressure (64).

Pathological Significance of UII

Atherosclerosis. Atherosclerosis is often regarded as a principal cause of most cardiovascular diseases and consequently as the leading cause of death in the Western world (95). Atherosclerosis is a process marked by chronic vascular inflammation of the arterial wall due to an infiltration of lipids, macrophages, and other inflammatory mediators. Foam cells, macrophage-derived cells filled primarily with cholesterol ester, become evident in the arterial wall. The resulting formation of plaques harden the vessel walls. Atherosclerosis often precedes cardiovascular events, such as myocardial infarction and stroke. The role that UII plays in the progression of atherosclerosis is a major and pressing area of current research (75).

In the apolipoprotein E gene knockout mouse model of atherosclerosis, quantitative RT-PCR analysis confirmed UT receptor expression to be increased in the aortic tissue of atherosclerotic mice (137). Using transgenic and crossbred mouse strains, we have recently demonstrated that selective induction of UT expression in mice VSMCs displayed far greater aortic lesions compared with wild-type mice, an effect that was even more pronounced in ApoE-knockout/UT transgenic crossbred mice (95). Interestingly, in the ApoE-knockout mice, an increased expression of UT was also observed. When VSMCs of normal and atherosclerotic human coronary arteries
were compared, no difference was observed (61). However, in human abdominal aortic aneurysm and carotid atherosclerotic extracts, UT and UII expression is increased (17).

UII is thought to play an autocrine/paracrine role in the formation of atherosclerotic plaques. Plasma UII levels are elevated in patients with confirmed atherosclerosis (118). Through regression analysis, UII plasma levels predicted a 1.6-fold higher risk of carotid plaque formation compared with the currently recognized risk factors (118). In addition, UII expression is increased in areas of atherosclerotic plaque as shown by immunocytochemistry (44) and real-time PCR (17). UII is upregulated throughout the vessel wall (in endothelial, myointimal, and medial smooth muscle layers) in human coronary atherosclerosis (44).

The well-known proliferative effect of UII on VSMCs might also contribute to atherosclerotic development. As mentioned, UII-mediated VSMC proliferation is known to be ERK and RhoA/Rho kinase-dependent (138). UII increases collagen-1 and decreases MMP-1 expression via these pathways in endothelial cells (135). Increased reactive oxygen species (ROS) levels also induce VMSC proliferation. Indeed, ROS production is an integral part of atherosclerotic development, as it converts low-density lipoproteins into oxidized low-density lipoproteins. UII plays direct effects on ROS levels by activating NADPH oxidase subunits p22phox and NOX4 (29, 138). ROS has also been shown to play a critical role in UII signal transduction (132). Thus, the roles of UII and ROS are intimately related along the progression of atherosclerosis. In addition to upregulating the expression of NADPH oxidase, UII also significantly upregulates acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT-1) expression. ACAT-1 is crucial in the aforementioned process of foam cell formation, as it converts intracellular free cholesterol into cholesterol ester.

Among the inflammatory cell population, lymphocytes appear to be the largest producers of UII, whereas monocytes and macrophages appear to express the UT receptor the most (17). Inflammatory compounds, such as LPS, TNF-α, and IFN-γ all upregulate UT receptor mRNA expression (105), alluding to the chemotactic and signaling roles that UII may play in the progression of atherosclerosis. Indeed, UII-mediated chemotaxis has been demonstrated in monocytes in a RhoA/Rho kinase-dependent manner (105). It is becoming increasingly clear through biochemical, animal, and human studies that UII may be a key player in the development of atherosclerosis.

**Congestive heart failure and other cardiac diseases.** UII expression and UII plasma levels are increased in many types of cardiac disease. In myocardial specimens from patients with congestive heart failure, immunohistochemical analysis demonstrated strong cardiomyocyte expression of UII (33). The presence of UII in the cardiomyocytes correlated significantly with left ventricular end-diastolic volume and was inversely correlated with ejection fraction. A subsequent study found that UII plasma levels are also significantly elevated in patients with CHF, and that UII levels are inversely correlated to ejection fraction (43). hUII immunoreactivity was increased in blood samples taken from the aortic root of CHF patients (102). Finally, both UII and UT receptor expression are increased in proportion to disease severity in heart failure (43, 152).

UII is also clearly implicated in coronary artery disease (46), left ventricular systolic (70) and diastolic (130) dysfunction, and myocardial infarction (65, 133). In fact, UII and UT receptor expression are increased in proportion to disease severity in infarct and noninfarct zones of patients with myocardial infarction (133). UII plays an active role in cardiac fibrosis, hypertrophy and remodeling (133). Lastly, plasma UII levels correlate positively with ET-1, adrenomedullin, and Nδ2-terminal brain natriuretic peptide (33, 35, 43).

