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ORIGINAL ARTICLE

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Effects of human urotensin II in isolated vessels of various species; comparison with other vasoactive agents

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Abstract Urotensin II is a cyclic undecapeptide which activates the GPR14 receptor and exerts potent vasoconstrictor effects in some species of fish and mammals.

The present study intended to investigate isolated vessels from various species in an attempt to find sensitive preparations to be used in studies of the human urotensin (hU-II)/GPR14 system.

Contractile responses evoked by noradrenaline (NA), angiotensin II (Ang II), endothelin 1 (ET-1) and hU-II were measured in large vessels (aorta and some large arteries and veins) of rats, guinea pigs, rabbits, pigs and humans. Relaxing effects of hU-II, bradykinin (BK) and substance P (SP) were measured in pig coronary arteries contracted with KCl 30 mM. The rat mesenteric vasculature was investigated from the arterial and venous site to establish the function of ET-1 and hU-II receptors. Results indicate that the only preparation showing high sensitivity to hU-II (pEC_{50}=8.27) is the rat aorta, whose contractions in response to hU-II develop slowly and persist for hours, similar to those of ET-1 (pEC_{50}=8.35). Effects of NA (pEC_{50}= 8.12) and Ang II (pEC_{50}=7.95) develop and reverse more rapidly. Tissues treated with ET-1 and hU-II show marked desensitization, in contrast to those treated with NA. Specific antagonists for α_1 (prazosin, pA_2=10.46), AT_1 (EXP 3174, pA_2=10.20), $5HT_2$ (ketanserine, pA_2=8.61) and ET_A-ET_B (bosentan, pA_2=6.88) receptors were shown to block the effects of the respective agonists, while being inactive against hU-II. In some vessels, hU-II behaved as an highly potent but scarcely effective contractile agent. It is concluded that: the hU-II/GPR14 is not a functional contractile system in vessels of several species, in contrast with NA/α_1, Ang II/AT_1, 5HT/$5HT_2$ and ET-1/ET_A-ET_B. The rat aorta appears however to be a sensitive and reliable preparation for evaluating biological activities of hU-II and related peptides.

Keywords Human urotensin II · Noradrenaline · Endothelin-1 · Angiotensin II · Isolated vessels · Rat aorta · Antagonists

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Introduction

In early studies, the Lederis group reported the amino acid composition of urotensin I (U-I) and urotensin II (U-II) from lower species and described the smooth muscle contractile as well as the osmoregulatory actions of these peptides. In 1980, a cyclic peptide (goby U-II) was isolated from the urophysis of the teleost fish (Pearson et al. 1980) and shown to contract mammalian vessels (Itoh et al. 1988). The human peptide (hU-II) was recently cloned (Coulouarn et al. 1998) and shown to be a cyclic peptide with the following primary structure:

$Glu^1 - Thr^2 - Pro^3 - Asp^4 - Cys^5 - Phe^6 - Trp^7 -$
$Lys^8 - Tyr^9 - Cys^{10} - Val^{11}$

Rat, mouse and porcine isoforms of U-II have also been cloned (Coulouarn et al. 1999; Mori et al. 1999). The cyclic region of the peptide (Cys^5-Cys^{10}) is fully conserved among different species suggesting that it might be critical for biological activity.

A G-protein-coupled receptor, named GPR14, predominantly expressed in the cerebellum, the cardiovascular system (heart, aorta, coronary artery, endothelia), and the kidney has been found to mediate the biological effects

(vasoconstriction, vasodilatation, depression of heart functions) of hU-II (Ames et al. 1999; Liu et al. 1999; Mori et al. 1999; Nothacker et al. 1999). Despite its similarities to somatostatin and opioid receptors (Marchese et al. 1995; Tal et al. 1995), GPR14 selectively binds hU-II and promotes Ca^{2+} mobilisation from CHO cells (Ames et al. 1999). U-II is particularly active in arteries from primates and the rat as a strong contractile agent, and is considered to be a long-acting vasoconstrictor, as potent as endothelin-1 (Ames et al. 1999). It also contracts primate airways (especially small bronchi) but is a vasodilator of the rat coronary and mesenteric districts (Bottrill et al. 2000; Douglas et al. 2000; Hay et al. 2000), and of human pulmonary and mesenteric arteries (Stirrat et al. 2001).

In vivo, hU-II markedly increases total peripheral resistance in anaesthetised non-human primates, in association with a profound cardiac contractile dysfunction (Ames et al. 1999). Katano and colleagues (2000) have also demonstrated, in rats and mice, that hU-II transiently decreases coronary flow, and causes sustained vasodilatation: these effects are attenuated by N^G-nitro-L-arginine, a nitric oxide synthase inhibitor.

The present study was focused on the myotropic effects of hU-II on vascular preparations (arteries and veins) from different species and several vascular districts. The effects of hU-II were systematically compared to those of NA, Ang II and ET-1. Experiments were carried out on vascular preparations of rats, rabbits, pigs, and guinea pigs and on a few human vessels. The major purpose was to find out if this new peptide, like other vasoconstrictors, finds functional sites in arterial and venous smooth muscle cells.

Human urotensin II was also tested in vitro in the perfused arterial and venous mesenteric beds of the rat prepared according to Warner (1990), to establish if and how it is active in resistance and capacitance vessels.

Materials and methods

Tissue preparation. Male New Zealand white rabbits (from Morini, Reggio Emilia, Italy) weighing 1.8–2.3 kg were killed by stunning and exanguination. The vessels used were the jugular vein (rbJV), thoracic aorta (rbA), pulmonary (rbPA), renal (rbRA), iliac (rbIA), femoral (rbFA), and carotid (rbCA) arteries, the saphenous vein (rbSV), mesenteric artery (rbMA) and superior vein cava (rbSVC). Each vessel was quickly removed and cut either in helical strips about 15–20 mm long and 3 mm wide (rbRA, rbIA, rbFA, rbSV), or in rings (rbJV, rbCA, rbMA), or in open rings 4–6 mm wide (rbA, rbPA, rbSVC), according to Furchgott and Bhadrakom (1953), Gaudreau et al. (1981), and Gobeil et al. (1996). Porcine heart, kidney, spleen and lung were obtained from adult pigs (weighing 160 kg) from the Negrini slaughterhouse (Salara, Rovigo, Italy) within 30 min of death and immediately immersed in cold (4°C) Krebs solution. In the laboratory, the left anterior descending coronary artery (pCA), lienal artery (pLA), pulmonary artery (pPA), renal artery (pRA) and lienal vein (pLV) were cut in helical strips; renal vein (pRV) was cut in open rings. Thoracic aorta (rA) as well as the mesentery (rMes) were taken from male Sprague-Dawley rats (Morini, Reggio Emilia, Italy and Charles River, Montreal, Canada) decapitated under ether anaesthesia. Vessels were cleaned of fat and connective tissues and cut in rings. Thoracic aorta (gpA), pulmonary artery (gpPA), inferior vena cava (gpIVC) and portal vein (gpPV) were rapidly dissected out from Dunkin-Hartley guinea pigs (200–250 g; from Morini, Reggio Emilia, Italy) decapitated under ether anaesthesia. The vessels were cut in rings (rA, gpPV, gpCV), open rings (gpPA) or strips (gpA).

