British Journal of Pharmacology (2006) 148, 884–901 © 2006 Nature Publishing Group All rights reserved 0007–1188/06 $30.00
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REVIEW

The role of urotensin II in cardiovascular and renal physiology and diseases

*,[1]Yi-Chun Zhu, [2,3]Yi-Zhun Zhu & [2]Philip Keith Moore

[1]Department of Physiology and Pathophysiology, Fudan University Shanghai Medical College, Shanghai, China; [2]Cardiovascular Biology Research Group, Department of Pharmacology, National University of Singapore, Singapore, Singapore and [3]Department of Pharmacology, School of Pharmacy, Fudan University, Shanghai, China

1 Urotensin II (U-II) is a cyclic neuropeptide that was first isolated from teleost fish some 35 years ago. Mammalian U-II is a powerful vasoconstrictor with a potency greater than that of endothelin-1.

2 Nevertheless, unlike endothelin-1, which constricts all or nearly all vascular beds, the vasoactive effects of U-II are reported to be dependent both on the species and on the regional vascular bed examined. Typical regional variability occurs in the rat in which vasoconstriction to U-II is most robust in thoracic aorta proximal to the aortic arch and decreases gradually towards the distal peripheral arteries. As small peripheral arteries but not larger arteries such as the aorta play a major role in regulating peripheral resistance and consequent blood pressure as well as workload on the heart, doubts have been raised concerning the importance of this peptide in cardiovascular physiology. Moreover, an interaction between U-II and other endogenous vasoactive molecules may add a level of complexity to the vascular actions of U-II.

3 On the other hand, recent experimental and clinical studies have revealed increased expression of U-II and urotensin receptor (UT receptor) in animals with experimentally induced myocardial infarction, heart failure, and in patients with hypertension, atherosclerosis, and diabetic nephropathy, which suggests a potential role for U-II in both cardiovascular and renal diseases. A series of peptidic and nonpeptidic UT receptor ligands have been shown to be effective in antagonizing the effects of U-II in the cardiorenal system.

4 This article aims to review recent advances in our understanding of the physiology and pathophysiology of U-II with particular references to its role in cardiovascular health and disease.
British Journal of Pharmacology (2006) **148,** 884–901. doi:10.1038/sj.bjp.0706800;
published online 19 June 2006

Keywords: Urotensin II; urotensin receptor; vasoactive effects; signalling mechanisms; interaction

Abbreviations: ERK, extracellular signal-regulated kinase; $ERK_{1/2}$, extracellular signal-regulated protein kinase; MAPK, mitogen-activated protein kinase; moxLDL, mildly oxidized LDL; NADPH, reduced nicotinamide adenosine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; PAI-1, plasma plasminogen activator inhibitor-1; PKC, protein kinase C; U-II, urotensin II; UT receptor, urotensin receptor; VSMCs, vascular smooth muscle cells

Introduction

Urotensin II (U-II) was first recognized some 30 years ago as an important teleost fish hormone. In more recent years, U-II has been identified as a vasoactive peptide in mammals, which acts by binding to the orphan G-protein-coupled receptor 14 (GPR14). Several recent reports have revealed the powerful vasocontractile effect of U-II (amounting to some eight- to 110-fold the potency of endothelin-1), which further testifies to the potential importance of this peptide in cardiovascular physiology and diseases (Douglas *et al*., 2000). However, the reported vasodepressor, and regionally (in terms of anatomical location) selective vasodilator effect (Bottrill *et al*., 2000; Gardiner *et al*., 2001; Stirrat *et al*., 2001) of U-II suggests that the effect of this peptide on the vasculature is likely to be more complex than was first envisaged. Unlike endothelin-1, which uniformly constricts most blood vessels, the vasoactive effects of U-II are both species- and vascular bed-dependent, which emphasizes the importance of understanding the intracellular signalling mechanisms underlying its actions and also its interactions with other vasoactive factors. Moreover, the peptidic U-II receptor (UT receptor) ligand, urantide, blocked the contractile effect of U-II on the isolated rat aorta, but was inactive against the hypotensive action of U-II *in vivo*, which is perhaps indicative of the existence of more than one receptor type for U-II (Gendron *et al*., 2005). However, to date, questions of this type remain largely an enigma.

On the other hand, based on its blood pressure-independent trophic and mitogenic actions, U-II has been suggested to function in the pathological processes such as myocardial hypertrophy and fibrosis (Tzanidis *et al*., 2003), vascular smooth muscle cell (VSMC) proliferation (Tamura *et al*., 2003; Djordjevic *et al*., 2005), atherosclerosis (Maguire *et al*., 2004), and diabetic nephropathy (Langham *et al*., 2004). The implied

*Author for correspondence at: Department of Physiology and Pathophysiology, Key Laboratory of Molecular Medicine of The Ministry of Education, Fudan University Shanghai Medical College, 138 Yi Xue Yuan Road, Shanghai 200032, People's Republic of China; E-mail: yczhu@shmu.edu.cn

role of U-II in such disease states is postulated largely on the basis of changes in the expression levels of this peptide (and/or its receptor) in the disease processes. Additionally, the synthetic U-II receptor agonists and antagonist, for example, the peptidic UT receptor ligand urantide (Gendron *et al*., 2005) and the nonpeptidic U-II receptor antagonist SB-611812 (Rakowski *et al*., 2005) may be used as pharmacological tools to elucidate the role of U-II in cardiovascular physiology and disease. All in all, these observations provide fertile ground to explore further the participation of U-II in the development of cardiovascular and renal diseases.

Cellular and organ distribution of U-II and its receptors

U-II has recently been cloned in several mammalian species including man. Isoforms of U-II isolated from different species such as human, monkey, rat, mouse, pig, goby, and frog share a conserved cyclic hexapeptide, that is, Cys, Phe, Trp, Lys, Tyr and Cys at the C-terminal of the peptides. Despite variability in the N-terminal region, the pharmacological properties of U-II isoforms are indistinguishable when assessed using either binding or functional studies in various species (Douglas *et al*., 2004).

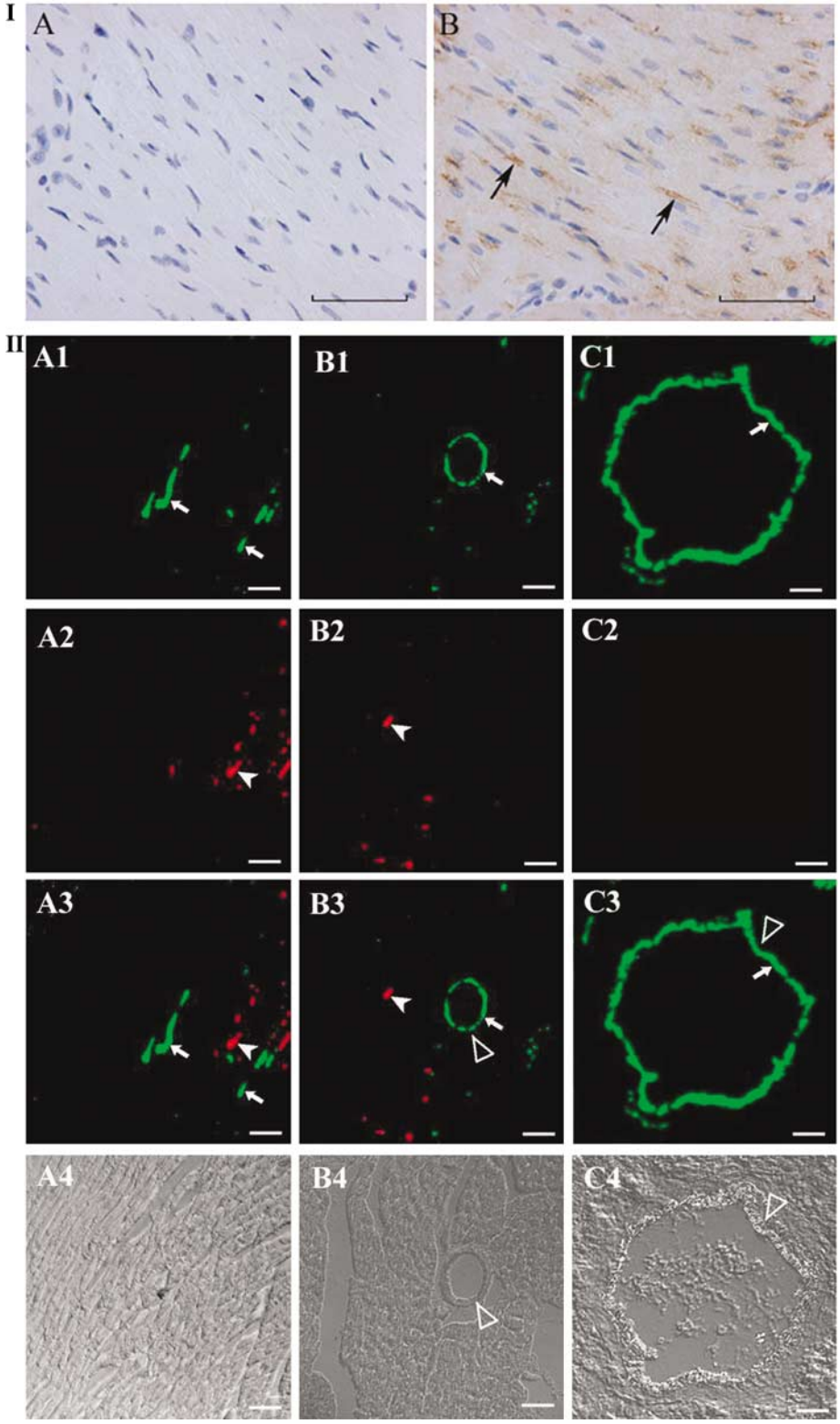
The mRNA transcripts for U-II were detected in the kidney and right atrium, but to a lesser extent in the vasculature in man (Matsushita *et al*., 2001). When examined using immunohistochemical staining, U-II was detected in the endothelium of normal human blood vessels from various organs (heart, kidney, placenta, adrenal, thyroid, and umbilical cord) as well as human kidney epithelial cells (Shenouda *et al*., 2002; Maguire *et al*., 2004), rat pancreas (Silvestre *et al*., 2004), and human urine (Matsushita *et al*., 2001). In normal human kidneys, U-II immunoreactivity was mostly found in the epithelial cells of tubules and ducts with greatest intensity in the distal convoluted tubules. Moderate U-II staining was apparent in endothelial cells of renal capillaries. However, in the glomeruli, only focal signals were identified in the endothelial cells. No staining was seen in the veins (Shenouda *et al*., 2002). The U-II in urine is assumed to be of renal origin as the fractional excretion of U-II exceeded the glomerular filtration rate (Matsushita *et al*., 2001). Moreover, more intense U-II staining was identified in the tubular epithelial cells in renal biopsy samples from patients with diabetic nephropathy in comparison with healthy tissue (Langham *et al*., 2004).