**Systemic arterial hypertension.** One of the major risk factors in the development of atherosclerosis is elevated blood pressure. Hypertension is an important part of the metabolic syndrome and also carries predictive value for the onset of myocardial infarction and stroke. In fact, the incidence of hypertension parallels the incidence of stroke (144). UII may play an important role in the etiology of essential hypertension. UII plasma levels and systolic blood pressure mirror one another (24). UII-like immunoreactivity concentrations were found to be high in patients with essential hypertension and in patients with hypertensive renal disease (87).

UII can act synergistically with angiotensin II to induce vasoconstriction in the endothelium-denuded rat aorta (136). Interestingly, this synergism is not so apparent in the endothelium-intact rat aorta, possibly due to a masking of the effect by NO (136). In what can be thought of as a cause-and-effect relationship, UII causes potent vasoconstriction that leads to hypertension. Hypertension, in turn, increases turbulent hemodynamic flow and shear stress on the endothelium, which may lead to endothelial damage and endothelial dysfunction. This endothelial dysfunction further compromises the arterial system having to handle synergistic UII-angiotensin II-mediated vasoconstriction. The vessel walls may also become more vulnerable to the development of atherosclerosis (91).

**Renal disease.** Renal disease is often associated with other diseases, such as diabetes and hypertension, and can also be complicated by the need for dialysis treatment (104). There is widespread evidence of UII expression alterations and changes in circulating UII levels in a number of renal pathologies, including renal failure, nephrotic disorders, and end-stage renal disease.

Plasma UII-like immunoreactivity was assessed in chronic renal failure. Compared with normal subjects, levels were twofold higher in nondialysis patients and threefold higher in patients on hemodialysis (129). Thus, in this disease, renal clearance may be impaired (145). Patients with hypertensive renal disease displayed an increased urinary UII-like immunoreactivity compared with normotensive renal disease patients, perhaps as a result of hypertensive target organ damage (87). Renal dysfunction in diabetes is associated with higher plasma and urinary levels of UII than in diabetics with normal renal function (128). UII and UT receptor mRNA is increased in renal biopsy tissue of patients with diabetic nephropathy compared with normal subjects by upward of 45- to 2,000-fold (69). In patients with renal tubular disorders, an increased UII-like immunoreactivity was observed, while those patients with renal glomerular disease were not different than normal subjects in this regard (87). In patients with renal carcinoma, immunohistochemistry assays demonstrated the presence of UII in renal tumors and in the surrounding inflammatory cells (106).

UII is also altered in patients with end-stage renal disease (ESRD) with interesting inverse correlations to risk. Plasma levels of UII in ESRD are elevated compared with age-
matched controls. However, in these patients, plasma UII levels were inversely correlated to many neurohumoral factors (84), to biomarkers of atherosclerosis, and to endothelial activation (83). Furthermore, elevated UII levels are associated with more favorable echocardiographic profiles (154) and actually predict lower overall cardiovascular risk in ESRD (155). These paradoxical findings hint at a potential protective role of UII in some forms of renal disease. This role is thought to stem from the interference of UII with sympathetic and NO systems (154).

Adrenal tumors and other cancer types. Basal endogenous expression of UII and of the UT receptor in renal carcinoma (106) and in certain rhabdomyosarcoma cell lines (31), respectively, lend support to the implicated role of UII in a variety of cancers. In particular, substantial UII and UT expression alterations were observed in a number of adrenal cancers (88). Secretion of UII was measured by radioimmunoassay in seven tumor cell lines: T98G glioblastoma cells, IMR-32 neuroblastoma cells, NB69 neuroblastoma cells, BeWo choriocarcinoma cells, SW-13 adenocortical carcinoma cells, DLD-1 colorectal adenocarcinoma cells, and HeLa cervical cancer cells (122). The UT receptor was expressed in all seven cell lines, and UII was expressed in all lines except NB69. Moreover, significant UII-like immunoreactivity by radioimmunoassay was detected in SW-13 cells, indicating UII secretion in this cell line (122). Treatment of the SW-13 line with UII (10⁻⁶ and 10⁻⁷ mol/l) for 24 h in a subsequent experiment resulted in a significant increase in SW-13 cell number (121). UII has also been shown to induce proliferation of human pheochromocytoma cells (149). Thus, UII is implicated once more as an autocrine/paracrine growth factor.

Recently, pheochromocytoma and aldosterone-producing adenoma samples were obtained and compared by using real-time PCR and immunohistochemistry (41). Relative to one another, UII expression is increased in pheochromocytoma, whereas UT receptor expression is increased in the adenoma tissue (41). This may account for the clinically observed relationship between pheochromocytoma and primary aldosteronism (41).

