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## **Urotensin II: its function in health and its role in disease**

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Short title: Urotensin II

## **Abstract**

Urotensin II (U-II) is the most potent vasoconstrictor known, even more potent than endothelin-1. It was first isolated from the fish spinal cord and has been recognized as a hormone in the neurosecretory system of teleost fish for over 30 years. After the identification of U-II in humans and the orphan human G-protein-coupled receptor 14 as the urotensin II receptor, UT, many studies have shown that U-II may play an important role in cardiovascular regulation. Human urotensin II (hU-II) is an 11 amino acid cyclic peptide, generated by proteolytic cleavage from a precursor prohormone. It is expressed in the central nervous system as well as other tissues, such as kidney, spleen, small intestine, thymus, prostate, pituitary, and adrenal gland and circulates in human plasma. The plasma U-II level is elevated in renal failure, congestive heart failure, diabetes mellitus, systemic hypertension and portal hypertension caused by liver cirrhosis. The effect of U-II on the vascular system is variable, depending on species, vascular bed and calibre of the vessel. The net effect on vascular tone is a balance between endothelium-independent vasoconstriction and endothelium-dependent vasodilatation. U-II is also a neuropeptide and may play a role in tumour development. The development of UT receptor antagonists may provide a useful research tool as well as a novel treatment for cardiorenal diseases.

[216 words]

Key words: urotensin II, hypertension, vasoactive peptides, vasoconstriction

## **Introduction**

Urotensin II (U-II) was first isolated from the fish spinal cord and has been recognized as a hormone in the neurosecretory system of teleost fish for over 30 years.<sup>1,2</sup> U-II is the most potent vasoconstrictor known and is even more potent than endothelin-1 (ET-1).<sup>2</sup> This brief review summarises what is known about the peptide and its receptor (UT), their physiological roles and relation to diseases.

## **Amino acid sequence and mRNA expression**

U-II is a cyclic peptide and shares a similar sequence with somatostatin (Table 1).<sup>1</sup> U-II isoforms from human, monkey, pig, rat, mouse and goby all contain a conserved C-terminal cyclic hexapeptide sequence (Cys-Phe-Trp-Lys-Tyr-Cys) that confers most of the biological activity. The N-terminus of U-II differs in length and sequence depending on the animal species.<sup>3,4</sup>

Human U-II (hU-II) is an 11 amino acid cyclic peptide and is derived from a large precursor molecule (prepro-U-II). The gene encoding the peptide, *UTS2*, is located at 1p36 and contains 5 exons (Figure 1). Human prepro-U-II mRNA has been found in the heart, aorta, vascular endothelial cells, leukocytes, brain, spinal cord, kidney, lung, liver, adrenal gland, pituitary, spleen, small intestine, colon, placenta and other tissues, with the highest intensity in the spinal cord.<sup>5-9</sup>

## **Receptor structure and expression**

The receptor for hU-II turned out to be the orphan G-protein-coupled receptor 14 (GPR14).<sup>2,10</sup> This receptor, now termed UT, is a 389-amino acid protein with seven transmembrane domains. It is homologous to rat GPR14 and is similar to the somatostatin

receptor  $sst_4$  in structure.<sup>2,10</sup> The gene coding for the human UT receptor is intronless and is located at 17q25.3.<sup>11</sup> The UT receptor is found in human brain, spinal cord, leukocytes, ventricular myocardium, vascular endothelial and smooth muscle cells, kidney cortex, adrenal gland, pituitary and thyroid, with the highest density in skeletal muscle and cerebral cortex.<sup>2,6,7,9,12</sup> The distribution of U-II and its receptor suggests that U-II may act as a local or circulating vasoactive hormone in cardiovascular regulation. Differential distribution of UT receptors may partly explain the variability in contractile responses to U-II. U-II was previously thought to be arterioselective because UT receptors have not been found in human veins except umbilical veins.<sup>2,10</sup> More recent studies showed that hU-II contracts epigastric, facial, saphenous and umbilical veins, suggesting the presence of the UT receptor.<sup>12,13</sup>