To identify UT receptor in man, Ames *et al*. (1999) cloned a human G-protein-coupled receptor similar to the rat GPR14, which selectively bound U-II. U-II has been demonstrated by numerous authors to act as the endogenous ligand for GPR14 (Liu *et al*., 1999; Mori *et al*., 1999; Nothacker *et al*., 1999). UT receptor mRNA is widely expressed in human cardiovascular tissue, including cardiac myocytes, VSMCs, and endothelial cells (Ames *et al*., 1999), as well as in the spinal cord (Coulouarn *et al*., 1998). In the mouse, UT receptor mRNA was also expressed in motor neurons of the spinal cord, smooth muscle cells of the bladder, and cardiomyocyte (Liu *et al*., 1999). It is noteworthy that the distribution patterns of U-II and UT receptor mRNA in man are not similar. For example, the level of mRNA transcripts for U-II was much higher in the homogenate of the kidney and right atrium than in the vasculature, including the aorta, left internal thoracic artery, and great saphenous vein, whereas mRNA for UT receptor were equally expressed in both cardiovascular and renal tissues (Matsushita *et al*., 2001). In addition to experiments with mRNA transcripts, localization of the U-II receptor protein has also been determined using radioligand binding assay. Using a fixed concentration of ^{125}I-U-II, highest ^{125}I-U-II binding density was seen in the abducens nucleus within the brainstem ($139.6 \pm 14\,\mu mol\,mm^{-2}$) with moderate levels in the dorsal horn of spinal cord ($85 \pm 14\,\mu mol\,mm^{-2}$) and lower levels in the aorta ($22.5 \pm 6\,\mu mol\,mm^{-2}$) in Sprague–Dawley rat (Maguire *et al*., 2000). Moreover, different ^{125}I-U-II binding characteristics were observed between rat strains. There were high-affinity ^{125}I-U-II binding sites in the kidney outer medulla of Sprague–Dawley rats (K_d 1.9 ± 0.9 nM and B_{max} $408 \pm 47\,\mu mol\,mm^{-2}$) compared to the low-density ($<20\,fmol\,mg^{-1}$ protein) and low-affinity ($>1\,\mu M$) ^{125}I-U-II binding sites in that of Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats (Disa *et al*., 2006). In man, the highest receptor density was identified in the skeletal muscle ($31.9 \pm 9\,\mu mol\,mm^{-2}$) and cerebral cortex ($32.6 \pm 13\,\mu mol\,mm^{-2}$), with lower levels in the kidney cortex ($14.5 \pm 4\,\mu mol\,mm^{-2}$) and myocytes in the left ventricle ($9.4 \pm 4\,\mu mol\,mm^{-2}$). Minimal binding sites were detected in the atria, conducting system of the heart, and lung parenchyma. The density in human coronary artery smooth muscle was less than that of rat aorta ($22.5 \pm 6\,\mu mol\,mm^{-2}$). ^{125}I-U-II bound with a single high-affinity (K_d 0.24 ± 0.17 nM) and a B_{max} of $1.97 \pm 1.1\,fmol\,mg^{-1}$ to sections of human skeletal muscle (Maguire *et al*., 2000). As radioligand binding assays fall short of identifying the type of cells in which U-II receptors are located, we have recently investigated the cellular distribution of UT receptor protein in the rat heart by using an immunohistochemistry and confocal microscopic immunofluorescence double-staining procedure. This study revealed the cardiomyocyte as the only cell type expressing UT receptor protein in rat left ventricle with no visible signal for UT receptor apparent in intramyocardial coronary arteries, capillaries, or in VSMCs (Figure 1) (Gong *et al*., 2004). These findings suggest that U-II may regulate cardiac function and cell growth by stimulating its receptors on cardiomyocytes. Certainly, the presence of UT receptor in rat coronary arteries cannot be excluded based on the evidence provided solely by this study, as UT receptor density may be too low to be detected by immunohistochemical staining. Functional evidence for the existence of UT receptors has been provided by Maguire *et al*. (2004) using isolated human coronary arteries and also by Bottrill *et al*. (2000) using isolated rat coronary arteries (see the following section). On the other hand, U-II may exert vasoactive effects indirectly *in vivo* by regulating the contractility of cardiomyocytes and consequent release of metabolites such as adenosine, which is known as a major factor in regulating vascular tone of intramyocardial coronaries.

Effect of U-II on peripheral vessels

Early in 1975, Medakovic *et al*. (1975) reported a U-II-induced increase in blood space in the cortex of kidney obtained from anaesthetized rats, which was assessed by measuring ^{51}Cr-labelled erythrocytes. About one decade later, the cardiovascular effect of U-II was further examined by Gibson *et al*.

(1986) in anaesthetized rats. These authors demonstrated that *gillichthys* U-II reduced blood pressure, with diastolic pressure being lowered to a greater extent than systolic pressure, suggesting a vasodilator effect of this peptide (Gibson *et al*., 1986). Analogous *in vitro* experiments were performed by the same group on rat aortic strips. Following precontraction with noradrenaline, low concentrations (0.1–0.5 nM) of *gillichthys* U-II caused a transient relaxation, whereas higher concentrations (1–10 nM) caused sustained contraction. In endothelium-denuded aortic strips, relaxations to U-II were absent while the contractile effect was potentiated (Gibson, 1987). The U-II-induced relaxation is apparently mediated by vasodilator



factors released from the endothelium, whereas the sustained contractile response to U-II may be ascribed to the continuous externalization and recycle of UT receptors in VSMC (Giebing *et al*., 2005). The vasoactive activity of U-II may therefore be a result of the counterbalance of U-II-induced contraction and relaxation. In vessels where sufficient UT receptors are located in the VSMC, U-II-induced sustained contractile effect may overwhelm the endothelium-dependent transient relaxation.

The cardiovascular effects of U-II have also been described in teleost and amphibian. For example, U-II isolated from the urophysis of the white sucker induced an increase in arterial blood pressure in the eel. Intra-arterial injections of trout U-II (25 pmol) elicited a pronounced hypertensive response with a concomitant decrease in heart rate in the conscious rainbow trout. In vascular rings from the trout celiacomesenteric and efferent branchial arteries, synthetic trout U-II (10^{-9}–10^{-7} M) produced sustained and concentration-dependent contractions. In the anaesthetized bullfrog, bolus injections of frog U-II (100 nmol kg^{-1}) into the left systemic arch caused a rapid and sustained fall in blood flow through the right branch of the truncus arteriosus (to 62±5% of preinjection values) (Conlon *et al*., 1996).

These novel and interesting findings have not attracted much attention from cardiologists until recent years. Currently, the hypothesis that U-II contributes to cardiovascular (patho)physiology has been based upon its ability to cause a sustained and potent contraction of isolated mammalian blood vessels from various species (Gibson *et al*., 1986; Ames *et al*., 1999; Douglas *et al*., 2000; Maguire *et al*., 2000; 2004). Thus, U-II contracts large isolated arteries from the rat (Gibson *et al*., 1986), rabbit (Douglas *et al*., 2000), dog (Douglas *et al*., 2000), pig (Douglas *et al*., 2000), non-human primates (Ames *et al*., 1999), and man (MacLean *et al*., 2000).

Probably, the most profound vasoconstrictile effect of U-II is seen in rat thoracic aortae ($-\log[EC_{50}]$ 9.09±0.19, E_{max} 143±21% 60 mM KCl) (Douglas *et al*., 2000). In main pulmonary arteries of the rat, U-II was also a potent vasoconstrictor ($-\log[EC_{50}]$ 8.55±0.08, E_{max} 57.0±6.1% 50 mM KCl) (MacLean *et al*., 2000). Also in rat, the contractile effect of U-II was observed in isolated rat carotid arteries ($-\log[EC_{50}]$ 8.84±0.21, E_{max} 67±26% 60 mM KCl) (Douglas *et al*., 2000) and left anterior descending coronary arteries (EC_{50} 1.3±0.8 nM, E_{max} 20.1±4.9% of control contraction induced by 10 μM serotonin (5-HT)) (EC_{50} 3.5±1.1 nM, E_{max} 103±10% 60 mM KCl), whereas no effect was observed in isolated small (third-generation) mesenteric arteries (Bottrill *et al*., 2000). When these vessels were first precontracted with methoxamine, U-II caused endothelium-dependent relaxation (Bottrill *et al*., 2000). In addition, in isolated rat renal arteries of ≈0.2 mm i.d., U-II acted as a vasodilator (Zhang *et al*., 2003). The overall effect of U-II on blood pressure may, to some extent, represent the integration of the variable vasoactive effects of this peptide on different regional vascular beds. As U-II dilates rat small resistance arteries (Bottrill *et al*., 2000; Zhang *et al*., 2003), which accounts for the major portion of total peripheral resistance, it is perhaps not surprising that a bolus injection of U-II (300 and 3000 pmol kg^{-1}) decreased blood pressure with a concomitant tachycardia, and mesenteric and hindquarters hyperaemic vasodilatations in conscious Sprague–Dawley rats (Gardiner *et al*., 2001). In anaesthetized Lewis rats, intravenous (i.v.) bolus injection of U-II (1–30 nmol kg^{-1}) resulted in a dose-dependent decrease in mean arterial pressure and cardiac contractility represented by $\pm dP/dt$, with no significant change in heart rate or diastolic pressure (Hassan *et al*., 2003), suggesting differences in U-II-induced haemodynamic responses between rat strains. Indeed, this strain-related difference may be ascribed to different experimental procedures – that is, Sprague–Dawley rats (Gardiner *et al*., 2001) were in conscious states, whereas the Lewis rats were anaesthetized (Hassan *et al*., 2003). Another example of strain-related difference was provided by Gendron *et al*. (2005), who showed that i.v. injection of U-II (0.1–10 nM kg^{-1}) decreased mean arterial pressure in anaesthetized SHR and WKY rats. The hypotensive effect was more prominent in SHR than WKY rats. On the other hand, the mode of U-II administration may influence the haemodynamic effect of the peptide, for example, when administered by sustained infusion over a period of 6 h, U-II (30, 300, and 3000 pmol kg^{-1} h^{-1}) caused dose-dependent tachycardia and hindquarters vasodilatation with a concomitant, slowly developing rise in blood pressure in conscious Sprague–Dawley rats (Gardiner *et al*., 2006) in contrast to U-II (300 and 3000 pmol kg^{-1}) bolus injection-induced decrease in blood pressure accompanied by tachycardia and hindquarters vasodilatation in same strain of conscious rats (Gardiner *et al*., 2001). The authors assumed that the sustained U-II infusion-induced increase in blood pressure is due to an increase in cardiac output (Gardiner *et al*., 2006). This is supported by Gong *et al*. (2004), who demonstrated a direct positive inotropic effect of U-II (10–1000 nmol l^{-1}) in left ventricular papillary muscles isolated from Sprague–Dawley rats. The model of sustained infusion, which may be necessary to uncover the inotropic effect of U-II, may be more suitable to mimic the change in U-II levels in