Metabolic syndrome. The metabolic syndrome consists of a number of metabolic disturbances that may influence one another. Collectively, these abnormalities are conducive to the development of type 2 diabetes, cardiovascular disease, and eventually, death (36). Despite the lack of a clear definition or set of guidelines (3), the metabolic syndrome is generally agreed to encompass hypertension, dyslipidemia, obesity, hyperglycemia, and insulin resistance (91). The cumulative effects of UII on glucose metabolism and insulin resistance may combine with the pressor, lipogenic, and inflammatory effects in a positive feedback mechanism. Although UII may not be implicated in the initiation of the individual factors comprising the metabolic syndrome, there is certainly evidence that UII plays a role in the development of each factor, particularly when coupled to endothelial dysfunction (124).

Dyslipidemia refers to the imbalance of lipids, namely increased levels of triglycerides and low-density lipoproteins and decreased levels of high-density lipoprotein, and is related to obesity. Increases in adipose tissue elevate the level of free fatty acids in the plasma, via both the hydrolysis of triglycerides and the lipolysis of lipoproteins (36), which further contribute to hypertension and hyperglycemia. UII plays a role in both dyslipidemia and obesity. In addition to systolic and diastolic blood pressure, plasma UII levels were found to correlate with body weight (24). UII has been shown to induce hyperlipidemia, at least in fish, by enhancing triglyceride lipase activity (107). UII may also contribute to obesity and dyslipidemia, as well as to hyperglycemia, via indirect means; as mentioned, adrenal secretions are influenced by UII (140).

Notably, the pathophysiology that stems from the metabolic syndrome is thought to be largely attributable to insulin resistance (36). Hyperglycemia and insulin resistance are two interrelated components of the metabolic syndrome, and both are related to UII. UII and the UT receptor are both expressed in the pancreatic islets (111). In salmon, UII increases glucose-6-phosphatase activity and reduces liver glycogen content (107). At high concentrations (100 nmol/l) frog UII inhibits glucose- and arginine-induced insulin responses in the rat pancreas (112). When synthetic rat UII was used on the same model, a dose-dependent inhibition of glucose-induced insulin release was observed once again (IC₅₀ = 0.12 nmol/l) (111). This insulinoostatic pathway of inhibition is independent of the somatostatin pathway (86). In humans, single nucleotide polymorphism analysis in the Hong-Kong Chinese population confirmed that certain UTS2 gene haplotypes are associated with insulin resistance and pancreatic β-cell function (92). Thus, UII likely plays an important and direct inhibitory role on β-cell insulin secretion in an autocrine/paracrine manner, and may contribute to insulin resistance in pathological settings (111).

Type 2 diabetes. The individual and separate actions of UII in the metabolic syndrome may act to promote the onset of type 2 diabetes (91). The pathological progression of diabetes in itself also likely involves UII. Plasma UII levels are almost twice as high in patients with type 2 diabetes without proteinuria compared with healthy subjects (7.8 fmol/ml vs. 4.4 fmol/ml) (127). The elevated UII levels do not appear to be a downstream result of diabetic hyperglycemia. Instead, UII may be responsible for hyperglycemia through the mobilization of glucose, an aforementioned contribution of UII to the metabolic syndrome (127). In the Han population, the UTS2 polymorphism rs2286848 is associated with type 2 diabetes (120, 151). In the Japanese population, the S89N polymorphism in the UTS2 gene was found to be associated with the development of type 2 diabetes (141). The allele frequency of 89N was greater in type 2 diabetics; and in the subjects with normal glucose tolerance, 89N was correlated to higher insulin levels during oral glucose tolerance testing (141).

Pulmonary hypertension. As aforementioned, the actions of UII in the pulmonary circulation are quite variable (78, 115). These variations at different levels of the pulmonary circulation make it difficult to understand the role of UII in pulmonary hypertension. As in atherosclerosis, proliferation, remodeling, and ROS production are vital processes in the progression of pulmonary hypertension. Thus, UII may play an important role in disease progression in this sense.

UII increased the activity of NADPH oxidase and plasminogen activator inhibitor-1 in human pulmonary VSMCs (29). Endothelial cells and VSMCs in the pulmonary arteries of rats with experimentally induced pulmonary hypertension display an increased expression of UII (99). Hypoxia is a well-known cause of pulmonary hypertension. Although one study in chronically hypoxic rats did not observe any increase in plasma UII...
levels (148), in another study, hypoxia was found to specifically increase UII in endothelial and smooth muscle cells in the pulmonary arteries of rats (50). Hypoxia may, therefore, cause pulmonary hypertension, at least in part, via UII-dependent activation of NADPH oxidase in the pulmonary vascular wall and via UII-dependent proliferative effects on VSMCs. However, with the current discrepancy in the literature, researchers are still largely uncertain of the role that UII plays in pulmonary hypertension.