Human umbilical vessels were obtained from women 23–40 years old after spontaneous delivery at term. They were used after storage in cold Krebs (4°C) solution for no longer than 12 h. The middle segment of the cord was cut and placed in petri dishes containing Krebs solution at room temperature, and within 30 min the human umbilical vein (hUV) and human umbilical artery (hUA) were dissected free of surrounding tissue. The arteries were cut into ring segments and the vein into helical strips. Healthy and pathological saphenous vein (hSV), splenic artery (hSA), inferior mesenteric artery (hMA), facial vein (hFV), epigastric vessels (hEV) and renal vein (hRV) were obtained from the Department of Surgery of our Medical School. All these vessels were cut into helical strips.

The endothelium was mechanically removed from all vessels, with the only exception of some pCA tissues, by gently rubbing the internal surface with moistened filter paper. All tissues were suspended in organ baths containing oxygenated (95% O_2 and 5% CO_2) Krebs solution of the following composition (in mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, $KHPO_4$ 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.2, glucose 10. The solution was maintained at 37°C and at pH 7.4. The tissues were stretched to a resting tension of 0.5 g for rbRA, rbSV, gpCV, gpPV, 0.7 g for gpA, gpPA, gpCV, 2 g for pCA, pLA, pRA, hUV, hMA, hAS, hRV, 4 g for hUA or 1 g for the other tissues. During the equilibration period (3 h for the hUV, hUA; 1.5 h for the rbJV, pCA and pLA; 1–1.5 h for the other tissues) fresh Krebs solution was applied and the tension readjusted at 20-min intervals. The contractions were converted in electrical signal by isometric transducers (Grass FT03) and recorded by a multichannel poligraph (Linseis L2005).

Rat mesenteries were prepared with the method of Warner (1990). Rats were killed by stunning and exanguination: the mesenteric artery and portal vein were cannulated with Portex (3FG) tubes, and warm (37°C), oxygenated Krebs solution (see above) containing heparin (100 units/ml) was perfused through the artery at 2 ml/min for 5 min to remove blood. The mesentery was then separated from intestine by cutting close to the intestinal border. Venous and arterial vasculatures were then perfused independently with Krebs (2 ml/min) containing indomethacin (5 μM) to block prostaglandin synthesis. In some preparations, L-NAME (100 μM) was applied to block the synthesis of NO. Changes of perfusion pressure induced by the agents were measured with a Statham transducer (P-23A) on a Grass recorder (7-D). hU-II and ET-1 were tested in this preparation.

Agents and drugs. hU-II was synthesised and purified by Dr. Remo Guerrini of the Department of Pharmaceutical Sciences of Ferrara University. The effects of this peptide were compared with a commercially available hU-II (Bachem, Switzerland) and with peptides supplied by S. Douglas, Smithkline Beecham Pharmaceuticals (USA) or by E. Kostenis, Aventis (Germany). Endothelin 1 was from Neosystem (Paris, France). Ketanserine (3-2-4-(4-fluorobenzoyl)-1-piperidinylethyl-2,41*H*,3*H*-quinazolinedione) was bought from Tocris (UK), bosentan (4-tert-butyl-*N*-[6-(2-hydroxyethoxy)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl]-benzenesulfonamide sodium salt) was from Roche (Switzerland). EXP 3174 (*n*-butyl-4-chloro-1-([2'-1*H*-tetrazol-5yl]biphenil-4-ylmethyl)imidazole-5-carboxylate) was made available by Aventis (Germany). Prazosin (1-[4-amino-6,7-dimethoxy-2-quinazolynil]-4-[2-furanylcarbonyl]-piperazine), acetylcholine, sodium nitroprusside, methoxamine, N^G-nitro-L-arginine and all other substances were from Sigma (USA).

Statistical analysis. The data are expressed as means ± standard error of the mean (SEM) of *n* experiments. Data were statistically analysed using Student's *t*-test for unpaired data or one-way ANOVA followed by Dunnett test for multiple comparison. The agonist potencies are given as pEC_{50}, which is the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect (E_{max}). The E_{max} is the maximal effect that an agonist can elicit in a given tissue and it is

expressed as grams of contraction (g) or as percent of the contraction induced by noradrenaline (NA) 1 μM. Antagonist potencies are expressed in terms of pA_2, calculated using the Gaddum-Schild equation: $pA_2=\log_{10}[(CR-1)/[Antagonist]]$.

Experimental protocols. Following the equilibration period, the preparations were exposed to high K^+ concentrations (30 mM for pCA and pLA and 100 mM for the other tissues), to assess the responsiveness of the preparation. The absence of functional endothelium was assessed with 1 μM acetylcholine (ACh) in tissues precontracted with 1 μM NA. About 30 min later, cumulative concentration-response curves (crc) to agonists were measured. Generally three crc to different agonists were tested consecutively within one preparation. In the rat aorta, 10 nM prazosin, 3 nM EXP 3174, 1 μM bosentan, 10 nM ketanserine or a solution containing all these antagonists were left in contact with the tissue for 20 min before measuring the crc to NA, Ang II, ET-1, 5-HT and hU-II in their presence, and the potencies of the antagonists were expressed in terms of pA_2. In some experiments performed in the rat aorta (summarized in Fig. 3), crc to hU-II and to the other agonists were repeated in the same tissue. Since the contractile effects of hU-II and ET-1 were scarcely reversible, several hours (generally 3–4 h) were necessary for the tissues to come back to the baseline. Then, 90 min after the tissue tension had come back to baseline a second crc to agonists was performed. As a whole, the period of time between the first and the second crc was about 5 h.