Figure 1 Cellular localization of the U-II receptor GPR14 in the left ventricle of the rat (reproduced with permission from Gong *et al*. (2004)). (I) GPR14-positive signals visualized with the avidin–biotin–peroxidase complex (brown signals) in the left ventricle of the rats with light microscopy. The immunoreactive signals for GPR14 protein (arrow) are localized in the left ventricle (B). (A) Negative control of the left ventricle using the nonimmune rabbit IgG instead of the primary rabbit anti-GPR14 antibodies. A and B, bar = 50 μm. (II) Photomicrographs show cellular localization of the GPR14 protein with immunofluorescence double staining in the left ventricle of the rats imaged with a confocal microscope. Sections were doubled stained with the endothelial cell type marker, MRC OX43 (A1, B1, and C1, green signal, arrow) and anti-GPR14 antibodies (A2, B2, and C2, red signal, arrow head). Panels A3, B3, and C3 are the superimpositions of A1 and A2, B1 and B2, and C1 and C2, respectively, showing that the cellular localization of GPR14 protein does not colocalize with the endothelial cells. Panels A4, B4, and C4 are the phase-contrast images of panels A1–A3, B1–B3, and C1–C3, respectively. Panels A1–A4, a double-stained left ventricular section showing GPR14 protein and the capillary endothelial cells. Panels B1–B4, a double-stained left ventricular section showing GPR14 protein, the capillary endothelial cells, and a cross-sectional intramyocardial coronary artery. Panels C1–C4, a feature view of a cross-sectional intramyocardial coronary artery. As can be taken from B3–B4 and C3–C4, the GPR14 protein (red signal, arrow head) is located neither on the endothelial cells (green signal, arrow) nor on the VSMC layer (open arrow head) of the intramyocardial coronary artery. A1–A4, B1–B4, bar = 40 μm; C1–C4, bar = 20 μm.

disease states such as heart failure and hypertension. In line with this hypothesis, chronic administration of U-II (300 pmol $kg^{-1} h^{-1}$) for 2 weeks caused an increase in the ratio of left ventricular collagen I : III and reduced myocardial contractility in rats (Kompa *et al*., 2004). On the other hand, U-II isoforms of rat and man had similar haemodynamic effects in rats (Gardiner *et al*., 2001). Nevertheless, the role of endogenous U-II in the regulation of vascular tone and blood pressure is yet to be defined.

In non-human primates, U-II caused vasoconstriction in large vessels such as aorta (Ames *et al*., 1999), coronary arteries (Ames *et al*., 1999), and pulmonary arteries (Zhu *et al*., 2004). However, until recently, there has been no direct evidence for an effect of U-II on small resistance arteries. In cynomolgus monkeys, a bolus injection of U-II induced a dramatic decrease in blood pressure and subsequent circulation collapse (Ames *et al*., 1999; Zhu *et al*., 2004) (Figure 2). In non-human primates, i.v. bolus injection of U-II (0.03 nmol and 0.3 nmol kg^{-1}) might therefore cause vasoconstriction in large arteries and a dramatic vasodilatation in small resistance arteries with the counter balance of these effects resulting in a final decrease in total peripheral resistance (Figure 2) (Zhu *et al*., 2004).

In man, arteries isolated from the heart (Maguire *et al*., 2000, 2004), lung (MacLean *et al*., 2000; Stirrat *et al*., 2001; Bennett *et al*., 2004), skin (Hillier *et al*., 2001), umbilical cord (Maguire *et al*., 2000), and abdominal tissues (Stirrat *et al*., 2001) have been used to examine the vasoactive effects of U-II. Despite the limitation of human samples available for such studies, evidence has been accumulated for the vasoactive effects of U-II in human vessels. For example, in six out of nine isolated human coronary arteries, U-II acted as a potent vasoconstrictor (data of responders: $-\log[EC_{50}]$ 10.05 ± 0.46, E_{max} $15.39 \pm 6.53\%$ 100 mM KCl) (Maguire *et al*., 2000). In five out of seven isolated human mammary arteries, Maguire *et al*. (2000) reported a vasocontractile effect of U-II (data of responders: $-\log[EC_{50}]$ 9.71 ± 0.90, E_{max} $16.41 \pm 6.15\%$ 100 mM KCl), whereas Paysant *et al*. (2001) observed contractile effect of U-II in three out of eight human mammary arteries examined and Hillier *et al*. (2001) failed to identify any contractile effect of U-II in human mammary arteries. In human radical arteries, U-II was vasoconstrictive (Maguire

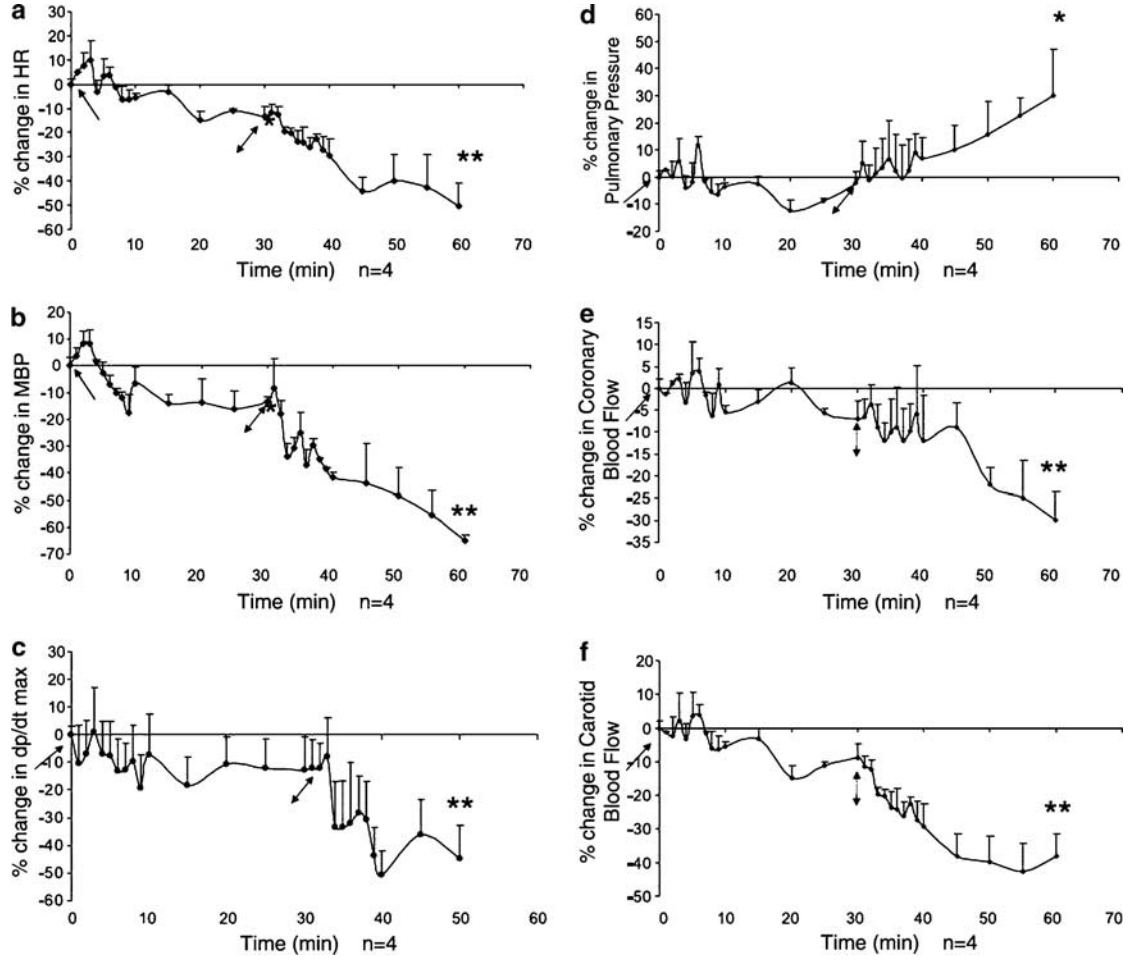


Figure 2 Changes in haemodynamics in anaesthetized cynomolgus monkeys after systemic administration of U-II (reproduced with permission from Zhu *et al*. 2004)). Urotensin II (U-II) produces a dose-dependent cardiovascular dysfunction in anaesthetized cynomolgus monkeys. Changes of hemodynamic parameters after systemic administration of 0.03 nmol/kg (↑) and 0.3 nmol/kg (↕) U-II ($n = 4$) are seen. Bolus i.v. applications of U-II are indicated by the arrows. Heart rate (HR) (a), mean arterial blood pressure (MBP) (b), maximum first derivative of left ventricular pressure (dp/dt_{max}) (c), pulmonary pressure (d), coronary blood flow (e), and carotid blood flow (f). $^*P < 0.05$, $^{**}P < 0.001$ relative to baseline measurement before U-II administration ($n = 4$). Values are means and vertical bars show s.d.

et al., 2000; Paysant *et al*., 2001). In human small resistance vessels such as abdominal resistance arteries (of ≈ 0.2 mm i.d.), Stirrat *et al*. (2001) observed an U-II-induced relaxation. In human skin microvasculature, there was no response to U-II (Hillier *et al*., 2001). The putative calibre-associated vasoactive effects of U-II in man were also suggested by the studies using isolated human pulmonary arteries of different diameter. In arteries of ≈ 0.25 mm i.d., three out of 10 vessels contracted in response to U-II (MacLean *et al*., 2000), whereas Bennett *et al*. (2004) failed to see any vasoactive effect of U-II in arteries of ≈ 0.5 mm i.d.. However, in very small human pulmonary arteries of ≈ 0.07 mm i.d., Stirrat *et al*. (2001) found a vasodilatation in response to U-II. In human heart, small coronary arteries exerted a greater maximum response to U-II than epicardial vessels (Maguire *et al*., 2004).

Indeed, the putative calibre association of the U-II effect in regulating vascular tone can be only suggested in several species such as rat and non-human primate. This provides fertile ground to investigate the underlying mechanism(s) of the calibre-associated U-II effects. For example, comparing the cellular UT receptor distribution in typical large and small vessels may shed some light on understanding the calibre-associated U-II effects. However, the cellular distribution of UT receptor (including receptor distribution in VSMCs and endothelial cells) in various peripheral vessels in rat, non-human primate, and man is largely unknown. Moreover, the intracellular signalling mechanisms downstream of UT receptor activation in VSMCs of large and small arteries may be different. Additionally, UT receptor in endothelial cells may be stimulated to release vasoactive factors to modulate the vasoactive effects of U-II on VSMCs (Bottrill *et al*., 2000; Zhang *et al*., 2003).