### **Signal transduction**

The UT receptor is coupled to the  $G\alpha_{q/11}$  signal transduction pathway, the activation of which leads to an increase in inositol triphosphate and mobilization of intracellular  $Ca^{2+}$  (Figure 2).<sup>2,14,15</sup> The mechanism by which U-II elicits smooth muscle contraction is complex.<sup>16,17</sup> It involves small GTPase RhoA and its downstream effector Rho-kinase<sup>18</sup>, phospholipase C, protein kinase C and tyrosine kinase<sup>19</sup>, PKC-independent phosphorylation of myosin light chain (MLC-2)<sup>20</sup> as well as the  $Ca^{2+}$ -calmodulin/MLC kinase system, extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase.<sup>21</sup> Rho signalling pathway and ERK may also be involved in U-II-induced vascular smooth muscle cell proliferation.<sup>18,22</sup>

### **Post-translational processing of U-II**

Human prepro-U-II, first cloned by Coulouarn et al.<sup>3</sup>, has a signal-peptide sequence at the N-terminal end (Figure 1). There are two alternative splicing variants of human prepro-U-II, isoforms a and b, with 139 and 124 amino acid residues respectively. They differ in the N-

terminal sequence.<sup>2,3,4</sup> Mature U-II is produced from the proteolysis of prepro-U-II at the putative tribasic site, K<sup>126</sup>K<sup>127</sup>R<sup>128</sup>, in the splice variant a and K<sup>111</sup>K<sup>112</sup>R<sup>113</sup> in the splice variant b (Figure 1).<sup>2,3</sup> The enzymatic cleavage confers biological activity.<sup>23</sup> A specific urotensin converting enzyme (UCE) has not been identified, but there are several enzymes that can perform the proteolytic cleavage. By studying the conversion of a 25-amino acid C-terminal fragment of prepro-U-II to mature U-II, Russell et al.<sup>24</sup> demonstrated that furin, an endoprotease which is expressed in most cell types and localized in the trans-Golgi network<sup>25</sup>, may function as an intracellular UCE. The same authors also showed that trypsin, a serine protease, may act on prepro-U-II in the circulation.<sup>24</sup>

### **U-II-like immunoreactivity**

As both prepro-U-II and mature U-II contain the Cys-Phe-Trp-Lys-Tyr-Cys cyclic motif, polyclonal antibodies may recognise other peptides containing this cyclic motif such as urotensin II-related peptide (URP) (Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val) (Table 1). URP is thought to be as the only peptide with U-II-like immunoreactivity in the rat brain and may be the endogenous ligand for the UT receptor in rat brain.<sup>8,26</sup> The seven C-terminal residues of URP are identical to those in hU-II. This may explain the large variations in the estimation of U-II-like immunoreactivity in different studies.<sup>8,23,27</sup> Human URP is derived from a 119-amino acid residue precursor protein encoded by a gene at 3q29, so the gene and the precursor are different from those of hU-II.<sup>8,26</sup> Although the physiological and pathological importance of URP is unknown at present, URP exhibits a slightly higher affinity for the human UT receptor and a slightly lower potency in the contraction of de-endothelialized aortic rings.<sup>8,26,28</sup>

Reverse-phase HPLC and radioimmunoassay of brainstem and spinal cord extracts contains additional U-II-immunoreactive peaks, which may be due to cleavage of prepro-U-II at two other putative sites (Arg<sup>84</sup>Lys<sup>85</sup> and Arg<sup>100</sup>Lys<sup>101</sup> in splice variant a and Arg<sup>69</sup>Lys<sup>70</sup> and Arg<sup>85</sup>Lys<sup>86</sup> in splice variant b) (Figure 1).<sup>29</sup> It is not known whether cleavage at these sites has any functional importance or is simply a process of protein degradation. Similar results were also observed in cultured human SW-13 adrenocortical carcinoma cells.<sup>30</sup> The anti-hU-II antibody cross-reacts with prepro-hU-II fragment.<sup>23</sup> Even using more specific monoclonal antibodies, Aiyar et al.<sup>27</sup> still found cross-reactivity and advised the cautious interpretation of U-II-like immunoreactivity.

