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Orphan-receptor ligand human urotensin II: receptor localization in human tissues and comparison of vasoconstrictor responses with endothelin-1

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1 We have determined the distribution of receptors for human urotensin-II (U-II) in human and rat CNS and peripheral tissues.

2 In rat, [^{125}I]-U-II binding density was highest in the abducens nucleus of brainstem (139.6 ± 14 amol mm^{-2}). Moderate levels were detected in dorsal horn of spinal cord and lower levels in aorta (22.5 ± 6 amol mm^{-2}).

3 In human tissues density was highest in skeletal muscle and cerebral cortex (~ 30 amol mm^{-2}), with lower levels (< 15 amol mm^{-2}) in kidney cortex and left ventricle. Little binding was identified in atria, conducting system of the heart and lung parenchyma.

4 Receptor density was less in human coronary artery smooth muscle (14.6 ± 3 amol mm^{-2}, $n = 10$) than rat aorta with no significant difference between normal and atherosclerotic vessels.

5 In human skeletal muscle [^{125}I]-U-II bound to a single receptor population with $K_D = 0.24 \pm 0.17$ nM and $B_{max} = 1.97 \pm 1.1$ fmol mg^{-1} protein ($n = 4$).

6 U-II contracted human coronary, mammary and radial arteries, saphenous and umbilical veins with sub-nanomolar EC_{50} values. U-II was 50 times more potent in arteries and < 10 times more potent in veins than endothelin-1 (ET-1). The maximum response to U-II ($\sim 20\%$ of control KCl) was significantly less than to ET-1 ($\sim 80\%$ KCl). In contrast, in rat aorta, U-II and ET-1 were equipotent with similar maximum responses.

7 This is the first report of high affinity receptors for [^{125}I]-U-II in human CNS and peripheral tissues. This peptide produces potent, low efficacy, vasoconstriction in human arteries and veins. These data suggest a potential role for U-II in human physiology.

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Keywords: Urotensin-II; [^{125}I]-U-II; orphan receptor; human; cardiovascular tissue; human brain; radioligand binding; vasoconstriction; *in vitro* pharmacology

Abbreviations: DCM, dilated cardiomyopathy; ET-1, endothelin-1; GPCR, G-protein coupled receptor; IHD, ischaemic heart disease; [^{125}I]-U-II, monoiodinated urotensin-II; U-II, urotensin-II; UT-II, human urotensin-II receptor

Introduction

Urotensin II (U-II) was characterized over 30 years ago as one of several peptides present in the urophysis of teleost fish (see Bern *et al*., 1995). This dodecapeptide originally isolated and sequenced from goby *Gillichthys mirabilis* (Pearson *et al*., 1980), shares some structural homology with somatostatin-14. Several forms of U-II have been identified in species of teleost, but all contain a conserved hexapeptide cyclic sequence within the C-terminal region that confers the majority of the biological activity (Itoh *et al*., 1987). In teleosts U-II produces general smooth muscle contraction, including vasoconstriction, plays a role in osmoregulation and acts as a prolactin inhibitory factor (see Bern *et al*., 1995).

Effects of fish U-II, usually goby, were subsequently reported in mammalian systems. Initial observations were of relaxation of the mouse anococcygeus muscle (Gibson *et al*., 1984) but in rat isolated blood vessels both endothelium-dependent vasorelaxation and endothelium-independent vasoconstriction have been detected (Gibson, 1987). Responses appear to be variable and highly dependent on vascular bed, although robust contraction to U-II was obtained in rat thoracic aorta (Itoh *et al*., 1987; 1988; Ames *et al*., 1999). Despite the ability to contract some arteries, infusion of goby U-II into rats produced a reduction in arterial blood pressure, with heart rate unaffected (Gibson *et al*., 1986; Hasegawa *et al*., 1992). Given the effects of fish U-II on mammalian tissues, it is not surprising that U-II was isolated and sequenced from frog brain (Conlon *et al*., 1992) and subsequently cDNAs were characterized encoding frog (Coulouarn *et al*., 1998), mouse, rat (Coulouarn *et al*., 1999) and human (Coulouarn *et al*., 1998) U-II precursors.