As reviewed above, the *in vitro* vascular reactivity of vessels varies in reports from different groups. For example, Maguire *et al*. (2000) reported a potent contractile effect of U-II (being 50 times more potent in arteries and <10 times more potent in veins than endothelin-1) in isolated human coronary, mammary and radial arteries, and saphenous and umbilical veins, whereas Hillier *et al*. (2001) did not find any vasoactive effect of U-II on vessels with comparable calibre, such as human mammary arteries and saphenous veins. This difference may be ascribed to different experimental procedures between the two studies – for example, the endothelium was denuded in Maguire's study while the endothelium was intact in Hillier's study. U-II may stimulate the endothelium to release vasodilators to counteract the direct contractile effect of U-II on the VSMC. This hypothesis is perhaps supported by the uncovering of the contractile responses to U-II in human pulmonary arteries by inhibition of nitric oxide synthase (MacLean *et al*., 2000).

Another interesting point was raised by the work of Gendron *et al*. (2005), who speculated the existence of two 'functional sites' for U-II with opposite effects. This hypothesis is based on the observation that, despite its capability to block U-II-induced vasoconstriction in thoracic aortae isolated from normotensive rats (Douglas *et al*., 2000), the UT receptor ligand urantide injected i.v. was not able to block the hypotensive effect of a bolus i.v. injection of U-II in 8-week-old anaesthetized SHR (Gendron *et al*., 2005). The hypothesis is interesting and remains to be tested. The evidence available to date is not sufficiently compelling to support the existence of novel UT receptor subtypes as no molecular evidence for the new receptor was provided (Gendron *et al*., 2005). On the other hand, the UT receptor ligand, urantide, used to block the effects of U-II in SHR (Gendron *et al*., 2005) has been shown to act as a low efficacy partial agonist in inducing calcium release in CHO cells overexpressing human UT receptors (Camarda *et al*., 2004; 2006) and is probably not the best tool to define receptor subtype. In contrary to Camarda *et al*., urantide has been shown to produce a concentration-related competitive inhibition of U-II-induced contractions in rat thoracic aortae ($pK_B = 8.3$) without any agonistic effect up to a concentration of 1 μM (Patacchini *et al*., 2003). It is worth noting that the partial agonistic effect of urantide in releasing calcium was observed only in CHO cells overexpressing human UT receptors but not in HEK293 cells overexpressing human UT receptors (Camarda *et al*., 2006). As the efficacy of a UT receptor ligand may be overestimated in a cell system expressing high levels of recombinant UT receptors (Camarda *et al*., 2002), the possibility that the partial agonist effects of urantide may be ascribed to the high levels of recombinant UT receptors, which may not be present in most (patho)-physiological conditions, cannot be excluded.

Recent *in vivo* studies in human, though limited in terms of methodology, have shed some light on our understanding of the (patho)physiological role of U-II in cardiovascular regulation (Bohm & Pernow, 2002; Wilkinson *et al*., 2002; Sondermeijer *et al*., 2005). In nine healthy volunteers, U-II (1–300 pmol min^{-1}) infusion into the brachial artery caused a dose-dependent reduction in forearm blood flow as determined by venous occlusion plethysmography (Bohm & Pernow, 2002). However, no change in systemic haemodynamics (i.e. blood pressure, heart rate, and cardiac output), forearm blood flow, and electrocardiogram was apparent during intra-arterial infusion of U-II (0.001–300 pmol min^{-1}) (Wilkinson *et al*., 2002). The contrasting results of these two studies may be ascribed to: (a) the healthy volunteers recruited in Wilkinson's study (mean age 37 ± 4 years, range 22–53) were older than Bohm & Pernow's (mean age 24 ± 1 years); (b) inherent heterogeneity of the volunteers recruited; (c) there was no placebo-treated group in Bohm & Pernow's, whereas the number of samples was too small in Wilkinson's study ($n = 4$ in 30 and 100 pmol min^{-1} U-II/placebo group and $n = 6$ in the 100 and 300 pmol min^{-1} U-II/placebo group); and (d) different sources of U-II used in the two studies.

Interestingly, iontophoresis-administered U-II (10^{-12}, 10^{-9}, 10^{-7} mol l^{-1}) induced a vasoconstrictor response in skin microvasculature assessed with a laser Doppler velocimetry in patients with either chronic heart failure (Lim *et al*., 2004) or essential hypertension (Sondermeijer *et al*., 2005), which is in sharp contrast to its vasodilator effect in normal subjects. The underlying mechanisms of the differential effects of U-II on vascular tone in normal subjects and patients with chronic heart failure and hypertension is interesting and remain to be clarified. It is possible, for example, that the diversity in the intracellular signalling mechanisms downstream of the U-II receptor, and the interaction between U-II and other vasoactive factors in disease states may modulate the vasoactive effect of U-II. The intracellular signalling mechanisms, which mediate the vasoactive effects of U-II in VSMCs, as well as its putative interactions with other vasoactive factors in physiology and disease remain largely unknown. On the other hand, endothelium dysfunction associated with heart failure and hypertension may unmask the contractile effects of U-II in contrast to normal subjects in whom endothelium may release

relaxing factors to counteract the contractile effects of U-II on VSMC. Moreover, the possibility that receptor externalization and recycling may also modulate the vasoactive effects of U-II should be considered in the light of a recent report showing that the long-lasting contractile effect of U-II in rat aorta may be due to continuous arrestin-independent externalization and recycle of UT receptors (Giebing *et al*., 2005).

In addition to its actions on regulating vascular tone, U-II (0.01–10 nmol kg^{-1}) has also been shown to dose-dependently increase plasma extravasation in mouse as examined using the Evans blue technique in various vascular regions, such as airways, and gastrointestinal and urogenital tract (Vergura *et al*., 2004). Similarly, the oedematogenic property of U-II (0.1–10 nmol kg^{-1}) was observed in specific organs from rats as assessed by the Evans blue method (Gendron *et al*., 2004). These findings suggest that U-II may also be involved in modulating interstitial fluid homeostasis across microvascular wall in specific tissues in addition to its role in regulating vascular tone.

The vasoactive effects of U-II on man, non-human primates, and the rat are summarized in Table 1.

In contrast to the rather complicated haemodynamic effects of U-II in man, non-human primate, and the rat, U-II induced a 'classical' systemic dose-dependent pressor response in the cat. I.v. injection of U-II (1 nmol kg^{-1}) doubled both mean blood pressure (from 99 ± 14 to 183 ± 15 mmHg) and systemic vascular resistance (from 0.36 ± 0.12 to 0.86 ± 0.20 mmHg ml min^{-1}) in the anaesthetized cat. U-II constricts all feline isolated arteries studied, including aortae, renal, femoral, carotid, and mesenteric conduit/resistance arteries ($-\log[EC_{50}]$'s 9.68 ± 0.24–8.73 ± 0.08) (Behm *et al*., 2004). The authors suggested that cat may be an 'ideal' model for determining the effects of U-II receptor antagonism on cardiovascular homeostasis. To achieve this goal, the difference between the U-II/UT receptor systems of cat and man deserves to be further investigated.

On the other hand, in contrast to the rather well-studied role of U-II in the regulation of vascular tone, the purported role of this peptide in regulating vascular morphology is largely unknown. In cultured VSMCs, U-II has been shown to promote cell proliferation (Sauzeau *et al*., 2001; Watanabe *et al*., 2001a, b; Tamura *et al*., 2003; Djordjevic *et al*., 2005). However, it is unknown whether U-II regulates vascular cell growth *in vivo*, for example, in physiological angiogenesis during organ development, thickening of vascular wall in hypertension, and development of arteriosclerotic lesions. Recently, Rakowski *et al*. (2005) reported that treatment with a selective nonpeptidic UT receptor antagonist SB-611812 significantly reduced intimal lesions in the carotid artery, which were induced by balloon angioplasty, suggesting a role of U-II in neointima formation (see the section of 'U-II and disease'). The study of the role(s) of U-II in endothelial cell growth are few and far between. In human umbilical vein endothelial cells, hU-II increased ^{3}H-thymidine incorporation into DNA and inhibited cell apoptosis induced by serum withdrawal (Shi *et al*., 2006). Additional *in vivo* studies may aid to demonstrate the (patho)physiological significance of these U-II effect on endothelial cell growth and apoptosis.

Effects of U-II on the heart

In spite of the accumulating evidence for a role for U-II in regulating peripheral vascular tone, little is known of the direct effect of this peptide on cardiac contractility. Several *in vivo* studies implied that U-II exhibited an inotropic effect, for example, systemic administration of U-II induced a decrease in left ventricular contractility with a concomitant decrease in blood pressure in the monkey (Ames *et al*., 1999; Zhu *et al*., 2004) and rats (Gardiner *et al*., 2001; Hassan *et al*., 2003; Gendron *et al*., 2005). As U-II has been shown to affect vascular tone (see preceding section), it is possible that the inotropic effect of U-II in these experiments is secondary to U-II-induced alterations in vascular tone and blood pressure, leading to subsequent modulation of cardiac contractility by baroreceptor reflex (Ames *et al*., 1999; Hassan *et al*., 2003; Zhu *et al*., 2004). Studies using isolated myocardial tissues may help to understand the direct inotropic effect of U-II. Certainly, in isolated human right atrial trabeculae (Russell *et al*., 2001) and rat left ventricular papillary muscles (Gong *et al*., 2004), U-II has been shown to be a positive inotropic factor possibly mediated *via* protein kinase C (PKC) activation (Russell *et al*., 2004).

U-II has also been suggested to affect the process of cell growth in the heart. Recently, Tzanidis *et al*. (2003) reported an upregulation of UT receptor expression in cardiomyocytes as well as in endothelial cells and fibroblasts in the rat heart after coronary artery ligation. In contrast, UT receptor was not identified in sham-operated rats. This study suggests that U-II receptors may be linked with the process of cardiac remodelling after myocardial infarction. Such an hypothesis has received some support from observations of the direct action of U-II on cardiac cells growth *in vitro*, that is, U-II treatment increased collagen mRNA and protein levels in cardiac fibroblast, and the hypertrophic response in neonatal cardiomyocytes expressing recombinant UT receptor. Onan *et al*. (2004) further showed that U-II promoted hypertrophy in cultured neonatal cardiomyocytes *via* extracellular signal-regulated protein $kinase_{1/2}$ ($ERK_{1/2}$) and p38 signalling pathways. In the rat, chronic infusion of U-II induced an increase in the ratio of left ventricular collagen I : III (which is usually observed in myocardial fibrosis) as well as a reduction in left ventricular contractility (Kompa *et al*., 2004). These studies indicate that chronic stimulation of U-II may result in deleterious effects such as cardiac hypertrophy and fibrosis *in vivo*. Moreover, U-II also stimulated the cultured rat cardiomyocytes to release certain peptides such as atrial natriuretic peptide and brain-derived natriuretic peptide (Zou *et al*., 2001) and cytokines such as interleukin-6 (Johns *et al*., 2004). It is worth noting that the hypertrophic effect of U-II on rat cardiomyocytes was usually not prominent in intact cardiomyocytes, and transfer and expression of recombinant UT receptors in cardiomyocytes 'unmasked' U-II-induced hypertrophy (Tzanidis *et al*., 2003; Johns *et al*., 2004). Whereas UT receptors was upregulated in the rat model of heart failure, U-II is assumed to induce myocyte hypertrophy in disease such as heart failure but not to modulate myocyte growth in physiology. Moreover, U-II-induced release of neurohumoral factors may modulate the U-II effects on the heart.