Results

Rat vessels

Rat aorta

Because of the high sensitivity of the rat aorta to hU-II documented in the literature (Bottrill et al. 2000; Douglas et al. 2000; Gibson 1987; Itoh et al. 1987, 1988), this tissue was given a great deal of attention. The contractile responses of the rat aorta to five agents, illustrated in Fig. 1, indicate that KCl and NA act rapidly and are quickly reversible: the contractions elicited by the two agents consist of an initial rapid phase (approximately 50% of the total) followed by a slow increase of tension which takes 15–20 min to reach its maximum. The contraction of the rA to Ang II cannot be separated into two phases and reaches the maximum in approximately 10 min: it is reversible more slowly than those of KCl and NA. The contractile effects of hU-II and ET-1 take 25–30 min to reach the maximum and are irreversible for several hours after

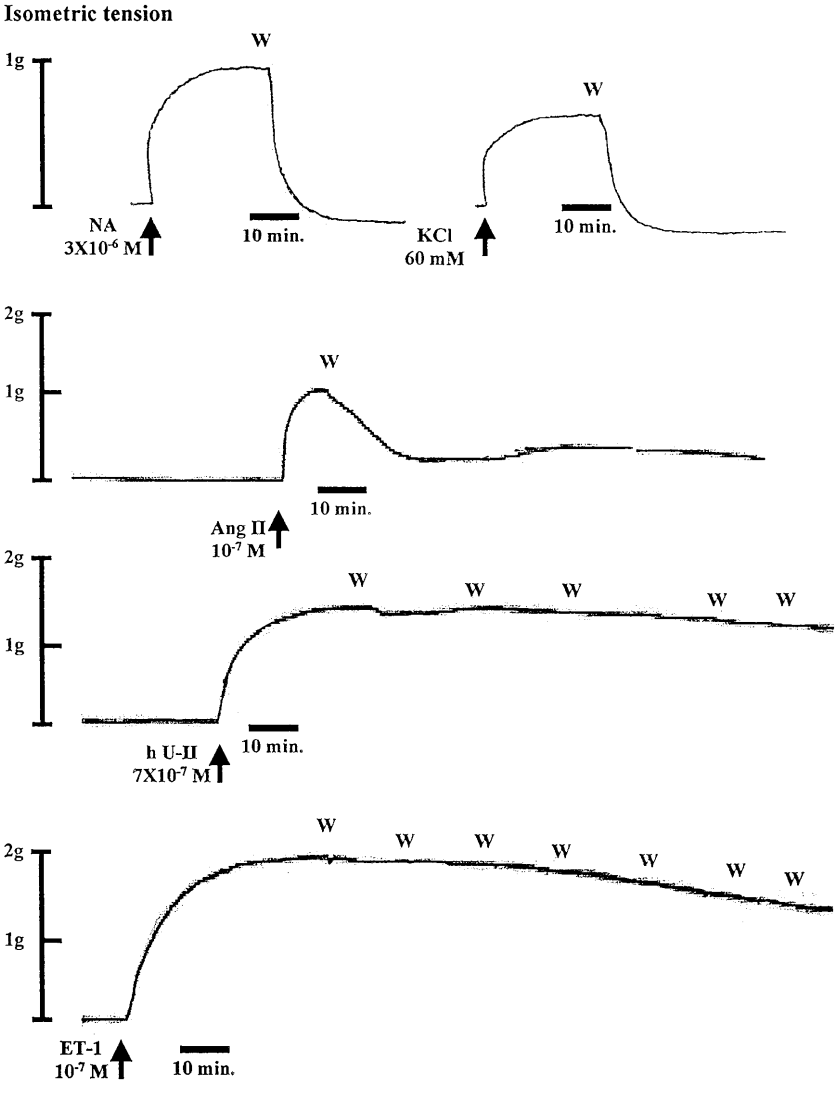

Fig. 1 *Tracings* showing the contractile effects of a single concentration (near maximal) of noradrenaline (*NA*, 3 μM), KCl (60 mM), angiotensin II (*Ang II*, 0.1 μM), human urotensin II (*hU-II*, 0.7 μM), and endothelin 1 (*ET-1*, 0.1 μM) in the rat thoracic aorta (*rA*). *Abscissa:* time in minutes (min). *Ordinate:* contraction in grams (g)

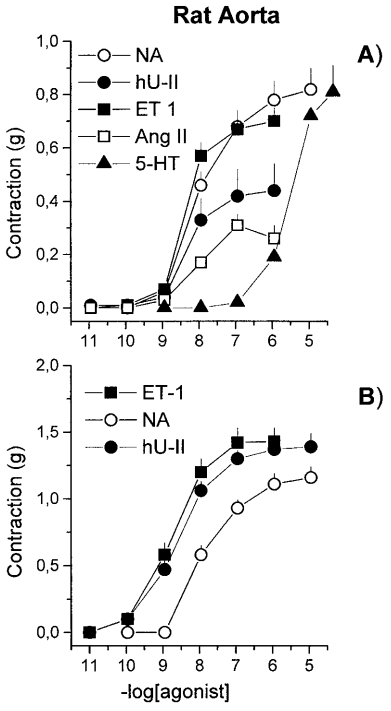

Fig. 2A,B Concentration-response curves (*crc*) obtained with noradrenaline (*NA*), endothelin 1 (*ET-1*), serotonin (*5-HT*), angiotensin II (*Ang II*) and human urotensin II (*hU-II*) in the rat thoracic aorta. **A** Data from Ferrara, Italy. **B** Data from Sherbrooke, Canada. *Abscissa:* –log of the molar concentration of agonist. *Ordinate:* contraction in grams (g). The data are means ± SEM of at least six experiments (2–4 tissues for each experiment)

Table 1 Comparison of the effects of noradrenaline, angiotensin II, endothelin 1, serotonin and human urotensin II, and their receptor antagonists in the rat thoracic aorta (pEC_{50} the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect, E_{max} the maximal effect that an agonist can elicit in a given tissue expressed as grams (g) of contraction, pA_2 antagonist-potency calculated using Gaddum-Schild equation). Cocktail of antagonist: prazosin 10 nM, bosentan 1000 nM, ketanserine 10 nM and EXP 3174 3 nM. The data are the means ± SEM of at least five experiments (2–4 tissues for each experiment)

	pEC_{50}	E_{max} (g)
Agonists from Ferrara, Italy		
hU-II	8.27±0.21	0.43±0.07
ET-1	8.35±0.12	0.69±0.04
NA	8.12±0.13	0.78±0.05
5-HT	5.54±0.24	0.83±0.14
Ang II	7.95±0.26	0.34±0.09
Agonists from Sherbrooke, Canada		
hU-II	8.81±0.17	1.39±0.10
ET-1	8.83±0.14	1.43±0.10
NA	7.94±0.13	1.18±0.07
Antagonists from Ferrara, Italy	pA_2	
Prazosin 10 nM vs. NA	10.46±0.28	
Bosentan 1000 nM vs. ET-1	6.88±0.21	
Ketanserine 10 nM vs. 5H-T	8.61±0.15	
EXP 3174 3 nM vs. Ang II	10.20±0.23	
Cocktail of antagonists vs. hU-II	Inactive	

washing, especially after high concentrations of the two agents.