The human homologue of fish U-II (Figure 1) is an eleven amino acid peptide (Coulouarn *et al*., 1998; Ames *et al*., 1999) that retains the highly conserved cyclic region of fish and frog homologues (see Davenport & Maguire, 2000). Using dot blot analysis of human tissues, the highest level of pre-pro-U-II mRNA expression was identified in spinal cord and medulla oblongata, with *in situ* hybridization studies implicating a sub-population of motor neurones and motor nuclei of the brainstem, including the abducens nucleus. In agreement with the molecular studies U-II-like immunoreactivity localized to motor neurones in the spinal cord of several species (Coulouarn *et al*., 1998; Ames *et al*., 1999) suggesting that U-II may modulate skeletal muscle activity

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and the receptor would be expected to be expressed in this tissue. Lower levels of mRNA were also detected in a number of peripheral tissues, including kidney (Coulouarn *et al*., 1998, Nothacker *et al*., 1999). The receptor mediating the actions of U-II was unknown, but was possibly one of the many orphan G-protein coupled receptors (GPCR) so far identified (see Marchese *et al*., 1999). These included rat GPR14 (SENR) which had some sequence homology with the somatostatin sst_4 and μ-opioid receptors (Marchese *et al*., 1995; Tal *et al*., 1995). Recent reports, published within weeks of each other, have identified human U-II as a ligand for GPR14 (Ames *et al*., 1999; Liu *et al*., 1999; Mori *et al*., 1999; Nothacker *et al*., 1999). Ames *et al*., (1999) additionally identified a human GPCR with 75% identity to rat GPR14 which when expressed in HEK-293 cells in a calcium-mobilization assay, was activated by human U-II. Functional data from this group in isolated blood vessels from the cynomolgous monkey were remarkable. Human U-II was 10 times more potent than endothelin-1 (ET-1) with an extremely slowly developing response. However, although U-II contracted all arteries tested with sub-nanomolar EC_{50} values, no response was obtained from venous tissues. The effects of systemic administration of U-II in the anaesthetized monkey were dramatic, resulting in markedly increased total peripheral resistance, reduced myocardial contractility and at the highest concentration, death. The changes in ECG in these animals were reminiscent of those that occur during myocardial ischaemia.

U-II clearly has potent cardiovascular effects in non-human primates but there is only one report of U-II effects in human vasculature. MacLean *et al*. (2000) have recently observed variable responses of human small pulmonary arteries to U-II, only in the presence of the NOS inhibitor L-NAME. The receptor distribution for U-II in human tissues has not been characterized. We have therefore localized binding sites for the novel radioligand human [^{125}I]-U-II in human and rat tissues where molecular studies have predicted the human UT-II and GPR14 receptors are expressed. We have quantified the ability of human U-II to constrict human arteries and veins from a range of vascular beds and compared this to rat aorta that is known to respond to human U-II (Nothacker *et al*., 1999). The magnitude of these responses was compared to endothelin-1 (Yanagisawa *et al*., 1988) the most potent vasoconstrictor of human blood vessels yet described (Maguire & Davenport, 1995).

Preliminary data have been presented to the British Pharmacological Society (Kuc *et al*., 2000, in press; Maguire & Davenport, 2000, in press).

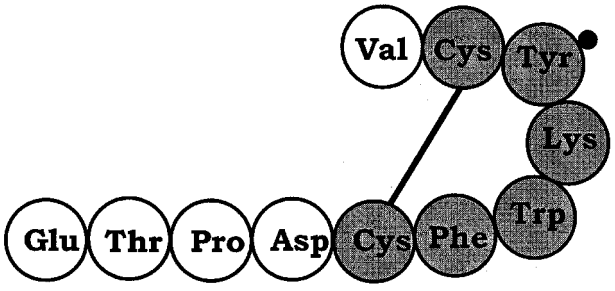

Figure 1 Deduced amino acid sequence of human U-II indicating the position of the iodinated tyrosine residue. Shaded residues are those that are conserved across all known species homologues of the peptide.