Central cardiovascular actions of U-II

In contrast to the hypotensive response in the rat (Gardiner *et al*., 2001; Hassan *et al*., 2003; Gendron *et al*., 2005) and non-human primate (Ames *et al*., 1999; Zhu *et al*., 2004) and no

Table 1 Vasoactive effects of U-II on the peripheral vessels

	Species					
	Human		*Non-human primate*		*Rat*	
	In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*
Aorta			Constriction (Ames *et al*., 1999)		Constriction (Bottrill *et al*., 2000; Douglas *et al*., 2000)	
Coronary artery	Constriction (Maguire *et al*., 2000; 2004)		Constriction (Ames *et al*., 1999)		Constriction (in isolated vessels, Bottrill *et al*., 2000; in perfused hearts, Gray *et al*., 2001) Dilatation (in perfused hearts, Katano *et al*., 2000)	
Pulmonary artery	Variable effects (in arteries of $\approx$0.25 mm i.d., three out of 10 vessels contracted, MacLean *et al*., 2000) No effect (in arteries of $\approx$0.5 mm i.d., Bennett *et al*., 2004) Dilatation (in arteries of $\approx$0.07 mm i.d., Stirrat *et al*., 2001)			Constriction (Zhu *et al*., 2004)	Constriction (in arteries of 2–3 mm i.d., MacLean *et al*., 2000)	
Renal artery					Dilatation (in arteries of $\approx$0.2 mm i.d., Zhang *et al*., 2003)	
Mesenteric artery					No effect (Bottrill *et al*., 2000)	Dilatation (Gardiner *et al*., 2001; 2004)
Mammary artery	Constriction (Maguire *et al*., 2000) Variable effect (three out of eight contracted, Paysant *et al*., 2001) No effect (Hillier *et al*., 2001)					
Radical artery	Constriction (Maguire *et al*., 2000) Variable effect (two out of three contracted, Paysant *et al*., 2001)		Constriction (Paysant *et al*., 2001)			
Skin microvasculature	No effect (Hillier *et al*., 2001)	Constriction in hypertensive (Sondermeijer *et al*., 2005) and heart failure (Lim *et al*., 2004) patients Dilatation in normotensive subjects (Sondermeijer *et al*., 2005)				
Abdominal resistance artery	Dilatation (in arteries of $\approx$0.2 mm i.d., Stirrat *et al*., 2001)					
Hindquarter artery						Dilatation (Gardiner *et al*., 2001; 2004)
Forearm vessel		Constriction (Bohm & Pernow, 2002) No effect (Wilkinson *et al*., 2002)				
Saphenous vein	Constriction (Maguire *et al*., 2000) No effect (Hillier *et al*., 2001; Paysant *et al*., 2001)					
Umbilical vein	Constriction (Maguire *et al*., 2000)					
Total peripheral resistance				Increase (Ames *et al*., 1999)		Decrease (Gardiner *et al*., 2001)

significant change in blood pressure in the sheep (Watson *et al*., 2003) upon i.v. administered U-II, intracerebroventricular (i.c.v.) injection of U-II elicited a dose-dependent increase in blood pressure and heart rate in the sheep (Watson *et al*., 2003; Hood *et al*., 2005) and rats (Lin *et al*., 2003a, b; 2004b). In the sheep, this hypertensive response (caused by i.c.v. U-II 0.2 nmol kg^{-1}) was accompanied by increases in myocardial contractility (Watson *et al*., 2003), cardiac output (Watson *et al*., 2003), as well as increases in coronary (Hood *et al*., 2005), renal (Watson *et al*., 2003), mesenteric (Watson *et al*., 2003; Hood *et al*., 2005), and iliac (Watson *et al*., 2003; Hood *et al*., 2005) blood flows and plasma glucose (Watson *et al*., 2003; Hood *et al*., 2005). In the rat, the increase in blood pressure in response to i.c.v. injection of U-II (10 nmol) was more pronounced in SHR than in WKY (Lin *et al*., 2003b). The haemodynamic changes induced by i.c.v. injection of U-II could be attenuated by pretreatment with the ganglion blocker pentolinium in the rat (Lin *et al*., 2003a), the β-adrenoceptor blocker propranolol in the sheep (Hood *et al*., 2005), and the extracellular signal-regulated kinase (ERK) inhibitor PD098059 or the Rho kinase inhibitor Y-27632 but not the phosphatidylinositol 3 kinase inhibitor wortmannin in the rat (Lin *et al*., 2004b). Hyperglycaemia induced by i.c.v. injection of U-II (0.2 nmol kg^{-1}) was prevented by ganglion blockade but not propranolol in the sheep (Hood *et al*., 2005). Two hours after i.c.v. infusion of U-II (0.2 nmol kg^{-1}), plasma adrenaline, and adrenocorticotropic hormone levels also increased dramatically in the sheep (Watson *et al*., 2003). These studies demonstrated that U-II can also act centrally to regulate cardiovascular function by stimulating the sympathoadrenal pathway.

Moreover, Lu *et al*. (2002) have suggested that U-II, in different brain regions, may play distinct roles in cardiovascular regulation. For example, in anaesthetized rats, microinjection of U-II into the A1 area caused dose-related depressor and bradycardic responses. In contrast, microinjection of U-II into either the paraventricular nucleus or arcuate nucleus caused significant increases in blood pressure and heart rate (Lu *et al*., 2002). These data suggest that U-II may affect neurons involved in cardiovascular regulation. High ^{125}I-U-II binding density has been identified in the abducens nucleus within the brainstem of Sprague–Dawley rat (Maguire *et al*., 2000). However, the abducens nucleus is probably not related to cardiovascular regulation. Thus, it remains to be clarified whether the elements of the U-II/UT receptor system colocalize with neurons in specific brain areas related to cardiovascular regulation, such as the rostral ventrolateral medulla, nucleus tractus solitarius, nucleus preopticus medialis, paraventricular nucleus, and arcuate nucleus.

However, in pre-eclamptic patients, U-II levels in maternal plasma or cerebrospinal fluid or umbilical vein did not alter significantly when compared with controls (Cowley *et al*., 2005). It is possible that U-II acts centrally as a local mediator/modulator in specific brain areas, which may not be reflected as a change in U-II levels in cerebrospinal fluid or plasma.

Taken together, the evidence available to date suggests that central U-II may also participate in the regulation of cardiovascular functions by modulating the sympathoadrenal pathway in addition to its direct effects on the heart and vessels. The purported pathways, including the neurotransmitters/modulators involved, for endogenous central U-II to regulated cardiovascular-related neurons is yet to be determined. Blockade of central UT receptors by pharmacological and molecular approaches may be a useful approach to test this hypothesis.

Role of U-II in renal physiology and body fluid homeostasis

A series of studies have suggested the ability of U-II to regulate transepithelial transport of ions and water across a variety of osmoregulatory surfaces such as skin, opercular membrane, urinary bladder, and anterior/posterior intestine in teleost fish. Thus, U-II may play a role in osmoregulation in fish, especially when the fish undergoes salinity transfer (i.e. between sea water and fresh water) (Winter *et al*., 2000).

A role for U-II in renal physiology in mammals was also suggested based on the detection of mRNA transcripts for U-II and UT receptors in human kidney (Matsushita *et al*., 2001), U-II-like immunoreactivity in human renal epithelial cells (Shenouda *et al*., 2002; Maguire *et al*., 2004), and U-II binding sites in human kidney cortex (Maguire *et al*., 2000). In the kidney outer medulla of Sprague–Dawley rats, high-affinity ^{125}I-U-II binding sites were identified, whereas low-affinity and low-density binding sites were present in medulla from WKY and SHR rats (Disa *et al*., 2006). Administration of U-II (5, 10, and 20 ng kg^{-1} min^{-1}) by continuous i.v. infusion for 1 h increased renal blood flow, glomerular filtration rate, and urinary water and sodium excretion in anaesthetized Sprague–Dawley rats. These effects were abolished by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (Zhang *et al*., 2003). In contrast, Balment *et al*. (2005) reported that i.v. bolus injection of U-II (10^{-10} and 10^{-9} M) caused decreases in glomerular filtration rate and urinary water and sodium excretion in anaesthetized Sprague–Dawley rats. The clear discrepancy between these studies indicates that the route of administration may have a profound influence on the physiological response to UII. In a rat model of volume overload heart failure induced by an arteriovenous fistula and its control, i.v. bolus injection of U-II at incremental doses (1, 10, and 100 nmol kg^{-1}) caused a decrease in mean arterial pressure in both control and heart failure rats at all doses tested. Renal blood flow was not altered in controls, but was significantly increased in heart failure rats (at doses of 10 and 100 nmol kg^{-1}). Glomerular filtration rate tended to decrease in control animals, but increased in the heart failure rats (at the dosage of 100 nmol kg^{-1}). Sodium excretion was not changed by U-II in both of the control and heart failure rats (Ovcharenko *et al*., 2006). This study suggests that the renal effect of U-II may be modulated by an ongoing disease state.

It remains to be investigated how U-II regulates renal function – by regulating vascular tone of small renal arteries as suggested by Zhang *et al*. (2003) or by a direct effect on the ion channels located in the tubules and collecting duct in the kidney. Evidence for a direct effect of U-II on renal tubule/duct has been provided by Matsushita *et al*. (2003), who showed that U-II stimulated cell growth *via* PKC and $ERK_{1/2}$ pathways as well as Ca^{2+} influx *via* voltage-dependent Ca^{2+} channels in cultured porcine renal epithelial cells. Taken together, this evidence indicates that the tubular epithelial cells are likely to be one of the targets for U-II to regulate renal function. Nevertheless, the precise role of U-II in regulating

renal tubular function, for example, with respect to the exchange of Na^+, H^+, K^+, Ca^{2+}, and Cl^- as well as in reabsorption of water, glucose, and amino acids are not clear. Localization of the cellular distribution of the U-II/UT receptor system in the kidney as well as specific approaches such as microinjection techniques to assess renal tubular function may help to address this intriguing question.