Two sets of experiments were performed in the present investigation in two different laboratories. Results of experiments carried out in Ferrara (Italy) are illustrated in Fig. 2A and summarised in Table 1, experiments performed in Sherbrooke (Canada) are depicted in Fig. 2B and summarized in Table 1. The concentration-response curves (crc) of Fig. 2A and the pEC_{50} values of Table 1 indicate that the three most potent agonists (NA, ET-1 and hU-II) have very similar potencies (pEC_{50} values from 8.12 to 8.35) but their maximum effects differ: in fact, NA and ET-1 are equally active while the maximal contractions evoked by hU-II are significantly lower. Ang II displayed high potency (pEC_{50} about 7.95) similar to ET-1 or NA but significantly lower E_{max} (Table 1); complete crc were obtained with 5-HT, a contractile agent that showed low potency (pEC_{50} 5.54) but high maximal effects, similar to those of NA or ET-1. Experiments performed in Sherbrooke (Canada; see Fig. 2B and Table 1) show results somewhat different from the previous ones, since hU-II is as active (in terms of E_{max}) as ET-1; maximal contractions induced by the two peptides are higher than those of NA, the peptides being more potent (by almost 1 log unit) than the catecholamine.

In other experiments, crc to ET-1, hU-II and NA were measured twice in the same tissues at intervals of about 5 h. As illustrated in Fig. 3, tissues treated with ET-1 and hU-II were completely (ET-1) or markedly (hU-II) desensitized, while those stimulated with the catecholamine recovered full response to this agent.

In other experiments, prazosin was used to block the α_1 receptor, ketanserine for the $5\text{-}HT_{2A}$, EXP 3174 for AT_1 and bosentan for ET_A and ET_B. The specificity of these compounds for their respective receptor was confirmed by the high potencies of antagonists obtained against their specific agonists and the absence of the effect of each compound against at least one of the other stimulants (Table 1). A cocktail containing the four antagonists was found to be inactive against hU-II (Table 1) suggesting that the effect of this peptide, in the rat aorta, may be mediated by a specific receptor.

The rat aorta was used to compare hU-II from different sources. The results of these experiments indicate that the four preparations of hU-II did not differ significantly in potency, since pEC_{50} values were very similar (preparation from: SKB pEC_{50}=8.03±0.18, Ferrara pEC_{50}=8.27±0.21, Aventis pEC_{50}=7.84±0.19 and Bachem pEC_{50}=7.97±0.11; these data are the means ± SEM of at least five experiments).

In an attempt to find out if peripheral arterial resistance or venous vessels have functional sites for hU-II, isolated and perfused mesenteries from rats (the species that express GPR14 in the aorta) were investigated. Results of six experiments indicate that hU-II has no constrictor effects in isolated rat mesenteries treated with indomethacin

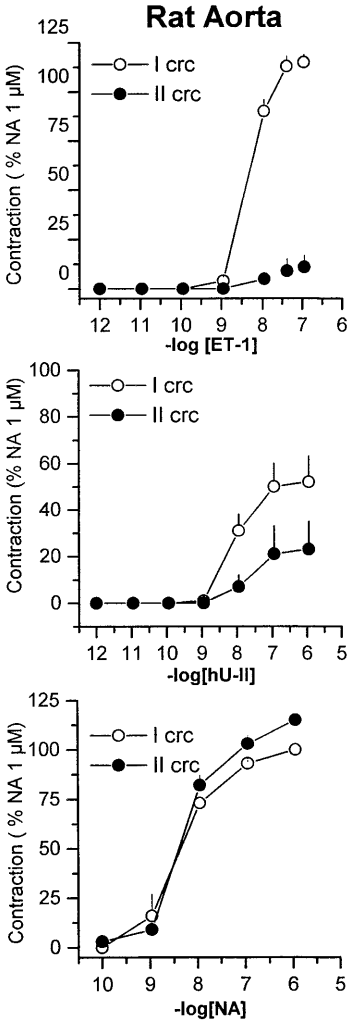

Fig. 3 I and II concentration-response curves (*crc*) to noradrenaline (*NA*), endothelin 1 (*ET-1*) and human urotensin II (*hU-II*) repeated in the same tissue after about 5 h. *Abscissa:* –log of the molar concentration of agonist. *Ordinate:* contraction in grams (g). The data are the means ± SEM of at least four experiments (2–4 tissues for each experiment)

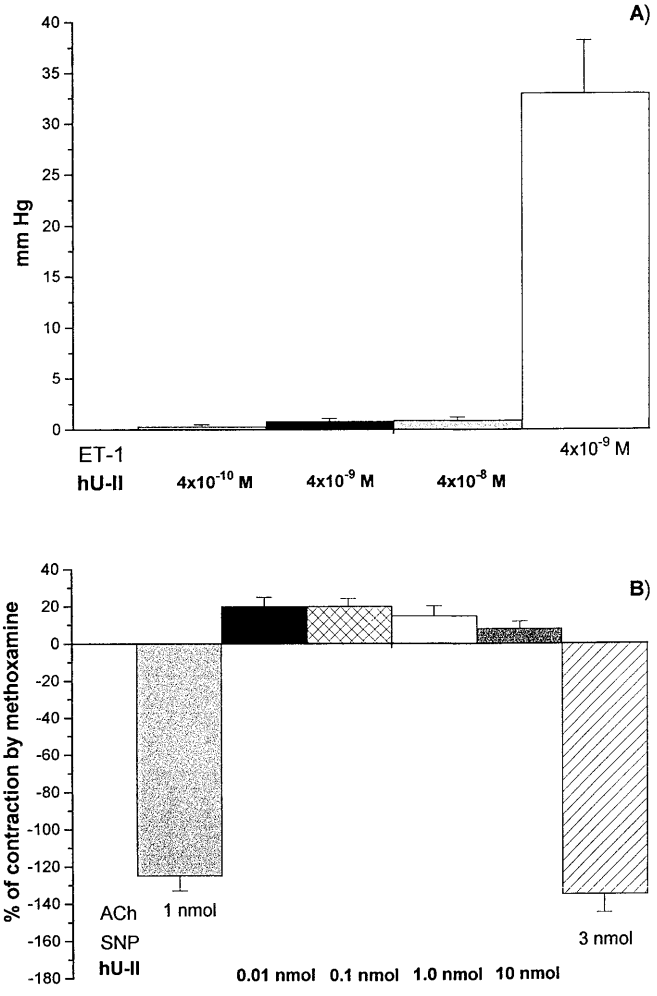

Fig. 4 **A** Effects of short application (3-min infusion at 2 ml/min i.a.) of hU-II and ET-1 in rat isolated mesenteries treated with L-NAME (100 µM). **B** Effects of hU-II, acetylcholine (*Ach*), or sodium nitroprusside (*SNP*) injected (bolus) in rat isolated mesenteries precontracted with methoxamine (400 µM). The data are means ± SEM of at least six experiments