Methods

Tissue collection

Human coronary artery, left ventricle and right atria were obtained from 25 patients transplanted for dilated cardiomyopathy (DCM), ischaemic heart disease (IHD) or whose hearts were not suitable for further transplantation (normal). Saphenous vein, mammary artery and radial artery were from 18 patients receiving coronary artery by-pass grafts. Skeletal muscle was removed with the mammary artery during these operations. Umbilical veins were from four cords obtained following normal deliveries. Histologically normal sections of kidney were obtained from four patients undergoing nephrectomy for non-obstructive carcinoma. Macroscopically normal cerebral cortex was obtained from three patients treated surgically for gliomas or epilepsy. Normal lung tissue was from three patients undergoing lobectomy for carcinoma. All human tissues were collected with local ethical committee approval.

Rat tissues (thoracic aorta, spinal cord and brain) were from seven female Sprague-Dawley rats (300–350 g). Animals were killed by an overdose of sodium pentobarbitone followed by exsanguination.

Saturation analysis of human [^{125}I]-urotensin II binding

Saturation binding experiments were carried out on cryostat-cut sections (20 μm) of normal human skeletal muscle. Sections were pre-incubated for 1 h in 20 mM Tris-HCl buffer, pH 7.4, containing 5 mM $MgCl_2$ and 0.2% BSA and then incubated with increasing concentrations (7.8 pM–4 nM) [^{125}I]-U-II for 1 h. Non-specific binding was determined using human U-II (1 μM). Sections were washed for 10 min in 50 mM Tris-HCl, pH 7.4 at 4°C, and counted for radioactive content. Data were analysed using the iterative, non-linear curve-fitting programmes EBDA and LIGAND (Munson & Rodbard, 1980) in the KELL package (Elsevier Biosoft, Cambridge, U.K.) to determine values of equilibrium dissociation constant (K_D) and receptor density (B_{max}). Protein concentration/tissue section was determined using a DC protein assay kit (Bio-Rad, Herts, U.K.).

Autoradiographical visualization of [^{125}I]-urotensin II binding

Autoradiography was carried out using cryostat-cut sections of rat thoracic aorta, spinal cord and brain and human tissues including coronary artery, left ventricle, right atria, kidney, cerebral cortex and skeletal muscle. Sections were pre-incubated as described above and then incubated with 0.25 nM [^{125}I]-U-II for 1 h. Dried sections were apposed, with standards, to radiation sensitive film for 3 days. The resulting autoradiograms were analysed using computer-assisted densitometry (Davenport *et al*, 1995). Binding densities (amol $mm^{-2} \pm$ s.e.mean) were compared using Student's 2-tailed *t*-test or one-way analysis of variance as appropriate using Minitab statistical package (Minitab Inc., PA, U.S.A.). The level of significance was set at 5% ($P<0.05$).

In vitro *pharmacology*

Human and rat arteries and veins were cleaned of connective tissue, cut into 4-mm rings and their luminal surface rubbed gently with a metal seeker to remove the endothelium (confirmed by the absence of staining with rabbit antiserum

to human von Willebrand factor as previously described (Davenport *et al*., 1996)). Vessels were set up for isometric force recordings in 5-ml organ baths containing oxygenated Kreb's solution (37°C) and allowed to equilibrate for 1 h. Contractile responses were then recorded to 100 mM KCl at incrementally increasing levels of basal tension until no further increase in KCl response was obtained. This determined the optimum resting tension for each preparation and was followed by a further 30 min equilibration period. Cumulative concentration response curves were then constructed to ET-1 ($10^{-10}-3\times10^{-7}$ M) or to U-II ($10^{-13}-10^{-7}$ M). In some experiments, ET-1 ($1-3\times10^{-7}$ M) was added at the end of the U-II curve and then all experiments were terminated by addition of 100 mM KCl to determine the maximum possible response for each preparation. Agonist responses were subsequently expressed as a percentage of this KCl maximum.