U-II and diseases

The first clinical observation linking U-II and human disease was reported by Douglas *et al*. (2002), who showed high levels of expression of U-II in cardiomyocytes and to a lesser degree in VSMCs, endothelial cells, and inflammatory cells of patients with end-stage congestive heart failure ($n=15$) compared with healthy controls ($n=8$). In patients with early-stage congestive heart failure ($n=5$), reduced U-II expression in the myocardium was noted. Myocardial U-II level correlated significantly with left ventricular end-diastolic dimension and inversely with ejection fraction in these patients. The density of the binding sites for fluorescein isothiocyanate-conjugated U-II was also significantly increased in the myocardium of patients with end-stage congestive heart failure (Douglas *et al*., 2002). U-II levels have also been reported to increase in plasma from patients suffering from heart failure (Ng *et al*., 2002; Richards *et al*., 2002; Russell *et al*., 2003; Heringlake *et al*., 2004). Ng *et al*. (2002) measured probrain natriuretic peptide and U-II levels in the plasma from 126 patients with heart failure and 220 age- and sex-matched controls. Both peptides were significantly increased in the plasma of heart failure patients. In contrast to probrain natriuretic peptide, plasma U-II levels did not correlate with the New York Heart Association class. However, the positive correlation between plasma U-II levels and disease phenotypes was not confirmed by several independent groups. No difference in plasma U-II levels was found between controls ($n=13$), patients with moderate ($n=10$) and severe ($n=11$) congestive heart failure, nor among different sites of blood sampling (pulmonary artery, left ventricle, coronary sinus, antecubital vein) within the single groups. Vasodilator therapy improving haemodynamics in the severe congestive heart failure group did not alter circulating U-II levels over 24 h in these patients (Dschietzig *et al*., 2002). In another study where patients and control subjects underwent cardiopulmonary exercise testing, plasma U-II levels were comparable in patients ($n=32$) with congestive heart failure and controls ($n=10$) at rest and peak exercise (Kruger *et al*., 2005). In patients with acute coronary syndromes ($n=54$), plasma U-II levels were significantly lower than patients with stable coronary artery disease ($n=51$) and healthy controls ($n=29$) (Joyal *et al*., 2006). The variable results for plasma U-II levels in heart failure patients reported by different investigators may be attributed to the number of samples examined (e.g. Ng *et al*. (2002) recruited 126 heart failure patients and 220 healthy controls, whereas Dschietzig *et al*. (2002) measured only 21 patients and 13 controls) as well as the methods used to measure U-II concentration.

Actually, reported human plasma U-II levels vary by $\sim$1000- to 10,000-fold between groups/assays. For example, Ng *et al*. (2002) reported the plasma U-II levels with mean values being in the range of 4.6–22.4 fmol ml^{-1} (1 fmol = 1.389 pg human U-II), which is much lower than that reported by Russell *et al*. (2003) ($\sim$300 pg ml^{-1}), Heringlake *et al*. (2004) (3065 $\pm$ 1541 pg ml^{-1}), and Aiyar *et al*. (2004) (12.4 $\pm$ 0.6 ng ml^{-1}). The methods used to measure plasma U-II levels are quite distinct from each other. Ng *et al*. (2002) purified plasma with C_{18} reverse-phase high-pressure liquid chromatography and then measured U-II levels with a noncompetitive immunochemiluminometric assay. However, U-II was measured by radioimmunoassay without prior purification of the plasma before measurement in the study of Russell *et al*. (2003). Richards *et al*. (2002) reported plasma U-II levels comparable to that described by Ng *et al*. (2002) by measuring U-II immunoreactivity with enzyme immunoassay (without purification of the plasma before measurement) and radioimmunoassay (with a plasma purification step of methanol extraction before measurement), respectively. Moreover, the antibodies against U-II used in these assays are from variable sources. Different antibodies may recognize different groups of U-II-related species such as U-II, U-II peptide fragments, pre–proU-II, urotensin-related peptide, and even some unknown peptides. Therefore, purification of the plasma with standard methods such high-pressure liquid chromatography as well as using specific antibodies may be critical points to make a precise measure of U-II levels by reducing nonspecific signals of crossreactivity.

Russell *et al*. (2003) further showed that the increased circulating U-II levels was attributed to cardiopulmonary production of the peptide as U-II levels were lower in the pulmonary artery than in the aortic root. In accordance with this study, significant arteriovenous gradients were observed across the heart (36%), liver (40%), and kidney (44%) in the sheep, suggesting an endogenous production of U-II in these organs (Charles *et al*., 2005). In a rat model of heart failure following myocardial infarction, postinfarction remodelling was associated with a 75% increase in U-II receptor gene expression in the heart (Tzanidis *et al*., 2003). Increased expression of U-II and its receptor in the circulation and myocardial tissue may contribute to the pathogenesis of myocardial remodelling in heart failure. This hypothesis was supported by *in vitro* studies, in which U-II stimulation of neonatal rat cardiac fibroblasts increased the level of mRNA transcripts for procollagens alpha1(I), alpha1(III), and fibronectin with a concomitant increase in collagen peptide synthesis. Transfection and expression of recombinant rat U-II receptor in neonatal cardiomyocytes caused significant U-II-dependent activation of hypertrophic phenotype (Tzanidis *et al*., 2003).

U-II expression has also been associated with hypertension. U-II levels increased significantly in cerebrospinal fluid (Thompson *et al*., 2003) and plasma (Cheung *et al*., 2004) in hypertensive patients. Blood pressure was significantly correlated with U-II levels in cerebrospinal fluid (Thompson *et al*., 2003) and plasma (Cheung *et al*., 2004). In rats exposed to chronic hypoxia for 4 weeks, pulmonary hypertension and right ventricular hypertrophy was accompanied by an increase in U-II and its receptor in the heart. U-II levels were increased by 97.5% in the right ventricle and 33.2% in the left ventricle. The density of U-II binding sites in the right ventricle was also upregulated to a greater extent than in the left ventricle (Zhang *et al*., 2002). U-II levels were significantly increased in patients with cirrhosis ($n=50$) compared to healthy controls ($n=15$), and were higher in ascitic than in nonascitic patients, suggesting a potential role for U-II in portal hypertension

(Heller *et al*., 2002). However, it is unclear whether U-II participates in the development of hypertension or is upregulated as a consequence of hypertension. *In vivo* chronic treatment using U-II receptor antagonist in hypertensive animal models may be valuable to define a role of U-II in the pathogenesis of hypertension.

U-II expression was identified in atherosclerotic lesions (Bousette *et al*., 2004; Maguire *et al*., 2004). In human aorta, there was a significant increase in the expression of U-II and its receptor in atherosclerotic lesions. Lymphocytes were identified as the predominant source of U-II mRNA. However, monocytes and macrophages were the main producers of UT receptor mRNA, with relatively little expression in foam cells, lymphocytes, and platelets (Bousette *et al*., 2004). In human coronary arteries with atherosclerotic lesion, immunoreactivity of U-II localized to regions of macrophage infiltration (Maguire *et al*., 2004). In human monocyte-derived macrophages, U-II increased the expression of mRNA transcript and protein and activity of acyl-coenzyme A : cholesterol acyltransferase-1, which converts intracellular free cholesterol into cholesterol ester, suggesting a role of U-II in inducing macrophage-derived foam cells (Watanabe *et al*., 2005). In apolipoprotein E−/− mice (with high serum levels of cholesterol and triglycerides compared with its wild-type control), ^{125}I-U-II binding to aorta (B_{max} 145 ± 18 fmol mg^{-1} protein) was increased by 64% compared its wild-type control (Wang *et al*., 2006). However, in both acute coronary syndromes and stable coronary artery disease patients, there was a negative relationship between plasma U-II levels and systemic arterial pressures (Joyal *et al*., 2006). U-II may participate in the pathogenesis of atherosclerosis as a local paracrine/autocrine factor and thus plasma U-II levels may not be in close correlation with the degree of atherosclerotic lesions. Whether U-II serves as a pathogeny or as a mediator of atherosclerosis remains to be clarified. Furthermore, it will be interesting to see whether U-II receptor antagonist can prevent arterial atherosclerosis.

On the other hand, changes of U-II levels in the urine (Matsushita *et al*., 2001) and plasma (Totsune *et al*., 2001) in patients with renal dysfunction imply a role of U-II in renal diseases. Accordingly, in patients with essential hypertension and those with renal tubular abnormality, but not with glomerular diseases, there was an increase in plasma U-II concentration (Matsushita *et al*., 2001).

Another U-II-associated disease is type II diabetes mellitus. Single-nucleotide polymorphism with one amino-acid substitution designated S89N in the coding region of the pre–proU-II gene has been shown to be associated with the development of type II diabetes in Japanese population. The allele frequency of 89N was higher in type II diabetic patients than in the control groups. Moreover, in the subjects with normal glucose tolerance, 89N was correlated with higher insulin levels in oral glucose tolerance test, suggesting reduced insulin sensitivity in subjects with 89N (Wenyi *et al*., 2003). Such nucleotide polymorphism analysis falls short in clarifying the (patho)physiological role of the mutant. On the other hand, to date, no nucleotide polymorphism of UT receptors has been reported. In patients with type II diabetes mellitus, there was a significant increase in U-II levels in both plasma and urine. In diabetic patients, U-II levels in plasma and urine significantly increased as renal function decreased, suggesting renal failure to be an independent factor associated with the increased U-II levels in type II diabetic patients (Totsune *et al*., 2004). In renal biopsy tissue samples from patients who suffered from diabetic nephropathy, mRNA transcripts of U-II and UT receptor were increased by 45- to almost 2000-fold when compared with control nephrectomy tissues, respectively. Immunohistochemical studies revealed intense U-II peptide staining in diabetic tissue distributed predominantly in tubular epithelial cells, and the binding sites for U-II were scattered with a similar tubular pattern of distribution (Langham *et al*., 2004). In perfused rat pancreas, U-II (IC_{50} 0.12 nmol l^{-1}) inhibited glucose-induced insulin release in a dose-dependent manner (Silvestre *et al*., 2004).