and L-NAME (100 µM), while ET-1 induces strong vasoconstriction (Fig. 4A). Treatment with L-NAME strongly potentiates the effects of ET-1 (>30 mmHg). When methoxamine (400 µM) was continuously infused to increase the arterial vessel tone, ACh (1 nmol) and sodium nitroprusside (3 nmol) exerted strong vasodilator effects, while hU-II was inactive or eventually induced small increase of vessel tone (Fig. 4B). The rat mesentery perfused from the venous side responded to ET-1 with strong increase of tension while hU-II was inactive in some preparations and had a very weak dilator effect in others (data not shown). This effect was not further investigated.

Isolated vessels from other species

Numerous experiments were performed in an attempt to detect and quantify contractile and relaxing (only in the pig coronary artery) effects of hU-II. Results obtained in rabbit, pig and guinea pig vessels, summarised in Table 2, indicate that: KCl is active in all vessels, although responses of large veins (rbJV, gpVC) are rather weak. NA is a moderately potent stimulant of all rabbit vessels, arterial and venous, and shows pEC_{50} values ranging from 6.4 to 7.7; it is however rather weak (pEC_{50} 5.2) in the rabbit mesenteric artery. Low potencies have been measured with NA in pig (pEC_{50} from 5.9 to 6.8) and guinea pig (pEC_{50} 5.8–6.3) vessels (Table 2). Ang II is a potent vasoconstrictor in many arteries and in some veins: pEC_{50} values are above 8.1 in rabbit vessels, with the exception of the mesenteric artery and the saphenous vein, which are almost insensitive to the octapeptide. High pEC_{50} values, ranging from 7.8 to 8.6, have been measured in porcine vessels, with the exception of the coronary artery and the renal vein, two preparations that do not respond to Ang II. Ang II is a potent stimulant of guinea pig arteries, but is weak on the veins (Table 2). ET-1 is a very potent stimu-

Table 2 Comparison of the effects of noradrenaline, angiotensin II, endothelin 1 and human urotensin II in vessels of rabbit, pig and guinea pig origin. KCl 100 mM (**x/n n*= number of tissues investigated and *x*= number of tissues responding to hU-II, *pEC$_{50}$* the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect, *E$_{max}$* the maximal effect that an agonist can elicit in a given tissue, expressed as grams of contraction). The data are the means ± SEM of at least five experiments (2–4 tissues for each experiment)

	KCl (g)	NA		Ang II		ET-1		hU-II		
		pEC_{50}	E_{max} (g)	pEC_{50}	E_{max} (g)	pEC_{50}	E_{max} (g)	pEC_{50}	E_{max} (g)	(*x/n)
Rabbit										
Arterial vessels										
Thoracic aorta	1.74±0.2	7.1	3.04±0.2	8.1	1.81±0.1	8.0	1.44±0.3	8.5	0.34±0.06	(34/39)
Pulmonary	2.58±0.2	6.9	3.29±0.4	8.1	1.48±0.7	7.7	2.01±0.3	7.8	0.15±0.05	(4/10)
Carotid	0.79±0.1	6.4	1.44±0.2	8.1	0.49±0.1	8.0	1.49±0.1	Inactive up to 1 µM		
Iliac	1.86±0.1	7.7	2.91±0.4	8.3	1.56±0.1	8.1	2.34±0.3	Inactive up to 1 µM		
Renal	0.51±0.1	7.1	0.98±0.2	8.4	0.46±0.1	8.0	0.84±0.2	Inactive up to 1 µM		
Mesenteric	1.06±0.3	5.2	1.18±0.3	100 nM: 0.06±0.02		8.0	0.98±0.2	Inactive up to 1 µM		
Venous vessels										
Jugular	0.35±0.2	6.9	1.18±0.3	8.4	1.01±0.2	8.9	2.42±0.5	8.1	0.13±0.07	(6/18)
Saphenous	1.02±0.1	6.6	1.91±0.2	10 nM: 0.03±0.02		8.2	1.88±0.2	Inactive up to 1 µM		
Superior cava	0.22±0.1	7.0	0.37±0.1	8.5	0.47±0.1	8.6	0.87±0.2	Inactive up to 1 µM		
Pig										
Arterial vessels										
Coronary	1.89±0.4	Inactive up to 1 µM		Inactive up to 1 µM		7.8	3.64±0.7	9.1	0.37±0.07	(10/19)
Pulmonary	0.88±0.1	6.2	0.63±0.1	8.0	0.54±0.1	8.1	2.37±0.3	Inactive up to 1 µM		
Lienal	1.65±0.2	5.9	11.8±2.7	7.8	3.55±0.9	7.6	7.20±0.9	Inactive up to 1 µM		
Renal	1.46±0.1	6.5	2.79±0.2	8.6	0.37±0.1	8.0	2.10±0.2	Inactive up to 1 µM		
Venous vessels										
Lienal	1.35±0.3	6.8	2.65±0.2	8.6	0.88±0.2	8.4	2.53±0.3	Inactive up to 1 µM		
Renal	1.29±0.1	6.4	0.99±0.4	Inactive up to 1 µM		8.2	2.05±0.1	Inactive up to 1 µM		
Guinea pig										
Arterial vessels										
Thoracic aorta	0.50±0.1	6.3	0.74±0.1	8.2	0.21±0.1	8.0	0.51±0.1	Inactive up to 1 µM		
Pulmonary	0.83±0.1	5.8	1.42±0.1	7.8	0.19±0.1	7.9	0.93±0.1	Inactive up to 1 µM		
Venous vessels										
Inferior cava	0.09±0.03	6.1	0.09±0.03	100 nM: 0.03±0.02		8.0	0.40±0.1	Inactive up to 1 µM		
Portal	0.34±0.1	5.8	0.56±0.1	100 nM: 0.08±0.04		7.9	0.70±0.2	Inactive up to 1 µM		