Data were analysed using the iterative curve-fitting programme Fig P (Biosoft, Cambridge, U.K.) to give values of pD_2 (negative $\log_{10}$ of the molar concentration producing 50% of the maximum response) and E_{max} (maximum agonist response as a percentage of the terminal KCl response). All data were expressed as mean ± s.e.mean and *n* values refer to the number of patients or rats from whom tissue was obtained. pD_2 and E_{max} values were compared using Student's 2-tailed *t*-test with a significance value of 5% ($P<0.05$).

Modified Kreb's solution had the following composition (mM): NaCl, 90; KCl, 5; $MgSO_4.7H_2O$, 0.5; Na_2HPO_4, 1; $NaHCO_3$, 45; $CaCl_2$, 2.25; glucose, 10; Na pyruvate, 5; fumaric acid, 5; L-glutamic acid, 5.

Materials

Human urotensin-II (Glu-Thr-Pro-Asp-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val) and endothelin-1 were purchased from Peptide Institute (Osaka, Japan). Iodinated human urotensin-II (2000 Ci mmol^{-1}) was from Amersham Pharmacia Biotech (Amersham, U.K.). All other reagents were from standard commercial sources and of Analar grade.

Results

Radioligand saturation binding and autoradiography

Human tissues were selected for autoradiographical analysis based on the reported expression of mRNA encoding either U-II peptide or receptors. Using a fixed concentration of [^{125}I]-U-II the highest density of receptors in the peripheral tissues analysed was present in skeletal muscle (31.9 ± 9 amol mm^{-2}, $n=5$; Figure 2) and this was used for further characterization by saturation analysis. [^{125}I]-U-II bound with a single high affinity ($K_D=0.24\pm0.17$ nM, $n=4$) and a B_{max} of 1.97 ± 1.1 fmol mg^{-1} to sections of human skeletal muscle. Hill slopes were close to unity ($n_H=1.02\pm0.03$) and a one site fit was preferred over a two site model.

In human cardiovascular tissue, binding was detected to vessels, including the media (smooth muscle layer) of epicardial coronary arteries (14.6 ± 3 amol mm^{-2}, $n=10$) and to myocytes in the left ventricle (9.4 ± 4 amol mm^{-2}, $n=4$) (Figure 2). There was no difference in the density of medial U-II receptors between normal (14.2 ± 5 amol mm^{-2}, $n=4$), DCM (12.6 ± 4 amol mm^{-2}, $n=3$) and atherosclerotic IHD (16.9 ± 6 amol mm^{-2}, $n=3$) arteries. Little or no specific binding (< 5 amol mm^{-2}) was detectable in the atria or to the conducting system of the human heart. In other peripheral tissues low densities were detected in kidney cortex (14.5 ± 4 amol mm^{-2}, $n=3$), but no binding was detected in lung parenchyma although low levels localized to pulmonary vessels of varying diameter (consistent with the constrictor response reported by MacLean *et al*., 2000) and bronchioles present within the lung sections (< 20 amol mm^{-2}). Binding was below the level for detection in fat. In human cerebral cortex densities were comparable to the highest levels in the peripheral tissues (32.6 ± 13 amol mm^{-2}, $n=3$) (Figure 2). Quantitative autoradiography using rat tissues indicated low density of specific binding (22.5 ± 6 amol mm^{-2}, $n=5$) localized to smooth muscle of aorta. In neuronal tissues, moderate levels (85 ± 14 amol mm^{-2}) were found in dorsal root ganglion of spinal cord with highest binding density observed in the abducens nucleus of the brain (139.6 ± 14 amol mm^{-2}, $n=4$) (Figures 2 and 3).