### **Analogues of U-II and their properties**

Analogues of U-II have been used to study the relationship between structure and function. The cyclic octapeptide, hU-II(4-11) generated by elimination of the Glu-Thr-Pro tripeptide in the N-terminal has a higher affinity to the UT receptor and higher vasoconstriction activity on the rat thoracic aorta than its full-length.<sup>19</sup> Thus, residues 4-11 confer biological activity and are conserved across species while the N-terminus confers species specificity.<sup>19</sup> The residues, Trp<sup>7</sup>-Lys<sup>8</sup>-Tyr<sup>9</sup> appear to be essential for biological activity.<sup>31,32,33</sup> Kinney et al.<sup>32</sup> suggested the presence of a tyrosine-binding pocket in the UT receptor and the substitution of Tyr<sup>9</sup> with (2-naphthyl)-L-alanine in U-II can improve the agonist activity slightly, perhaps due to enhanced hydrophobic interaction. The Phe<sup>6</sup> of U-II may also interact with Met184 and Met185 of the fourth transmembrane domain of the UT receptor.<sup>34</sup> The disulphide bridge of U-II is not essential for biological activity, as it can be replaced by a lactam ring.<sup>35</sup> The replacement of Cys<sup>5</sup> by penicillamine in hU-II(4-11) generates a potent agonist that has a 3-fold higher affinity for the receptor and 20-fold more potent in contracting the rat aorta than full-length hU-II<sup>36</sup>. Camarda et al.<sup>37</sup> generated a partial UT receptor agonist by replacing Lys<sup>8</sup>

with Orn. This [Orn8]U-II acts as a full agonist in calcium mobilization assay with a maximal effect similar to U-II, but acts as a competitive antagonist in the rat aorta assay, with a small and consistent residual agonist activity at high concentration.<sup>37</sup> Urantide ([Pen5, DTrp7, Orn8]hU-II(4-11)) is the most potent antagonist in the rat aorta assay but an agonist in the calcium mobilization assay in cultured CHO cells transfected with the human UT receptor.<sup>38,39</sup>

Based on the sequence similarity between U-II and somatostatin, Rossowski et al.<sup>19</sup> reported that somatostatin analogues PRL-2882, PRL-2903 and PRL-2915 can act as rat UT receptor antagonists. The somatostatin antagonist, SB-710411, is also a rat UT receptor antagonist.<sup>40</sup> However, it potentiates the contractile response to endothelin-1, limiting its usefulness in pharmacological experiments.<sup>41</sup> Interestingly, it is a full agonist at both monkey and human UT receptors, indicating that the functional response to UT receptor modulators may vary with species.<sup>42,43</sup> The neuromedin B receptor antagonist, BIM-23127, with sequence similarity to SB-710411, has also been identified as a potent competitive antagonist of both human and rat UT receptors.<sup>44</sup>

The development of UT receptor antagonists can advance the understanding of the pathophysiological role of U-II and the design of new drugs. Using a functional mammalian cell-based assay to screen a library of 180,000 small organic molecules, a highly selective non-peptide human UT receptor agonist with an EC<sub>50</sub> of 300nM, AC-7954, was discovered.<sup>45</sup> Using a pharmacophore model based on the structure-function relationship data and the NMR solution structure, Flohr et al.<sup>31</sup> identified by virtual screening 10 out of 500 compounds that can inhibit U-II induced calcium mobilization. Clozel et al.<sup>46</sup> reported a new potent and specific non-peptide UT receptor antagonist, palosuran (ACT-058362) which can inhibit U-



II-induced calcium mobilization, mitogen-activated protein kinase phosphorylation and constriction of rat aortic rings without any antagonistic effect on the actions of other vasoconstrictive agents. Intravenous administration of palosuran in a rat model of renal ischaemia improved renal glomerular and tubular dysfunction.<sup>46</sup> Clinical studies of palosuran in renal diseases are currently in progress.

### **Role in the cardiovascular system**

In human, hU-II can cause the vasoconstriction of coronary, mammary and radial arteries as well as saphenous and umbilical veins.<sup>12</sup> It is about 50 times more potent than ET-1 in causing contraction of these arteries and just under 10 times more potent than ET-1 in contracting veins. However, the maximum response is significantly lower than that achieved by ET-1, and approximately 30% of coronary and mammary arteries respond to ET-1 but not to U-II.<sup>12</sup> This may be due to the low density of high-affinity receptor in vascular smooth muscle cell of these vessels. The contractile effect of U-II, like ET-1, is of slow onset and long duration when compared with other vasoactive agents such as potassium chloride, noradrenaline and angiotensin II.<sup>13,47,48</sup> The UT receptor is involved because isolated thoracic aortic rings from UT receptor knockout mice do not respond to hU-II.<sup>49</sup>

U-II causes vasoconstriction in rat pulmonary artery but not the small pulmonary arteries of both rat and human.<sup>50</sup> The effect is enhanced by endothelium removal, raised vascular tone, nitric oxide (NO) synthase inhibition and in pulmonary hypertension.<sup>50</sup> However, Bennett et al.<sup>51</sup> did not find any vasoconstrictive activity of U-II in isolated perfused human lungs and isolated human pulmonary arteries in endothelial dysfunction.