lant of all arterial and venous vessels investigated in the present study without exception; pEC_{50} values for the bicyclic peptide are very homogenous and reach 8.0 or more in 16 of the 19 vessels examined. As illustrated in Fig. 1 for the rat aorta, ET-1 induces increase of vessel tone that persists for minutes and hours, depending on the agonist concentration, and has therefore to be considered as a potent and long-acting vasoconstrictor. Maximal effects of ET-1 are always higher than those of Ang II and are equivalent to or even higher than those of NA. Moreover, ET-1 is a potent contractor of coronary vessels (e.g. in the pig) and of large veins. Few positive results obtained with hU-II are indicated in the last column of Table 2. Weak contractions, amounting to only 10%–20% of those induced by NA or ET-1, were observed in a variable number of rabbit vessels (e.g. 34 out of 39 in the rbA, 6 out of 18 in the rbJV) and in the pCA (10 out of 19). All other tissues did not respond to hU-II. In a few experiments, the potential relaxing effect of hU-II, in comparison with bradykinin (BK) and substance P (SP), was evaluated in the pCA with endothelium (Fig. 5). Both SP and BK induced concentration-dependent relaxations of pCA contracted with KCl (30 mM) with pEC_{50} respectively of 8.76 and 8.73 and maximal effects reaching 92%–99%, while in 11 of 17 hU-II induced weak relaxations averaging 13% of the maximum (Fig. 5).

Human vessels

All human vessels investigated (see Table 3) are sensitive to KCl 100 mM, which evokes the highest maximal effects in umbilical vessels and the mesenteric artery. NA is able to contract all preparations examined with moderate potency (pEC_{50} from 5.7 to 7.0). It is always less potent than ET-1 (7.6–8.5) and Ang II (7.2–8.7) but evokes max-

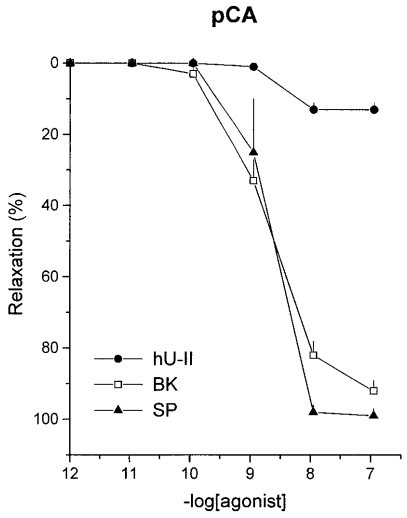

Fig. 5 Concentration-response curves to bradykinin (*BK*), substance P (*SP*) and human urotensin II (*hU-II*) in pig coronary arteries contracted with KCl (30 mM). *Abscissa:* –log of the agonist concentration. *Ordinate:* relaxation in percent of the contraction induced by KCl 30 mM. The data are means ± SEM of at least five experiments (2–4 tissues for each experiment)

imal effects that are higher than those of Ang II and similar to those of ET-1. Ang II is a potent stimulant in most vessels with exception of hUA and hUV which are insensitive to the peptide. ET-1 acts as potent vasoconstrictor in all vessels; in healthy saphenous veins, it is less potent than Ang II and is always more potent than NA. hU-II induced contractile responses in most umbilical veins (8/9) and in some umbilical arteries (2/4), in epigastric veins (2/7) and facial veins (4/10). Despite the high potency (pEC_{50} 8.6, 10.2 or 9.9 in hUA, hUV and hEV, respectively) hU-II produced very small maximal effects (from 0.06 g to 0.16 g), which correspond to about 5%–10% of those induced by KCl 100 mM.

Discussion

In addition to metabolic and endocrine functions (Bern et al. 1985) U-II exerts potent vasoconstrictor effects in some fishes and mammals (Gibson 1987) and stimulates arterial and venous vessels to contract in vitro (Ames et al. 1999; Douglas et al. 2000; MacLean et al. 2000; Maguire et al. 2000; see also the review by Douglas and Ohlstein 2000).

To find out if the recently identified cyclic undecapeptide hU-II (Coulouarn et al. 1998) modulates cardiovascular functions through receptors localized in arterial and venous smooth muscle cells, hU-II was compared to other vasoconstrictors in a variety of isolated vessels from several species. We tested the peptide of human origin in tissues from different species: therefore we can not rule out that some variations between species can be attributed to the use of the human form of the peptide. However, this possibility seems unlikely since the cyclic region of the peptide which is critical for biological activity is fully conserved among the different species (Douglas and Ohlstein 2000). In line with this, Gardiner et al. (2001) recently reported no differences in the cardiovascular effects of the human and the rat U-II peptides in conscious rats.

Results summarised in Tables 2 and 3 indicate that the hU-II is very weak or inactive in vessels of the rabbit, the pig and guinea pig, in contrast to NA and ET-1 (with some exception also Ang II) which are potent vasoconstrictors in practically all vessels investigated by us and other workers (for review see Douglas and Ohlstein 2000). The same is true for putative vasodilator effects of hU-II in coronary vessels (Bottrill et al. 2000; Katano et al. 2000):

Table 3 Comparison of the effects of noradrenaline, angiotensin II, endothelin 1 and human urotensin II in vessels of human origin. KCl 100 mM. Inactive up to 1 µM (**x/n n*= number of tissues investigated and *x*= number of tissues responding to hU-II, pEC_{50} the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect, E_{max} the maximal effect that an agonist can elicit in a given tissue, expressed as grams of contraction). The data are the means ± SEM of at least three experiments (2–4 tissues for each experiment)

	KCl (g)	NA		Ang II		ET-1		hU-II		
		pEC_{50}	E_{max} (g)	pEC_{50}	E_{max} (g)	pEC_{50}	E_{max} (g)	pEC_{50}	*E*max (g)	(**x*/*n*)
Arterial vessels										
Umbilical	2.48±0.1	6.0	0.54±0.1	Inactive up to 1 µM		7.9	3.30±0.3	8.6	0.16	(2/4)
Splenic[a]	0.97±0.2	6.2	1.86±0.3	7.6	1.69±0.1	7.6	2.75±0.2	Inactive up to 1 µM		
Mesenteric inferior[a]	2.58±0.3	5.7	1.27±0.8	7.2	0.77±0.4	7.6	1.61±0.6	Inactive up to 1 µM		
Venous vessels										
Umbilical	3.56±0.2	6.9	4.82±0.5	Inactive up to 1 µM		8.2	5.10±0.6	10.2	0.14±0.04	(8/9)
Saphenous healthy	0.98±0.2	6.9	1.73±0.3	8.7	1.22±0.3	7.7	2.43±0.3	Inactive up to 1 µM		
Saphenous C2	1.39±0.3	6.6	2.45±0.9	7.7	1.36±0.3	7.6	2.73±0.4	Inactive up to 1 µM		
Epigastric	0.54±0.3	6.5	1.49±1.0	8.2	1.11±0.7	8.1	2.46±1.8	9.9	0.06	(2/7)
Facial	0.45±0.1	7.0	0.67±0.1	8.5	0.64±0.1	8.5	0.98±0.2	0.1 nM:	0.06±0.04	(4/10)
Renal[a]	0.60±0.1	6.4	1.30±0.3	8.7	1.07±0.1	8.3	1.12±0.2	Inactive up to 1 µM		

[a]Only one experiment (*n*=4 strips)

the present investigation was limited to the pCA in which BK and SP induced concentration-dependent relaxation while hU-II was very weak or inactive.