In vitro *pharmacology*

In rat the response to U-II in aorta was confined to an approximately 2-cm segment of thoracic vessel proximal to the carotid bifurcation of the aortic arch. Consistent responses were obtained to U-II in the first four aortic rings (4 mm) taken from this region, with loss of maximum response in more distal segments (Figure 4). No response was obtained in abdominal aorta (data not shown). U-II contracted proximal thoracic aorta from six out of seven rats with a pD_2 value in responders of 8.87 ± 0.28 and an E_{max} of 68.25 ± 11.59% KCl 100 mM. These were not significantly different from data for ET-1; $pD_2=8.80\pm0.31$, $E_{max}=92.90\pm6.93$% KCl 100 mM ($n=6$) (Table 1; Figure 5A).

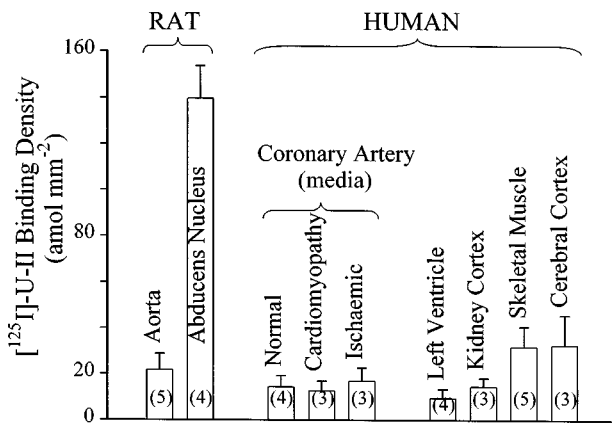

Figure 2 Relative densities of [^{125}I]-U-II binding determined autoradiographically in 20-μm sections of rat and human tissues. Data are mean ± s.e.mean. *n*-values are given in parentheses.

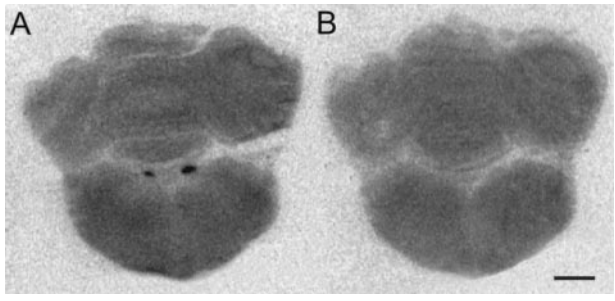

Figure 3 Autoradiographical localization of [^{125}I]-U-II binding to 20-μm coronal sections (Bregma −10.3) of rat brain showing (A) total binding to abducens nuclei and (B) non-specific binding in adjacent section. Scale bar = 2 mm.

All human blood vessels, arteries and veins, responded to ET-1 (Table 1). However, approximately 30% of coronary and mammary arteries were not contracted by any concentration of U-II tested, although ET-1 added after U-II contracted these vessels. For human arteries U-II was approximately 50 times more potent than ET-1, but because responses to U-II were very variable between individuals this difference only reached significance for coronary artery. In contrast the maximum response to U-II (approximately 20% of KCl response) was significantly lower than that achieved by ET-1 (approximately 80%) in all arteries tested (Figure 5B). The significant difference in maximum response between ET-1 and U-II was also observed in preparations of human veins. However, compared to arteries there was less than a 10 fold increase in potency of U-II compared to ET-1, which did not reach significance in either venous preparation (Table 1).

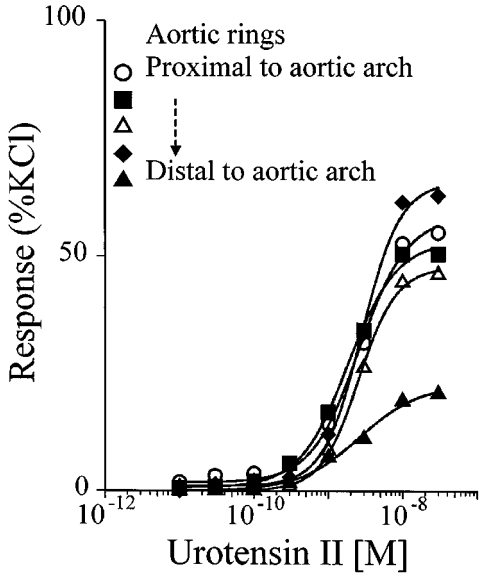

Figure 4 Example of response to U-II in consecutive 4-mm rings of rat thoracic aorta: effect of distance of aortic ring from carotid bifurcation on maximum U-II response.