The vascular actions of U-II vary with species, vascular beds and even regions of the same vascular beds.<sup>13,52,53,54,55</sup> U-II does not have any effect on human small subcutaneous resistance arteries and veins, human skeletal muscle small resistance arteries or mouse isolated thoracic and abdominal aortae.<sup>52,53,54</sup>

The species and regional variations of U-II responses may be due to differences in receptor density, enzymatic conversion of the peptide, and the activity of endothelium derived relaxing factors, in which receptor density seems to be the predominant factor. U-II contracts rat thoracic aorta much more than abdominal aorta which is related to the higher UT receptor density in rat thoracic aorta as demonstrated by radioligand binding assay and RT-PCR.<sup>53,56,57</sup>

The human coronary artery has a lower receptor density compared to rat aorta and this may account for the greater effect of U-II on the rat aorta compared to human coronary artery *in vitro*.<sup>12</sup> Douglas et al.<sup>53</sup> argued that the effect of endothelium derived relaxing factors could not explain the variations of U-II response in *in vitro* studies as regional and species differences still exist in endothelium-denuded vessels and instead suggested a spare receptor reserve hypothesis. In this hypothesis, most of the UT receptors are occupied by U-II in a “pseudo-irreversible manner” with a very slow receptor dissociation rate. Thus when there is a lack of spare receptor reserve, there may only be a small number of unoccupied UT receptors available for binding U-II. In such tissue, the response could be very variable or low. Moreover, different extent of UT receptor desensitization in different species or tissues may also contribute to the regional and species variations of U-II response.

Human U-II induces a biphasic response in perfused rat heart, a transient decrease in coronary flow followed by sustained vasodilatation that can be inhibited by a cyclooxygenase inhibitor and an NO synthase inhibitor.<sup>58</sup> Endothelium-dependent vasodilation was also

observed in methoxamine-precontracted small mesenteric arteries and phenylephrine-precontracted renal artery<sup>57,59</sup>, and ET-1-precontracted small pulmonary arteries and abdominal resistance arteries.<sup>55</sup> This might be due to the release of the NO or endothelium-derived hyperpolarizing factor from an intact endothelium.<sup>50,57</sup> NO plays an important role in the regulation of cardiac function and vascular tone.<sup>60</sup> It is possible that the vasoconstricting effect of U-II is unmasked in endothelial dysfunction in which NO production is impaired. U-II upregulates endothelial nitric oxide synthase (eNOS).<sup>61</sup> In rat renal artery, hU-II induces NO synthesis in the intact endothelium, resulting in vasodilatation.<sup>59</sup>

U-II also exhibits cardiostimulant effects in human heart *in vitro*.<sup>62</sup> In a concentration-dependent manner, hU-II increases the contractile force without changing the contraction duration in right atrial trabeculae from non-failing hearts, and causes a small increase in contractile force in right ventricular trabeculae from explanted hearts.<sup>62</sup> It also enhances plasma extravasation in specific vascular territories, and may therefore be involved in the development of oedema in heart failure.<sup>63</sup>

A biphasic haemodynamic response was observed after bolus injection of hU-II in conscious rats.<sup>64</sup> The initial response was a prostanoïd-mediated mesenteric and hindquarter vasodilatation, tachycardia and a small fall in blood pressure. After 30-60 min of injection, a second phase response was observed, including tachycardia and NO-dependent hindquarters vasodilatation with a modest rise in blood pressure.<sup>64,65</sup>

Intravenous administration of U-II at low dose ( $<30\text{pmolkg}^{-1}$ ) in anaesthetized monkeys increases cardiac output and reduces peripheral resistance while a higher dose decreases myocardial function, cardiac output, stroke volume, heart rate, carotid and coronary blood

flow with an increase in vascular resistance which culminates in severe pulmonary hypertension, myocardial depression and fatal circulatory collapse.<sup>2,66</sup> In anaesthetized rats, intravenous bolus hU-II injection decreases cardiac contractility, mean arterial blood pressure and left ventricular systolic pressure.<sup>67</sup> Recently, the cat has been found to be a useful model to study as isolated feline arteries are highly responsive to U-II.<sup>68</sup> Infusion of U-II in the cat doubles the systemic vascular resistance and blood pressure without marked changes in the heart rate or cardiac output.<sup>68</sup>