Worthy of mention is the fact that, while the other vasoactive agents are found to be active in a large majority of isolated tissues, responses to hU-II show marked interindividual variation, as if only some individuals of a species would express contractile GPR14 receptors in a sufficient number to produce measurable responses. In this respect, our findings are in accord with the recent report by Douglas et al. (2000). Moreover, when the response is detectable, it is generally very weak (5%–20% of those of ET-1 or NA): these considerations are based on the present data as well as on findings by other authors (Bottrill et al. 2000; Douglas et al. 2000; Maguire et al. 2000; Nothacker et al. 1999). This variability in U-II responses could possibly be attributed to differences in the peptide metabolism among tissues and/or species. However, little, if any, information are available on U-II metabolism and the enzymes involved for supporting or discarding this possibility. Further investigations are therefore required to address this issue.

It is also to be noticed that GPR14, in contrast to ET_A, AT_1 and the α-adrenergic receptor, mediated contractile responses only in some large vessels: for instance in the rat, it has been shown that U-II-induced contractions can be obtained in the isolated aorta and carotid arteries, but not in other large vessels as mesenteric, renal, femoral arteries (Gendron et al. 2001) in accord with early (Itoh et al. 1987, 1988) and recent findings (Douglas et al. 2000). When tested in isolated perfused organs to assess the potential effects of hU-II in peripheral resistance vessels (e.g. in the rat mesentery) both as vasoconstrictor (in the presence of L-NAME) and as vasodilator (after increasing the arteriolar tone with methoxamine), the new peptide was extremely weak as a constrictor and inactive as vasodilator, when compared with the appropriate reference agents (e.g. ET-1). This is in line with data published recently by Hillier et al. (2001) who studied human arteries and veins of varying calibre. These findings lead us to conclude that the GPR14 receptor induces contractions only in functional large vessels and in the heart (Ames et al. 1999; Davenport and Maguire 2000; Liu et al. 1999; Mori et al. 1999; Nothacker et al. 1999) where it may modulate cardiovascular functions. This could explain why hU-II, injected intravenously in rats, is almost inactive on blood pressure (Gendron et al. 2001).

Alternatively, the peptide may constrict some vascular districts and relax others with the result of no change of mean arterial pressure. It has been recently reported that both human and rat U-II promote regionally selective vasodilator effect in conscious rats (Gardiner et al. 2001). The peculiar behaviour of this new agent, compared to well-known vasoconstrictors and vasodilators, may explain the large discrepancies that are found in recent literature with regard to the rat aorta (Ames et al. 1999; Bottrill et al. 2000; Douglas et al. 2000; Maguire et al. 2000; Nothacker et al. 1999). Such discrepancies have been confirmed in the present study, by comparing two sets of data, obtained with the same compound in Ferrara (Italy) and Sherbrooke (Canada; see Fig. 2); the maximal response of the rat aorta to hU-II, averaged 68% of that of NA in Ferrara, and 118% in Sherbrooke. Such differences have been reported before by Nothacker et al. (1999; 32% of NA), Maguire et al. (2000; 68% of KCl), Ames et al. (1999; 143% of KCl), Bottrill et al. (2000; 103% of KCl) and reveal that the UII-GPR14 system, unlike other vasoactive systems, is characterized by marked individual variations.

hU-II was found to be a potent contractor of the rat aorta (in 100% of tissues), confirming early and recent reports (Ames et al. 1999; Douglas et al. 2000; Gibson 1987; Itoh et al. 1987). It is therefore assured that contractile GPR14 receptors are operative in the rat cardiovascular system, especially in some large vessels, but not in the peripheral arteries and vein of the mesentery, in contrast to ET_A. Such a receptor distribution could be important for the overall function, if any, of the hU-II/GPR14 system, which could modulate the compliance of large vessels rather than the peripheral resistance vessels. Myotropic effects of hU-II are similar to those of ET-1 in their slow onset, long duration and consistent tachyphylactic effect in isolated vessels (Figs. 1, 3). This indicates that, when activated, the GPR14 receptor might exert prolonged stimulation of Ca^{2+} accumulation (Gibson et al. 1988; Itoh et al. 1987) in vascular smooth muscle cells and, in some district also in the endothelium, to increase smooth muscle tone or promote the release of NO (or other vasodilators) from the endothelium (Bottrill et al. 2000; Katano et al. 2000). While the contractile effect could be well quantified and analysed in the present and in other studies (Ames et al. 1999; Maguire et al. 2000; Douglas et al. 2000), the vasodilator action, if any, is variable and elusive (Douglas et al. 2000); however, the absence of changes in blood pressure and heart rate observed in rats (Gendron et al. 2001) indicates that hU-II is not a peripheral vasoconstrictor: hU-II may act preferentially on large vessels that take part in vascular compliance (aorta, pulmonary, carotid arteries) and increase cardiac post load. In summary, the new hU-II/GPR14 system may play a role in the regulation of cardiovascular functions by intervening with large vessels and with the heart. Vascular peripheral actions remain to be demonstrated, eventually in the kidney. Similar to the ET-1/ET_A system, the new peptide and its receptor appear to exert strong and long-lasting effects that might be important in cardiac and vascular syndromes of the pulmonary and the general circulation. If reduced arterial compliance will associate with cardiac dysfunction, the activation of the hU-II/GPR14 system could be deleterious for the cardiovascular homeostasis.