Discussion

This is the first report of the distribution of [^{125}I]-U-II binding in human tissue. [^{125}I]-U-II binding was saturable to human skeletal muscle with an affinity constant in the sub-nanomolar range. Hill slopes were close to unity indicating that [^{125}I]-U-II bound with a single affinity, with no evidence for receptor subtypes. The K_D for human [^{125}I]-U-II, not previously determined in native human tissues, was within the range reported for goby [^{125}I]-U-II binding to rat cardiac membranes (0.35 nM) or expressed human UT-II receptors (0.43 nM; Ames *et al*., 1999) and human [^{125}I]-U-II binding to expressed rat GPR14 receptors (0.07 nM, Nothacker *et al*., 1999), under comparable binding conditions. The maximum density of receptors measured in human skeletal muscle using human [^{125}I]-U-II was similar to that reported for goby [^{125}I]-U-II in rat arteries or cardiac membranes, 2–20 fmol mg^{-1} (Itoh *et al*., 1988;

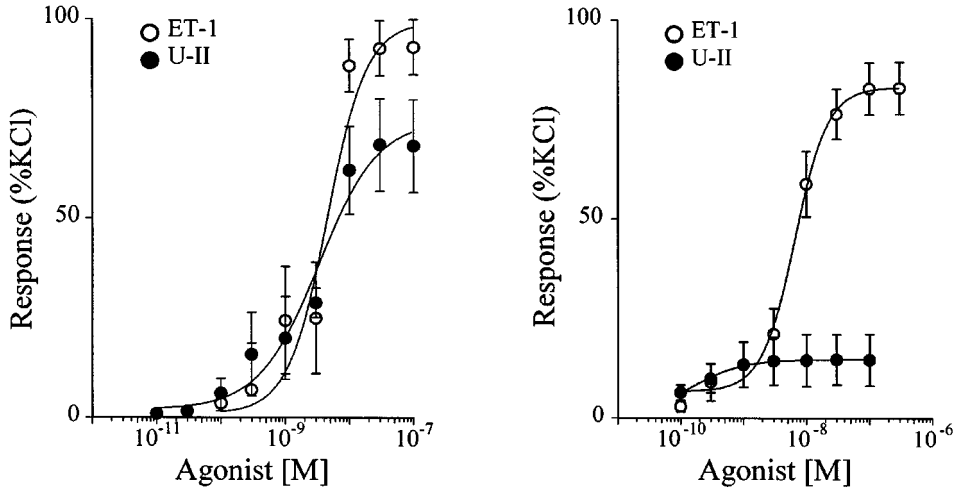

Figure 5 (A) Concentration-response curves to ET-1 and U-II in endothelium-denuded rat thoracic aorta. Data points are mean±s.e.mean, $n=6$. (B) Concentration-response curves to ET-1 ($n=9$) and U-II ($n=6$) in endothelium-denuded human coronary artery as an example of the relative response of the two peptides in human arteries and veins. Data points are mean±s.e.mean.

Table 1 Comparison of vasoconstrictor activity of endothelin-1 and urotensin-II in rat aorta and human arteries and veins

	Endothelin-1			*Urotension-II*		
	pD_2	E_{max} (% KCl)	n	pD_2	E_{max} (% KCl)	n
Rat aorta	8.80±0.31	92.90±6.93	6	8.87±0.28	68.25±11.59	6/7*
Human coronary artery	8.35±0.13	83.78±6.02	9	10.05±0.46‡	15.39±6.53‡	6/9*
Human mammary artery	8.14±0.15	82.11±6.90	6	9.71±0.90	16.41±6.15‡	5/7*
Human radial artery	7.89±0.04	57.47±10.14	4	9.52±0.83	19.65±6.26†	4
Human saphenous vein	8.49±0.40	94.83±3.42	3	9.43±0.57	31.48±9.54‡	5
Human umbilical vein	8.41±0.38	82.55±8.24	3	9.29±0.40	16.61±9.99‡	4

*x/n; n=number of animals or individuals, x=number responding to U-II. Only data from responders included in table. Significantly different from ET-1 control: †$P<0.05$ ‡$P<0.005$ Student's 2-tailed t-test.