*In vivo*, U-II causes potent vasoconstriction in man with a dose-dependent reduction in forearm blood flow.<sup>69</sup> However, in another study, an increase in blood pressure or peripheral resistance was not observed following the infusion of U-II in healthy men.<sup>70</sup> Moreover, intravenous U-II infusion did not affect systemic haemodynamics or arterial stiffness, even with a 100-fold increase in plasma U-II levels.<sup>71</sup> As U-II can cause both endothelium-independent vasoconstriction and endothelium-dependent vasodilatation, the net effect can be variable, depending on the balance between vasoconstriction and vasodilatation. In the studies of Wilkinson et al.<sup>70</sup> and Affolter et al.<sup>71</sup>, the vasodilatation effect of U-II may mask its vasoconstriction effect. The vasoconstriction effect in the study of Bohm et al.<sup>69</sup> may probably be due to possible loss of vasodilator capacity and loss of NO. This loss of vasodilator capacity has been observed in the skin vessels heart failure patients, which may be due to endothelial dysfunction.<sup>72</sup>

### **Role in the kidney**

In fish, U-II affects sodium transport, lipid and glucose metabolism.<sup>1</sup> The urinary hU-II concentration is about 3 orders of magnitude greater than the plasma concentration.<sup>5</sup> U-II may play a role in the regulation of GFR via the tubuloglomerular feedback and the reflex

control of glomerular filtration rate (GFR).<sup>73</sup> In the kidney, U-II has vasodilator and natriuretic effects. Increases in renal blood flow and GFR were observed after the infusion of synthetic hU-II into the renal artery of anesthetized rats and this can be completely inhibited by a nitric oxide synthase inhibitor.<sup>59</sup>

### **Role in the nervous system**

The presence of U-II-like immunoreactivity and the UT receptor in the motor neurons in the spinal cord and the brain stem suggests a potential role of U-II in the central nervous system.<sup>3,6,10</sup> Prepro-U-II expression in the ventral horn of the spinal cord and facial nucleus motor neurons is reduced in neurons expressing androgen.<sup>74</sup> How androgen interferes with U-II expression is unclear but androgen can cause the promotion of motor neuron growth and regeneration as well as the prevention of normally occurring cell death in the sexually dimorphic spinal nucleus.<sup>75,76,77</sup>

hU-II is interestingly expressed in the motor neurons of the human spinal cord motoneurons, like calcitonin gene-related peptide.<sup>3,78</sup> U-II stimulates spontaneous neurotransmitter release from the motor nerve terminal in frogs.<sup>79</sup> U-II induces *c-fos* in the cingulate cortex and periaqueductal grey.<sup>80</sup> These areas integrate cognitive and emotional responses, and control motor, endocrine and autonomic functions. Intracerebroventricular (ICV) administration of hU-II in rats increased rearing and grooming, and motor activity as well as the plasma levels of thyroid stimulating hormone and prolactin without changing the levels of dopamine or serotonin (5-HT) levels, showing that U-II has behavioural and endocrine effects in the central nervous system.<sup>81</sup>

Intracerebroventricular administration of U-II elicits a pressor and tachycardic response via activation of the sympathetic nervous system.<sup>82</sup> hU-II has different effects in different parts of the brain. Microinjection of U-II into the A<sub>1</sub>, but not A<sub>2</sub> area of the rat medulla causes a dose-dependent hypotensive and bradycardiac response while microinjection into either the paraventricular or arcuate nucleus increases the arterial blood pressure and heart rate slightly and transiently.<sup>83</sup> In conscious unstressed sheep, intracerebroventricular administration of U-II leads to secretion of adrenocorticotrophic hormone (ACTH) and epinephrine by stimulating the sympathoadrenal medullary and the hypothalamic-pituitary-adrenal axes.<sup>84</sup> This is then accompanied by increased cardiac output, raised arterial pressure, peripheral vasodilatation and hyperglycemia. In contrast, intravenous administration of U-II produces only a positive chronotropic effect.<sup>84</sup>

Whereas U-II causes Ca<sup>2+</sup> influx from intracellular stores in vascular smooth muscle through L-type Ca<sup>2+</sup> channels via protein kinase C, it causes Ca<sup>2+</sup> influx in the spinal cord neuron through N-type Ca<sup>2+</sup> channels via protein kinase A.<sup>18,19,85</sup>

### **Plasma U-II levels in human diseases**

U-II circulates in human plasma and its plasma level is elevated in renal failure<sup>6</sup>, congestive heart failure<sup>23,86,87,88,89</sup>, diabetes mellitus<sup>7</sup>, systemic hypertension<sup>90</sup> and portal hypertension caused by liver cirrhosis<sup>91</sup> (Table 2).