**Hepatic disease.** Vasoactive substances have long been known to be important in liver pathologies, such as portal hypertension (64). It is noteworthy that UII is structurally similar to both somatostatin and to its synthetic analog octreotide (64). Direct evidence for the pathological role of UII in chronic liver disease has recently been found (63). In patients with chronic liver disease, serum UII is elevated compared with controls. In addition, UII levels correlate with the severity of disease and with the extent of portal hypertension. Finally, baseline UII levels can even carry predictive value for determining survival or future portal complications (63). Similar reports of increased UII levels have been observed in the human cirrhotic population (45), with disease severity being related to the degree of UII plasma elevation. Patients with ascites display even higher levels of UII. It appears as though elevated levels of UII, among other things, increases mesenteric blood flow via local vasodilation (64). This effect, in turn, increases blood pressure and causes portal hypertension.

**Protective effects of UII**

An emerging concept proposes that the observed increases in UII levels in a number of cardiovascular and renal diseases may actually be protective in nature. Although UII levels are increased in many diseases compared with the physiological condition, those diseases individualized with the highest UII levels appear to be better protected against adverse events than diseased individuals with lower UII levels. For example, compared with patients with stable coronary artery disease and with healthy subjects, those patients with acute cardiac ischemia displayed lower circulating levels of UII (59). There may also be a protective effect from high UII levels in post-MI patients, as higher levels are associated with a lower risk of adverse events (65). The protective role of UII is also observed in renal disease. In those patients with ESRD, an inverse association was found between UII levels and clinical outcome (155). Specifically, patients with higher UII levels appear to be better protected against adverse events than the reported UT affinity for palosuran in mammals (8).

Importantly, in another study, UII was shown to promote survival, increased insulin levels, and slowed the increase in glycemia in a rat model of type I diabetes (26). Palosuran was also shown to reduce portal pressure, increase splanchic vascular resistance, and decrease the portal inflow in a rat model of cirrhosis and in bile duct-ligated rats (131). Thus, palosuran may help to alleviate portal hypertension (131). Despite the results, it was proposed that in the rat study on renal ischemia-reperfusion, only approximately half the dose necessary for efficacy was given (8), as a dose of 10 mg/kg translates to a plasma concentration of only 5 μM (123). In addition, it should be noted that the binding affinity of palosuran in rats is very low (123).

Importantly, palosuran also blocks human UTs (25). Unlike uratide, palosuran has been used in humans. A very recent entry to humans study tested palosuran in healthy adult males with favorable tolerability (108). Unfortunately, to date, human studies using palosuran have been largely inconclusive. Oral palosuran treatment of hypertensive type 2 diabetic nepropathy patients with 125 mg, twice daily for 13.5 days when combined with ACE inhibitors or ARBs, decreased 24-h urinary albumin excretion rate (110). However, palosuran did not affect important renal hemodynamic parameters, such as glomerular filtration rate and renal blood flow (110). In a subsequent study in type 2 diabetics, palosuran demonstrated no clinically significant effects on β-cell secretory capacity and no effects on first- and second-phase insulin response (109). The reason for these disappointing results has been largely ascribed to a poor dosing regimen, as 125 mg of palosuran twice daily translates to a peak plasma concentration of < 260 nM, > 10-fold lower than the reported UT affinity for palosuran in mammals (8). Other accounts of poor study design, such as low number of participants and lack of a control group, implies that palosuran...
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suran, if well administered, may still hold great promise in treating human disease.

Positive results obtained with other, more reliable UT receptor antagonists cement the notion that UII targeting is indeed of benefit in treating many diseases. Novel synthetic UII antagonists continue to emerge (77), bringing safe and potent UT receptor blockers ever closer to clinical use. Perfecting this intervention is a principal focus, today and in the future.

Perspectives and Significance
It is an exciting era in the growing field of research on UII. Many landmark discoveries have been made since the initial discovery of this potent somatostatin-like peptide some 30 years ago, and the specific role and mechanisms of action of UII in a variety of diseases is beginning to unravel. UII antagonism may become a significant therapy for a number of diseases in the years to come. However, with the strong evolutionary conservation of this compound, caution must be used in employing UII antagonists too loosely. Basic and clinical research assessing the physiological roles and pathophysiological mechanisms of UII continue to expand our knowledge of this interesting compound.

GRANTS
A. Giaid is supported by the Canadian Institutes of Health Research.

DISCLOSURES
No conflicts of interest are declared by the authors.

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