Ames *et al*., 1999). These densities correspond to B_{max} values in human arteries for other vasoactive peptides such as angiotensin II (Wharton *et al*., 1998), thromboxane A_2 (Katugampola & Davenport, 2000, in press) and ET-1 (Davenport *et al*., 1995; Bacon *et al*., 1996). Our data are therefore consistent with [^{125}I]-U-II binding to human UT-II receptor, the human homologue of rat GPR14.

Ames and colleagues (1999) have recently deduced the sequence of the human isoform of the rat GPR14 receptor. This human UT-II receptor (Davenport & Maguire, 2000) comprises 389 amino acids. Previous reports suggest that human UT-II receptor mRNA can be detected in tissue homogenates of human heart (cardiac myocytes in atria and ventricle) (Ames *et al*., 1999; Liu *et al*., 1999), skeletal muscle and bladder (Liu *et al*., 1999), pancreas, brain, arterial (but not venous) smooth muscle and endothelial cells (Ames *et al*., 1999). In agreement with these findings we detected highest levels of human [^{125}I]-U-II binding in human skeletal muscle, compatible with the proposed distribution of U-II in motorneurones of the spinal cord. Although some binding was detected in human cerebral cortex it was not possible to investigate if motor nuclei of human brain express very high densities of receptors as seen in the rat abducens nucleus. Consistent with predictions from dot blot analysis we also localized low levels of receptors to coronary artery smooth muscle and kidney cortex. However we found very low levels of receptor expression in human left ventricle and could not detect specific binding in atria and conducting system of the human heart. This finding is perhaps not unexpected as Ames group used quantitative RT–PCR to detect the low levels of transcript present in human cardiovascular tissues. Our observation of binding of human [^{125}I]-U-II to rat aorta, spinal cord and abducens nucleus is also consistent with previous reports of a neural and sensory localization of this receptor in rat (Tal *et al*., 1995; Liu *et al*., 1999; Nothacker *et al*., 1999).

In both rat and human nervous system prepro-U-II mRNA was consistently identified in spinal cord and motor nuclei (including the abducens nucleus) of the medulla oblongata (Coulouarn *et al*., 1998; 1999; Ames *et al*., 1999; Nothacker *et al*., 1999). However the source of U-II peptide interacting with the UT-II receptor in peripheral tissues is unclear and there is some discrepancy between molecular studies. Whilst Ames and colleagues (1999) reported U-II-like immunoreactivity in the human vasculature, with diffuse staining in the heart, mRNA encoding the peptide was not detected in homogenates of human tissue, including heart and aorta, by dot blot analysis. Nothacker *et al*. (1999) using similar techniques, also failed to detect message in most of the peripheral tissues they examined although they found high expression of human prepro-U-II mRNA in human kidney. Coulouarn *et al*. (1998) also reported highest expression of U-II mRNA in human spinal cord and medulla oblongata but found a more widespread distribution (by dot blot analysis) of human prepro-U-II mRNA in peripheral tissues, including the adrenal glands, kidney and spleen, which may be suggestive of some local synthesis and release. However, it is possible that mRNA may be below the level for detection or the peptide may circulate in the plasma and is synthesized some distance from target organs.