### **Renal dysfunction**

The plasma U-II concentration is 2-fold higher in patients with renal dysfunction not on haemodialysis and 3-fold higher in patients on haemodialysis compared to healthy individuals.<sup>6</sup> Although there is no correlation between blood pressure and urinary U-II level,

a higher urinary U-II level was observed in patients with essential hypertension, patients with both glomerular disease and hypertension, and patients with renal tubular disorders, but not in normotensive patients with glomerular disease.<sup>5</sup> Abundant U-II-like immunoactivity is observed in the distal convoluted tubules and the epithelial cells of tubules and ducts in the normal kidney as well as renal clear-cell carcinoma.<sup>73</sup>

In Type 2 diabetic patients, plasma and urinary U-II levels are higher in those with renal dysfunction than those with normal renal function.<sup>92</sup> This may be due to increased production of U-II by various organs as well as by renal tubular cells as a result of renal damage.<sup>5,6</sup> In diabetic nephropathy, there are dramatic increases in the expressions of U-II and UT receptor in the tubular epithelial cells.<sup>93</sup>

### **Diabetes**

The elevation in plasma U-II level in diabetic patients is independent of the level of blood glucose.<sup>7</sup> Insulin secretion in the rat pancreas in response to glucose and arginine can be inhibited by U-II.<sup>94,95</sup> A single nucleotide polymorphism (SNP) at rs228648 (T21M) in the *UTS2* gene is correlated with genetic susceptibility to type 2 diabetes in Han people.<sup>96</sup> It is noteworthy that the SNP at rs2890565 (S89N) has been associated with increased insulin resistance and susceptibility of developing type 2 diabetes in Japanese.<sup>97,98</sup>

### **Systemic hypertension**

As U-II is a potent vasoconstrictor, its role in hypertension is worthy of investigation. In the anaesthetised cat, intravenous administration of hU-II induces a classical systemic hypertensive response with increases in mean blood pressure and systemic vascular resistance.<sup>68</sup> In a small pilot study, 10 normotensive and 10 hypertensive patients have similar

cerebrospinal fluid (CSF) and plasma concentrations of U-II, although the average mean arterial blood pressure and CSF U-II concentration show a positive correlation in the hypertensive patients.<sup>99</sup> However, in a study of 62 hypertensive patients and 62 normotensive sex-age-matched controls, plasma urotensin II level is raised in hypertensive patients compared to normotensive controls and is directly related to the systolic blood pressure.<sup>90</sup>

### **Pulmonary hypertension**

Since endothelial dysfunction has a central role in the initiation and progression of pulmonary hypertension, the vasoconstricting effect of U-II on pulmonary artery may be unmasked in pulmonary hypertension.<sup>50,100</sup> In rats with pulmonary hypertension, U-II-like immunoreactivity levels in pulmonary artery endothelial and smooth muscles cells are raised.<sup>101</sup> In chronic hypoxic rats that have pulmonary hypertension and right ventricular hypertrophy, there is up-regulation of UT receptor in the right ventricle.<sup>102</sup> At present, not much is known about the role of U-II in human pulmonary hypertension. Bosentan, an ET-1 antagonist, has been used for the treatment of human pulmonary hypertension with considerable success. It would be of interest to study if modulation of U-II is of benefit in patients with pulmonary hypertension.

### **Atherosclerosis**

There is increased expression of U-II in atherosclerotic carotid arteries and aortae.<sup>9</sup> The observation of U-II-like immunoreactivity in the lipid-laden smooth muscle and macrophage-rich regions of in human coronary atherosclerotic plaque suggests a role of U-II in the development of atherosclerosis.<sup>2</sup> U-II acts synergistically with mildly oxidized low density lipoprotein in inducing vascular smooth muscle cell (VSMC) proliferation.<sup>103</sup> Serotonin (5-HT), contained in platelets, also interact synergistically with U-II to induce VSMC



proliferation that may contribute to the **rapid** development of atherosclerosis in hypertensive vascular disease.<sup>104</sup> Thus, U-II expression in atherosclerotic plaques may stimulate VSMC proliferation. Moreover, locally released U-II may cause coronary vasoconstriction and induce myocardial ischaemia.<sup>105</sup>