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References

Ames RS, Sarau HM, Chambers JK, Willette RN, Aiyar NV, Romanic AM, Louden CS, Foley JJ, CF S, Coatney RW, Ao Z, Disa J, SD H, Stadel JM, Martin JD, Liu WS, Glover GI, Wilson S, McNulty DE, Ellis CE, Elshourbagy NA, Shabon U, Trill JJ, Hay DW, Douglas SA (1999) Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. Nature 401:282–286

Bern HA, Pearson D, Larson BA, Nishioka RS (1985) Neurohormones from fish tails: the caudal neurosecretory system. I. "Urophysiology" and the caudal neurosecretory system of fishes. Recent Prog Horm Res 41:533–552

Bottrill FE, Douglas SA, Hiley CR, White R (2000) Human urotensin-II is an endothelium-dependent vasodilator in rat small arteries. Br J Pharmacol 130:1865–1870

Coulouarn Y, Lihrmann I, Jegou S, Anouar Y, Tostivint H, Beauvillain JC, Conlon JM, Bern HA, Vaudry H (1998) Cloning of the cDNA encoding the urotensin II precursor in frog and human reveals intense expression of the urotensin II gene in motoneurons of the spinal cord. Proc Natl Acad Sci USA 95: 15803–15808

Coulouarn Y, Jegou S, Tostivint H, Vaudry H, Lihrmann I (1999) Cloning, sequence analysis and tissue distribution of the mouse and rat urotensin II precursors. FEBS Lett 457:28–32

Davenport AP, Maguire JJ (2000) Urotensin II: fish neuropeptide catches orphan receptor. Trends Pharmacol Sci 21:80–82

Douglas SA, Ohlstein EH (2000) Human urotensin-II, the most potent mammalian vasoconstrictor identified to date, as a therapeutic target for the management of cardiovascular disease. Trends Cardiovasc Med 10:229–237

Douglas SA, Sulpizio AC, Piercy V, Sarau HM, Ames RS, Aiyar NV, Ohlstein EH, Willette RN (2000) Differential vasoconstrictor activity of human urotensin-II in vascular tissue isolated from the rat, mouse, dog, pig, marmoset and cynomolgus monkey. Br J Pharmacol 131:1262–1274

Furchgott RF, Bhadrakom S (1953) Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J Pharmacol Exp Ther 108:129–143

Gardiner SM, March JE, Kemp PA, Davenport AP, Bennet T (2001) Depressor and regionally-selective vasodilator effects of human and rat urotensin II in conscious rats. Br J Pharmacol 132:1625–1629

Gaudreau P, Barabè J, St-Pierre S, Regoli D (1981) Pharmacological studies of kinins on venous smooth muscles. Can J Physiol Pharmacol 59:371–379

Gendron G, D'Orleans Juste P, Regoli D (2001) Comparison of pharmacological effects of ET-1 and Urotensin II in vivo and in vitro in the rat. In: ET-7 Meeting, Edinburgh, 16–19 September

Gibson A (1987) Complex effects of Gillichthys urotensin II on rat aortic strips. Br J Pharmacol 91:205–212

Gibson A, Conyers S, Bern HA (1988) The influence of urotensin II on calcium flux in rat aorta. J Pharm Pharmacol 40:893–895

Gobeil F, Filteau C, Pheng LH, Jukic D, Nguyenle XK, Regoli D (1996) In vitro and in vivo characterization of bradykinin B-2 receptors in the rabbit and the guinea pig. Can J Physiol Pharmacol 74:137–144

Hay DW, Luttmann MA, Douglas SA (2000) Human urotensin-II is a potent spasmogen of primate airway smooth muscle. Br J Pharamcol 131:10–12

Hillier C, Berry C, Petrie MC, O'Dwyer PJ, Hamilton C, Brown A, McMurray J (2001) Effects of urotensin II in human arteries and vein of varying caliber. Circulation 103:1378–1381

Itoh H, Itoh Y, Rivier J, Lederis K (1987) Contraction of major artery segments of rat by fish neuropeptide urotensin II. Am J Physiol 252:R361–R366

Itoh H, McMaster D, Lederis K (1988) Functional receptors for fish neuropeptide urotensin II in major rat arteries. Eur J Pharmacol 149:61–66

Katano Y, Ishihata A, Aita T, Ogaki T, Horie T (2000) Vasodilator effect of urotensin II, one of the most potent vasoconstricting factors, on rat coronary arteries. Eur J Pharmacol 402:R5–R7

Liu Q, Pong SS, Zeng Z, Zhang Q, Howard AD, Williams DL, Davidoff M, Wang R, Austin CP, McDonald TP, Bai C, George SR, Evans JF, Caskey CT (1999) Identification of urotensin II as the endogenous ligand for the orphan G-protein-coupled receptor GPR14. Biochem Biophys Res Commun 266:174–178

MacLean MR, Alexander D, Stirrat A, Gallagher M, Douglas SA, Ohlstein EH, Morecroft I, Polland K (2000) Contractile responses to human urotensin-II in rat and human pulmonary arteries: effect of endothelial factors and chronic hypoxia in the rat. Br J Pharmacol 130:201–204

Maguire JJ, Kuc RE, Davenport AP (2000) Orphan-receptor ligand human urotensin II: receptor localization in human tissues and comparison of vasoconstrictor responses with endothelin-1. Br J Pharmacol 131:441–446

Marchese A, Heiber M, Nguyen T, Heng HH, Saldivia VR, Cheng R, Murphy PM, Tsui LC, Shi X, Gregor P, et al (1995) Cloning and chromosomal mapping of three novel genes, GPR9, GPR10, and GPR14, encoding receptors related to interleukin 8, neuropeptide Y, and somatostatin receptors. Genomics 29: 335–344

Mori M, Sugo T, Abe M, Shimomura Y, Kurihara M, Kitada C, Kikuchi K, Shintani K, Kurokawa T, Onda H, Nishimura O, Fujino M (1999) Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14). Biochem Biophys Res Commun 265:123–129

Nothacker HP, Wang Z, McNeill AM, Saito Y, Merten S, O'Dowd B, Duckles SP, Civelli O (1999) Identification of the natural ligand of an orphan-protein-coupled receptor involved in the regulation of vasoconstriction. Nat Cell Biol 16:383–385

Pearson D, Shively JE, Clark BR, Geschwind II, Barkley M, Nishioka RS, Bern HA (1980) Urotensin II: a somatostatin-like peptide in the caudal neurosecretory system of fishes. Proc Natl Acad Sci USA 77:5021–5024

Stirrat A, Gallagher M, Douglas SA, Ohlstein EH, Berry C, Kirk A, Richardson M, MacLean MR (2001) Potent vasodilator responses to human urotensin-II in human pulmonary and abdominal resistance arteries. Am J Physiol 280:H925–H928

Tal M, Ammar DA, Karpuj M, Krizhanovsky V, Naim M, Thompson DA (1995) A novel putative neuropeptide receptor expressed in neural tissue, including sensory epithelia. Biochem Biophys Res Commun 209:752–759

Warner TD (1990) Simultaneous perfusion of rat isolated superior mesenteric arterial and venous beds: comparison of their vasoconstrictor and vasodilator responses to agonists. Br J Pharmacol 994:427–433