It has been known for some time that fish U-II will contract rat arteries, particularly the thoracic aorta. We used this preparation as a control to compare the constrictor activity of human U-II to the potent vasoconstrictor ET-1 (Yanagisawa *et al*., 1988; Maguire & Davenport, 1995). We found that U-II and ET-1 were equipotent, with EC_{50} values of approximately 1.5 nM. This is consistent with that observed for human U-II in rat aorta of 2.4 nM reported by Nothacker and colleagues (1999). The maximum response to ET-1 was greater than that to U-II, but due to the greater variability of the U-II data this difference was not significant. Indeed one rat aorta failed to respond to U-II and we observed that only vessel rings removed from the portion of thoracic aorta proximal to the carotid bifurcation responded well, with a drastic reduction in efficacy seen in more distal tissue (Figure 4). As reported by others we found no response to U-II in rat abdominal aorta. As predicted by the lower receptor density in human coronary artery media compared to rat aorta the responses to U-II in human blood vessels were less robust. In human coronary, mammary and radial arteries U-II was approximately 50 times more potent than ET-1, however whereas all arteries contracted to ET-1 approximately 30% did not respond to U-II. This is likely to be due to the low receptor density and the greater variability of human compared to rat tissues. The low receptor density perhaps also may explain the low efficacy of U-II compared to ET-1 in these blood vessels. Ames *et al*. (1999) also reported U-II to be more potent than ET-1 in monkey arteries. However they found a very clear restriction of U-II-mediated vasoconstriction to arterial vessels with no response in venous tissue. In contrast we found that human U-II contracted human saphenous and umbilical vein and although all veins responded (in contrast to the arterial preparations), U-II was less than 10 times more potent than ET-1 in these vessels and the maximum response to U-II was again significantly attenuated compared to ET-1. Therefore in human blood vessels we find that human U-II is extremely potent, but has low efficacy which may reflect a low density of high affinity receptors localized to the vascular smooth muscle. The effect of U-II on human coronary artery is much less dramatic than that observed in monkey and it is possible therefore that the spectacular cardiac collapse elicited by U-II *in vivo* in this animal (Ames *et al*., 1999) would not be observed in man. Interestingly U-II-like immunoreactivity was reportedly present in atherosclerotic human coronary artery (Ames *et al*., 1999) suggesting the peptide may have a potential role in human cardiovascular disease. In our investigations we could detect no alteration in the density [^{125}I]-U-II binding in the media of healthy compared to atherosclerotic epicardial coronary arteries. This does not however preclude an increase in the synthesis of the peptide in this disease.

It has been reported that in human pulmonary artery *in vitro* constrictor responses to human U-II were not observed in vessels with an intact endothelium but were observed in 3/10 adjacent rings treated with the NOS inhibitor L-NAME (MacLean *et al*., 2000). These authors suggested that U-II-mediated release of NO may physiologically antagonize its direct vasoconstrictor activity and that U-II constrictor activity may be of greater relevance in pathophysiological conditions in which endothelial dysfunction occurs. The more robust response to U-II observed in our preparations may reflect the absence of endothelium in these experiments and certainly endothelium-dependent relaxation to fish U-II has previously been reported in mammalian vascular smooth muscle (Gibson *et al*., 1987). However, the relative importance of human U-II as constrictor or dilator of the human vasculature and whether this is altered in disease remains to be determined.

In conclusion this is the first report of the localization of high affinity binding sites for the novel radioligand human [^{125}I]-U-II in human and rat tissues. The receptor for this ligand was present in human arteries and veins, left ventricle, kidney cortex, skeletal muscle and cerebral cortex. Receptor density in these tissues was comparable to that in rat aorta but lower than detected in rat spinal cord and abducens nuclei of the rat brainstem. It will be of interest if high receptor densities

localize to these same regions in man. In functional assays we find that U-II potently constricts both human arteries and veins suggesting that this peptide may behave as a ubiquitous vasoconstrictor, similar to ET-1. However, while U-II was more potent than ET-1, the magnitude of response to U-II was considerably less than this peptide. Intriguingly, greater densities of UT-II receptors were found in tissues such as human skeletal muscle with highest densities in discrete regions of rat brain and spinal cord suggesting that the peptide may have other and perhaps more important physiological roles in the periphery and central nervous system.

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