### **Ischaemic heart disease**

U-II may play a role in myocardial ischaemia and acute myocardial infarction. In the myocardium of chronic hypoxic rats, there is increased UT receptor expression.<sup>102</sup> There are increased expressions of U-II and its receptor in both infarct and noninfarct zones of rat left ventricle after myocardial infarction.<sup>15</sup> U-II also induces the expression of procollagens  $\alpha 1(I)$  and  $\alpha 2(III)$  and fibronectin in neonatal cardiac fibroblasts.<sup>15</sup>

Hypertrophy can be induced by hU-II *in vitro* in cultured neonatal rat cardiomyocytes.<sup>15,106,107</sup> hU-II stimulates the expression of atrial natriuretic peptide and brain natriuretic peptide, protein synthesis and morphological changes in cardiomyocytes.<sup>106,107</sup> The hypertrophy of cultured cardiomyocytes is enhanced significantly when UT receptor is over-expressed and can be inhibited by the UT receptor antagonist, BIM-23127.<sup>15,108,109</sup> The hypertrophy of cardiac myocytes is mediated by the mitogen-activated protein kinases, ERK1/2 and p38 in an epidermal growth factor receptor-dependent signalling pathway.<sup>108</sup> It may also be mediated by IL-6, the release of which can be stimulated by U-II.<sup>109</sup>

### **Heart failure**

U-II is one of several neurohormonal systems activated in congestive heart failure.<sup>23,86,87,88,89</sup> In the diseased hearts of patients with end-stage heart failure, expressions of U-II and its receptor are upregulated in cardiomyocytes, endothelial cells and vascular smooth muscle

cells.<sup>89</sup> U-II is also expressed in macrophages and myofibroblasts in patients with ischaemic heart disease and its expression in subendocardial myocytes suggests a role in myocardial contractility.<sup>89</sup> An inverse correlation is observed between U-II expression or plasma U-II and ejection fraction.<sup>88,89</sup> In heart failure, U-II may also be elevated in diastolic myocardial dysfunction.<sup>110</sup> U-II may increase cardiac contractility<sup>62</sup> and the peripheral vascular tone.<sup>72</sup> Although increased contractility might be beneficial in the short term, prolonged activation might lead to myocardial remodelling. Indeed, U-II induces cardiac fibroblast proliferation, increases collagen type I gene expression and decreases matrix metalloproteinase-1 gene expression.<sup>15,111,112</sup> It is of interest to note that U-II causes vasodilatation in the skin vessels of normal healthy subjects but vasoconstriction in heart failure patients, which may be due to the unmasking of the vasoconstriction effect of U-II in endothelial dysfunction, common in heart failure diseases.<sup>72</sup>

### **Mitogenesis**

U-II may also act as a growth stimulating factor in tumors in an autocrine/paracrine manner.<sup>113,114</sup> U-II is mitogenic and induces arterial smooth muscle cell proliferation via the RhoA/Rho-kinase pathway.<sup>18</sup> There are expressions of U-II and UT receptor in various human tumour cell lines, such as T98G glioblastoma cells, IMR-32 neuroblastoma cells, BeWo choriocarcinoma cells, SW-13 adrenocortical carcinoma cells, DLD-1 colorectal adenocarcinoma cells and HeLa cervical cancer cells.<sup>30</sup> Cultured SW-13 adrenocortical carcinoma cells secrete adrenomedullin, ET-1 and U-II, all of which can promote tumour cell growth.<sup>30</sup> Indeed, U-II stimulates the proliferation of cultured SW-13 cells and VMRC-RCW human renal carcinoma cells.<sup>113</sup> U-II stimulates DNA synthesis in a dose-dependent manner and induces c-myc expression in quiescent renal epithelial (LLCPK1) cells.<sup>114</sup>

### **Therapeutic potential**

A UT receptor antagonist may have clinical use in the treatment of systemic, pulmonary and portal hypertension, and cardiac and renal failure. Palosuran is a non-peptide UT receptor antagonist. Intravenous administration of palosuran protects against renal ischaemia in a rat model<sup>46</sup>, perhaps by inhibiting U-II mediated renal vasoconstriction. Clinical studies of palosuran are now in progress to examine its effect on diabetic nephropathy.

### **Conclusions**

U-II is the most potent vasoconstrictor known, causing endothelium-independent vasoconstriction and endothelium-dependent vasodilatation. There is increasing evidence that U-II is associated with cardiovascular diseases, atherosclerosis, diabetes, renal dysfunction and hypertension although the results of some studies are ambiguous. More research is needed to elucidate the physiology and pathophysiology of U-II and its receptor. The role of URP in the cardiovascular and nervous system and its relationship with U-II is worthy of investigation. The development of UT receptor antagonists may provide a useful research tool as well as a novel treatment for cardiorenal diseases.