Several *in vivo* studies of therapeutic intervention with selective nonpeptidic U-II receptor antagonists have been published. Clozel *et al*. (2004) reported the discovery of a selective nonpeptidic UT receptor antagonist, ACT-058362 (1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulphate salt) (also known as Palosuran). Binding experiments performed by incubating 20 pM human ^{125}I-U-II for 8 h in the presence of increasing concentration of ACT-058362 showed IC_{50} values of 86 ± 30 and 3.6 ± 0.2 nM with intact CHO cells or membrane preparations carrying human UT receptors, respectively. Compared with the human UT receptor, the binding inhibitory potency of ACT-058362 against the rat UT receptor was much lower in intact cells (IC_{50} >10,000 nM) and in membrane preparation (IC_{50} 1475 ± 70). The inhibitory potency of ACT-058362 (IC_{50}) for Ca^{2+} mobilization induced by 30 nM U-II was 17 ± 0.63 and >10,000 nM in recombinant CHO cells carrying human or rat U-II receptor. In a rat model of renal ischaemia–reperfusion, i.v. infusion of ACT-058362 (10 mg kg^{-1} h^{-1} i.v., resulting in a plasma concentration of 5 μM), which was initiated 30 min before renal artery occlusion and continued during the 45 min of renal ischaemia and the 60 min of reperfusion, prevented the decrease in renal blood flow, which follows renal artery clamping, increased creatinine clearance, and decreased the severity of tubulointerstitial lesions examined 48 h after renal artery occlusion. Blood pressure, heart rate, and baseline renal blood flow were not altered by ACT-058362 treatment (Clozel *et al*., 2004). This study suggests that U-II may be involved in the pathogenesis of renal ischaemia–reperfusion. However, the mechanisms underlying the UT receptor antagonist-induced beneficial effect in renal ischaemia–reperfusion remain unknown. The sticking point is how U-II may participate in renal ischaemia–reperfusion injury – simply by constricting the renal arteries after ischaemia and the subsequent damage of renal function and morphology is just a consequence of reduced renal blood flow, or by a direct stimulation on the renal tubules in addition to its vasoactive effects. This hypothesis still remains to be testified as neither U-II nor UT receptors was examined in the renal arteries, glomeruli, and tubules in this study (Clozel *et al*., 2004). Additionally, as ACT-058362 has a much lower inhibitory potency on rat UT receptors than human UT receptors *in vitro*, it is surprising to see this compound to provide protection in a rat model of renal ischaemia–reperfusion injury with plasma concentrations of the drug reaching 5 μM. To date, no evidence has been available for the *in vivo* binding characteristics of ACT-058362 to rat UT receptors.

In a rat model of type I diabetes induced by streptozotocin treatment marked with pancreatic β-cell destruction, hyperglycaemia, dyslipidaemia, and renal dysfunction, 25-week oral treatment with ACT-058362 (300 mg kg^{-1} day^{-1}) improved

survival, increased insulin levels, and slowed the increase in glycaemia, glycosylated haemoglobin, and serum lipids. Renal blood flow was increase with concomitant delayed development of proteinuria and renal damage in diabetic rats treated with ACT-058362 (Clozel *et al*., 2006). The data provided in this study implies a role of U-II in both insulin production and the development of renal complications of diabetes. Although the study provides evidences of the beneficial effect of chronic UT receptor blockade in diabetic rats, it falls short in elucidating the mechanisms of U-II in mediating the pathological processes of diabetes. For example, U-II/UT receptors were not examined in the related tissues such as the β-cells of pancreatic islet, and glomeruli and tubules in the kidney, it is therefore unable to validate the hypothesis that endogenous U-II is involved in the two pathways, that is, regulation of insulin production and involvement in the pathological processes of renal damage in type I diabetes. It is also uncertain about how the UT receptor antagonist protects the pancreatic β-cells against the damage induced by streptozotocin and stimulates insulin release from survived β-cells. As discussed in the preceding paragraph, the *in vitro* inhibitory potency of ACT-058362 against the rat UT receptor is quite low. Whether the dose ($300\,mg\,kg^{-1}\,day^{-1}$) used in this particular study resulted in plasma/tissue levels high enough to block rat UT receptor *in vivo* is not certain. On the other hand, the type I diabetic model induced by streptozotocin is probably not the best model to represent human diabetes. Type II diabetic rats with insulin resistance may be more representative to investigate the role of U-II in the development diabetic nephropathy. The purported therapeutic effects of UT antagonists deserve to be further investigated in various disease models. To date, ACT-058362 is the first UT antagonist that has been tested in human. In November 2003, proof-of-concept trials (phase IIa) to test the efficacy of ACT-058362 in human diabetic nephropathy were commenced. Close to 100 patients have been studied so far and the results obtained failed to support the initiation of a full-fledged clinical development program for this drug in treating human diabetic nephropathy (see, http://www.actelion.com/uninet/www/www_main_p.nsf/Content/Develoment+Palosuran+).

In a rat model of carotid artery restenosis, Rakowski *et al*. (2005) treated the rat with a selective nonpeptidic UT receptor antagonist, SB-611812. Intimal lesions in the carotid artery were induced by balloon angioplasty. At day 14, there was extensive intimal thickening with concomitant strong expression of U-II. However, in the carotid arteries of uninjured rats, there was only weak expression of U-II in endothelial cells and little to no expression in VSMCs. Treatment with SB-611812 significantly reduced intima-to-media area ratio by 60% when compared to vehicle treatment. This study suggests that U-II is upregulated by balloon angioplasty and hence promotes intimal thickening including proliferation of VSMCs, and U-II may play a more predominant role in certain disease than in physiology. This hypothesis needs to be testified in more disease models and their normal controls by chronic treatment of selective UT receptor antagonists or molecular approaches such as UT receptor knockout.

Taken together, most of the published studies show merely an association of the gene expression levels of U-II/receptor with diseases such as heart failure, hypertension, atherosclerosis, arterial restenosis, and type II diabetes mellitus and accompanying nephropathy (as summarized in Table 2). The role of the U-II ligand–receptor system in the pathogenesis of the diseases remains to be further clarified by therapeutic intervention using U-II receptor antagonists and molecular approaches such as gene targeting for U-II receptor.

UT receptor ligands available as pharmacological tool

A series of peptide and nonpeptide small-molecule antagonists of the UT receptor have also been developed in recent years (for a review, see Douglas *et al*., 2004). Herein, we focus on those UT receptor ligands that have been shown to antagonize U-II effects in the cardiorenal system. Among the peptide ligands, [Pen5,DTrp7,Orn8]hU-II(4–11) (urantide) has been suggested as the most powerful antagonist for the UT receptor. This compound competitively antagonized hU-II-induced contractions in isolated rat thoracic aorta with $pK_B = 8.3 \pm 0.09$ while not modifying the noradrenaline- or endothelin 1-induced contraction. (Patacchini *et al*., 2003). However, urantide has been reported to act as an agonist (pEC_{50} 8.11) in a calcium mobilization assay performed in CHO cells overexpressing recombinant human UT receptors, suggesting perhaps a partial agonist action (Camarda *et al*., 2004). Indeed, the agonist effect may be overestimated in recombinant cells, which express extremely high (nonphysiological) levels of UT receptors. Whether urantide can antagonize U-II effects in *in vivo* models is unknown.

Another UT receptor ligand assumed to be an antagonist is [Pen^5,DTrp^7,Dab^8]U-II(4–11) (UFP-803). In rat isolated rat aorta, UFP-803 alone, up to $10\,\mu M$, did not evoke a contractile response, but concentration dependently displaced the contractile response curve to U-II to the right indicative of competitive antagonism with a pA_2 value of 7.46. In the fluorometric imaging plate reader $[Ca^{2+}]_i$ assay, performed at room temperature in HEK293 cells carrying recombinant human and rat UT receptors, both urantide and UFP-803 were inactive alone, but reduced, in a concentration-dependent manner, the maximal effects of U-II with apparent pK_B values in the range of 8.45–9.05. When the experiments were performed at 37°C using a cuvette-based $[Ca^{2+}]_i$ assay and CHO cells overexpressing human UT receptors, urantide exerted a stimulatory effect with an intrinsic activity ($\alpha = 0.80$), whereas UFP-803 displayed a small ($\alpha = 0.21$) but consistent residual agonist activity. *In vivo* in mice, UFP-803 ($10\,nmol\,kg^{-1}$) inhibited U-II ($1\,nmol\,kg^{-1}$)-induced increase in plasma extravasation in various vascular beds while being inactive alone. Thus, UFP-803 is a potent UT receptor ligand displaying antagonist characters, and is less potent as an agonist than urantide in certain recombinant cells (Camarda *et al*., 2006).

A somatostatin antagonist SB-710411 (Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide) ($10\,\mu M$) has also been shown to inhibit U-II-induced contraction in rat isolated thoracic aorta resulting in a significant, parallel shift of the contractile response curve (pK_B 6.28 ± 0.11). SB-710411 did not alter the contractile actions of angiotensin-II, phenylephrine, or KCl. However, SB-710411 potentiated the contractile response to endothelin-1 (pEC_{50} 8.02 ± 0.16). This effect may be ascribed to its affinity for somatostatin receptors as the somatostatin agonist somatostatin-14 and antagonist cyclosomatostatin also

Table 2 U-II and diseases

Disease	*Changes in U-II/UT receptor*	*Species*	*Comments*	*References*
Heart failure	Increased plasma U-II levels in heart failure patient	Human	Increase in U-II was attributed to cardiopulmonary production of the peptide	Ng *et al.* (2002), Richards *et al.* (2002), Russell *et al.* (2003), Heringlake *et al.* (2004)
Heart failure	Strong expression of U-II and UT receptors in the cardiomyocytes	Human	Myocardial expression of U-II correlated significantly with left ventricular end-diastolic dimension	Douglas *et al.* (2002)
Heart failure	No change in plasma U-II levels	Human	No difference in plasma U-II levels was found between controls and patients with congestive heart failure	Dschietzig *et al.* (2002), Kruger *et al.* (2005)
Heart failure	Significant increase in UT receptor gene expression in the heart	Rat	*In vitro* studies showed a growth-stimulating effect of U-II on cardiac fibroblasts and myocyte hypertrophy	Tzanidis *et al.* (2003)
Hypertension	Increased U-II levels in cerebrospinal fluid and plasma in hypertensive patients	Human	Blood pressure were directly related with U-II levels in cerebrospinal fluid and plasma	Thompson *et al.* (2003), Cheung *et al.* (2004)
Pulmonary hypertension	Increased U-II content and binding sites in both right and left ventricles	Rat	Increase in U-II and its receptor in the right ventricle was greater than that in the left ventricle in rats exposed to chronic hypoxia for 4 weeks	Zhang *et al.* (2002)
Coronary atherosclerosis	U-II-like immunoreactivity present in regions of macrophage infiltration	Human	U-II may participate in the pathogenesis of coronary atherosclerosis	Maguire *et al.* (2004)
Aortic atherosclerosis	Increased expression of U-II and UT receptor in atherosclerotic lesions	Human	Lymphocytes were the predominant source of U-II mRNA. However, monocytes and macrophages were the main producers of UT receptor mRNA	Bousette *et al.* (2004)
Restenosis of carotid artery following balloon angioplasty	Pronounced expression of U-II mRNA in myointimal cells	Rat	Treatment with the selective nonpeptidic U-II receptor antagonist SB-611812 resulted in a significant 60% reduction in intimal lesion following balloon angioplasty	Rakowski *et al.* (2005)
Type II diabetes mellitus	Elevate plasma immunoreactive U-II levels Increased urinary immunoreactive U-II excretion	Human	Concomitant renal failure is another independent factor associated with the increased plasma-immunoreactive U-II levels in Type II diabetic patients	Totsune *et al.* (2004)
Susceptibility to Type II diabetes mellitus	Single-nucleotide polymorphisms with amino-acid substitutions designated S89N in a coding region of the pre–proU-II gene	Human	Subjects with S89N in the U-II gene may be more insulin-resistant and thus more susceptible to type II diabetes mellitus development	Wenyi *et al.* (2003)
Diabetic nephropathy	Dramatic increase in mRNA transcripts of U-II and UT receptor in renal biopsy tissue samples	Human	Intense U-II peptide staining and U-II binding sites in diabetic tissue localized predominantly to tubular epithelial cells	Langham *et al.* (2004)

potentiated endothelin-1-induced contraction (Behm *et al.*, 2002).

Nonpeptidic small-molecule UT receptor ligands may perhaps be more effective as experimental tools and also as potential therapeutic agents. ACT-058362 has been shown to inhibit the binding of ^{125}I-U-II (20 pM, 8 h incubation) to intact CHO cells or membrane preparations carrying human UT receptors with IC_{50} values of 86 ± 30 and 3.6 ± 0.2 nM, respectively (Clozel *et al.*, 2004). Paradoxically, however, the binding inhibitory potency of ACT-058362 against the rat UT receptor was >120-fold lower in intact cells and 400-fold lower in membrane preparation, compared to that against human UT receptor (see preceding section). Therefore, it is surprising that an i.v. infusion of ACT-058362 ($10\,mg\,kg^{-1}\,h^{-1}$ i.v., resulting in a plasma concentration of $5\,\mu M$) provided protection in a rat model of renal ischaemia–reperfusion (Clozel *et al.*, 2004). Similarly, long-term oral treatment with ACT-058362 ($300\,mg\,kg^{-1}\,day^{-1}$) improved survival, renal function, and morphology in a rat model of type I diabetes induced by streptozotocin treatment (Clozel *et al.*, 2006).

Another nonpeptidic UT receptor antagonist tested in *in vivo* experiments is SB-611812, which significantly reduced intima-to-media area ratio by 60% in a rat model of carotid artery restenosis (Rakowski *et al*., 2005). Additionally, SB-706375 has been reported to act as a potent, competitive nonpeptidic U-II antagonist across species with pK_B 7.29–8.00 in a calcium mobilization assay performed in HEK293 cells carrying UT receptors, and in rat isolated aorta (inhibition of contraction) with pK_B 7.47. SB-706375 also reversed the tone established in the rat aorta by prior exposure to U-II (K_{app} approximately 20 nM) (Douglas *et al*., 2005).

Taken together, several peptide and nonpeptide UT receptor ligands appear to antagonize the effects of U-II in the cardiorenal system. To date, *in vivo* studies to examine the effects of UT receptor antagonism in various disease models are rare. In such studies, the mechanisms of the purported therapeutic effects of the UT receptor antagonists warrant further investigation.

Intracellular signalling mechanisms of U-II receptor activation

Most studies carried out to investigate the intracellular signalling mechanisms following U-II stimulation have been performed in VSMCs. The biological effects of U-II on VSMCs identified, to date, are contraction and proliferation. However, the intracellular signalling mechanism of the peptide remains largely unknown. Most published studies to investigate the intracellular signalling mechanisms of U-II to induce VSMC contraction were performed on isolated aortic rings using synthetic inhibitors to distinguish the pathways involved. In isolated rabbit thoracic aorta, the contractile effect of U-II was significantly inhibited by a phospholipase C inhibitor, 2-nitro-4-carboxyphenyl-*N*,*N*′,-diphenylcarbamate, but not by the cyclooxygenase inhibitor, indomethacin. Meanwhile, U-II increased phosphoinositide hydrolysis, and this effect was also inhibited by the phospholipase C inhibitor, suggesting a role of the phospholipase C-dependent/inositol phosphates pathway in mediating U-II-induced vasoconstriction (Saetrum *et al*., 2000). In isolated rat aortic rings, U-II increased cytosolic Ca^{2+} level. Complete inhibition of Ca^{2+} increase could only prevent part of the U-II-induced vasoconstriction. Likewise, PKC inhibitor (Go6983), mitogen-activated protein kinase kinase inhibitor (U0126), p38 mitogen-activated protein kinase (MAPK) inhibitor (SB203580), or myosin light chain kinase inhibitor (wortmannin) partially inhibited the U-II-induced vasoconstriction, suggesting the involvement of multipathways (Tasaki *et al*., 2004). For U-II-induced VSMC proliferation, recent studies suggest three signalling pathways, that is, U-II receptor/NADPH/reactive oxygen species/MAPKs and protein kinase B (Akt)/PAI-1 (Djordjevic *et al*., 2005), Rho A/Rho kinase (Sauzeau *et al*., 2001), and ERK-mediated pathways (Tamura *et al*., 2003). The suggested signalling mechanism of U-II stimulation in VSMCs is illustrated in Figure 3.

In cultured neonatal cardiomyocytes transfected with adenovirus-mediated UT receptors, U-II potently activated the MAPKs, $ERK_{1/2}$, and p38, and blocking these kinases with PD098059 and SB203580, respectively, significantly prevented U-II-induced hypertrophy. The activation of $ERK_{1/2}$ and p38 as well as myocyte hypertrophy was dependent on U-II-induced *trans*-activation of the epidermal growth factor receptor (Onan *et al*., 2004).

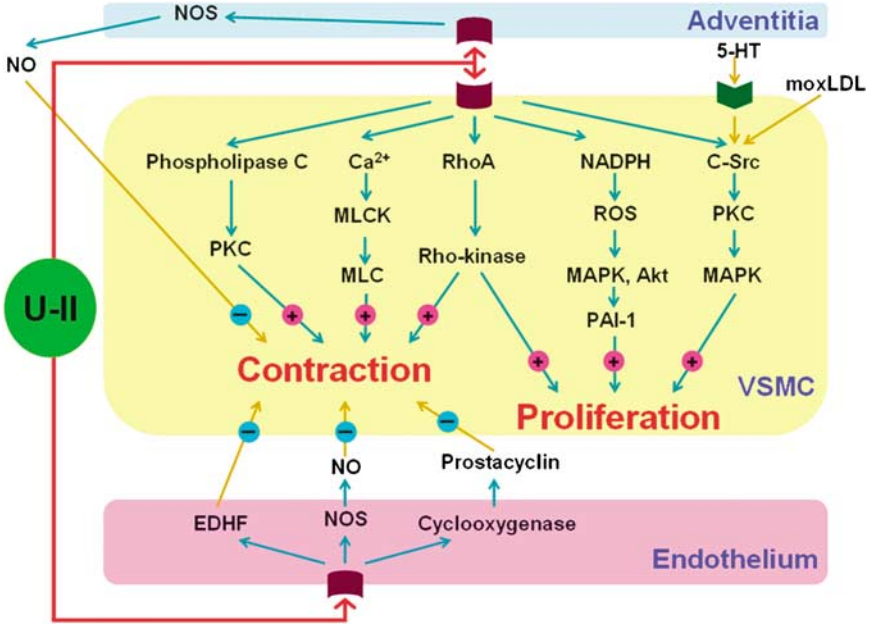


Figure 3 Schematic illustration of the suggested intracellular signalling mechanisms in VSMCs and the interaction between U-II and other vasoactive molecules.U-II, urotensin II; VSMCs, vascular smooth muscle cells; PKC, protein kinase C; MLC, myosin light chain; MLCK, myosin light-chain kinase; NADPH, nicotinamide adenosine dinucleotide phosphate; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; Akt, protein kinase B; PAI-1, plasma plasminogen activator inhibitor-1; 5-HT, serotonin; moxLDL, mildly oxidized LDL; EDHF, endothelium-derived hyperpolarizing factor; NOS, nitric oxide synthase; NO, nitric oxide.

Interaction between U-II and other endogenous vasoactive molecules

U-II-induced nitric oxide formation has been suggested to modulate the vasoactive effects of U-II in thoracic aorta (Bottrill *et al*., 2000), left anterior descending coronary arteries (Bottrill *et al*., 2000), perfused heart (Katano *et al*., 2000; Gray *et al*., 2001), and mesenteric and hindquarter vessels (Gardiner *et al*., 2004) of the rats. U-II induced the release of nitric oxide from the endothelium (Bottrill *et al*., 2000; Zhang *et al*., 2003) and adventitia (Lin *et al*., 2004a) of the arteries, which act antagonistically with U-II itself on the underlying smooth muscle to regulate vascular tone. Additionally, U-II has been suggested to trigger the release of prostacyclin (Gray *et al*., 2001; Gardiner *et al*., 2004) and endothelium-derived hyperpolarizing factor (Bottrill *et al*., 2000), which similarly relaxes blood vessels and hence opposes the contractile effect of U-II.

Additionally, U-II may also interact with other factors in regulating cell growth. In cultured VSMCs, U-II interacted synergistically with 5-HT (Watanabe *et al*., 2001b) and mildly oxidized LDL (moxLDL) (Watanabe *et al*., 2001a) in inducing VSMC proliferation *via* the c-Src/PKC/MAPK pathway. VSMC proliferation contributes to the pathogenesis of atherosclerosis and hypertension, while moxLDL is a well-established risk factor for atherosclerosis (Watanabe *et al*., 2001a). On the other hand, 5-HT plays a pivotal in pulmonary hypertension (Doggrell 2003). Therefore, the mitogenic effect of U-II on VSMCs may be significantly potentiated in diseases such atherosclerosis and pulmonary hypertension, suggesting a potential role for U-II in the pathogenesis of these diseases.

Interaction between U-II and other vasoactive factors may contribute to the complex anatomical location-, species-, and disease status-dependent vasoactive effects of U-II. The U-II-related interaction in the vessels is illustrated in Figure 3.

Conclusions

In summary, U-II and its receptor are abundantly distributed in cardiovascular and renal tissues in mammals including man. Changes in the expression levels of U-II and its receptor are apparent in subjects suffering cardiovascular and renal diseases. The vasoactive effects of U-II seem to be dependent on the calibre of the vessels, species, and the interaction with other vasoactive molecules released under various (patho)physiological conditions. Generation of nonpeptide U-II receptor antagonist may help to elucidate the role of U-II in the control of cardiovascular and renal homeostasis. Moreover, exploration for the putative new receptors for U-II may help to clarify the complex cardiovascular effects of U-II as well as to develop specific antagonists.

This work was supported by a grant from the National Natural Science Foundation of China (30470628).

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(Received December 29, 2005
Revised March 31, 2006
Accepted May 4, 2006
Published online 19 June 2006)