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Table 1. Amino acid sequences of somatostatin, U-II in different species and URP.

The conserved amino acid residues are underlined. The two cysteine residues in U-II and URP are linked by a disulphide bond to form a ring structure. **U-II: urotensin II; URP: urotensin-related peptide**

Table 2. Plasma U-II levels in different diseases in man.

Plasma U-II levels are expressed as mean  $\pm$  SD or median (ranges). **Plasma U-II levels** originally expressed in pg/ml are converted to pmol/l.

Figure 1. Post-translational processing of the human U-II gene product.

Alternative splicing of exon 2 produces a 7-amino acid fragment as isoform a and a 34-amino acid fragment as isoform b. **SP: signal peptide.**

**Figure 2. The signal transduction pathways involved in vasoconstriction, vasodilatation, cell proliferation and hypertrophy caused by urotensin II (U-II).**

In vascular smooth muscle cell (VMSC), U-II binds to a G-protein-coupled UT receptor, leading to hydrolysis of phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>2</sub>) to inositol 3,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG) by phospholipase C (PLC). IP<sub>3</sub> increases the release of Ca<sup>2+</sup> from the sarcoplasmic reticulum **or endoplasmic reticulum**. U-II also mediates Ca<sup>2+</sup> influx through activation of a voltage-gated Ca<sup>2+</sup> channel and a La<sup>3+</sup>-sensitive non-selective cation channel. DAG stimulates protein kinase C (PKC) which phosphorylates CPI-17 (protein kinase C-potentiated inhibitor protein of 17kDa), leading to inhibition of myosin light chain phosphatase (MLCP) which catalyses the dephosphorylation of phosphorylated **regulatory myosin light chain (MLC-2)**. Rho kinase also inhibits MLCP by phosphorylation. Stimulation of **myosin light chain (MLC)**

kinase by  $\text{Ca}^{2+}$ -calmodulin **complex** and inhibition of MLCP leads to increase in phosphorylated **MLC-2**. The increases in phosphorylated **MLC-2**, intracellular  $\text{Ca}^{2+}$  and phosphorylation of the actin-binding protein, caldesmon, by extracellular signal-regulated kinase (ERK) or p38 mitogen-activated protein kinase (p38MAPK) lead to contraction of VMSC. In endothelial cells, U-II stimulates the production of prostacyclin and nitric oxide (NO) which then diffuses into VMSC, leading to increase in cGMP and relaxation of VMSC. U-II also mediates cell proliferation and hypertrophy through activation of PKC and ERK 1/2 as well as RhoA and its downstream kinase system **possibly via guanine nucleotide exchange factor (GEF)**.

<i>Peptides</i>	<i>Amino acid sequence</i>
Human somatostatin-14	AGCKNFWKTFTSC
Human/monkey U-II	ETPDCFWKYCV
Mouse U-II	QHGAAP <del>E</del> CFWKYCI
Rat U-II	QHGTAP <del>E</del> CFWKYCI
Goby U-II	AGTAD <del>C</del> FWKYCV
Dogfish	NNFSDCFWKYCV
Frog U-II	AGNLSE <del>C</del> FWKYCV
Porcine U-II(A)	GPTSE <del>C</del> FWKYCV
Porcine U-II(A)	GPPSE <del>C</del> FWKYCV
Human/rat/mouse URP	ACFWKYCV

Disease	Number of subjects (control:patient)	Control (pmol/l)	Patient (pmol/l)	p-value	Reference
Heart failure	88:74	1.9 ± 0.9	3.9 ± 1.4	<0.0001	87
Heart failure	220:126	6.6 (3.1-42.6)	22.1 (3.1-49.2)	0.001	86
Congestive heart failure	18:21	16.3 ± 4.4	166.2 ± 49.5	<0.001	23
Renal dysfunction	24:12	4.4 ± 1.0	13.1 ± 3.1	<0.0001	6
Diabetes mellitus:					
With proteinuria	22:6	4.4 ± 2.0	7.3 ± 0.9	0.0018	7
Without proteinuria	22:10	4.4 ± 2.0	7.8 ± 0.6	<0.0001	
Cirrhosis and portal hypertension	15:50	2592 (72-8640)	8856 (1,152-29,808)	<0.001	91
Essential hypertension	62:62	8.8 ± 0.9	13.6 ± 1.4	0.005	90



