Novel insights into the role of urotensin II in cardiovascular disease

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Urotensin II (UII) is a vasoactive peptide that interacts with a specific receptor called the UT receptor. UII has been implicated in cardiovascular regulation, with promising therapeutic applications based on UT receptor antagonism. The endogenous ligands of the UT receptor: UII and urotensin-related peptide (URP), differentially bind and activate this receptor. Also, the receptor localization is not restricted to the plasma membrane, possibly inducing different physiological responses that could support its inconsistent, but potent, vasoactive activity. These properties could explain the disappointing outcomes in clinical studies, in contrast to the positive preclinical results regarding heart failure, pulmonary hypertension, atherosclerosis and diabetes mellitus. These aspects should be considered in future investigations to a better comprehension of the role of UII as a potential therapeutic target.

Introduction

Urotensin II (UII) is a peptide originally found in the caudal neurosecretory system of the teleost fish *Gillichthys mirabilis*, in a structure named the urophysis, in which other peptides with vasoactive properties coexist [1]. Although it was initially thought to be exclusive to fish [2–5], its cardiovascular effects were also described in amphibians [6] and in other vertebrates, namely in humans, suggesting that it appeared early during evolution. Nowadays, the role of UII in cardiovascular pathophysiology has been extensively reviewed [7–12] because of its vasoactive properties and significant expression of the peptide and its receptor in patients with cardiovascular morbidities [13,14]. However, several unanswered questions remain because the development of compounds that modulate the urotensinergic system reveal promising but inconsistent actions, potentially explained by certain characteristics related to the receptor localization and selective signaling pathways induced by its ligands.

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Molecular structure and biosynthesis of urotensin II

Human UII (hUII) is derived from a protein precursor coded by the *UTS2* gene located on chromosome 1p36 [15]. In humans, two of those proteins were identified, formed by 124 and 139 residues that result from alternative splicing [13,16]. However, there is no full knowledge about the metabolic pathway that contributes to hUII mature peptide production, namely the enzymes involved in the proteolytic cleavage of the protein precursors or the exact location where maturation occurs. There is evidence of a possible urotensin-converting enzyme (UCE), detected by a mass-spectrometry-assisted enzyme-screening system, in porcine renal tissue [17]. In another *in vitro* study, intracellular enzymes with furin-like characteristics and serine proteases (such as trypsin) were found in human blood and plasma samples, which are involved in the maturation process of pro-UII [18].

The mature peptide (Fig. 1) is formed by 11 amino acids, with a C-terminal region that includes a cyclic hexapeptide sequence (Cys-Phe-Trp-Lys-Tyr-Cys) as a result of a disulfide bond between cysteine residues. That cyclic sequence is highly conserved from fish to mammals, whereas the N-terminal region is highly variable in length and constitution [10,11]. The conserved sequence, re-

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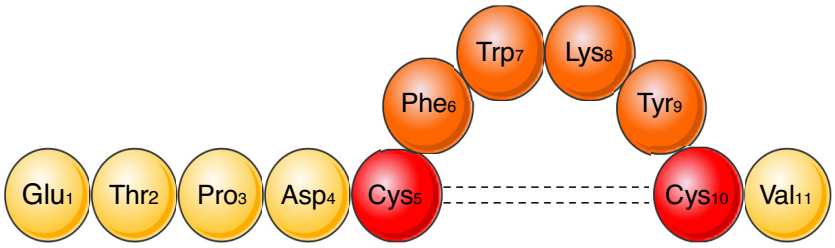

UII	Human and monkey	H-Glu-Thr-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH
	Mouse	<Gln-His-Lys-Gln-His-Gly-Ala-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Ile-OH
	Rat	<Gln-His-Gly-Thr-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Ile-OH
	Goby fish	H-Ala-Gly-Thr-Ala-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH
URP	Human and monkey	H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH
	Mouse	H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH
	Rat	H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH

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FIGURE 1

Representation of amino acid sequence for mature human urotensin II (hUII) and comparison of primary structures of urotensin II (UII) and urotensin-related peptide (URP) from various species. The conserved cyclic hexapeptide is highlighted in red. Illustration used elements from Servier Medical Art (http://smart.servier.com).

sponsible for its biological activity, explains why the non-native UII (species-nonspecific form) is active in different species. Nevertheless, it was also found that non-native UII can produce different biological responses compared with its native form, suggesting that the N-terminal region is essential to receptor activation [19].

Distribution and origin of urotensin II

In the central nervous system (CNS), hUII pre-pro-hormone mRNA was found by northern dot blot in the spinal cord, more exactly in motoneurons, and in the medulla oblongata, although with lower expression compared with the signal found in the spinal cord [16]. These results were later confirmed in a study that showed UII-like immunoreactivity confined to a subset of motoneurons in the ventral horn [20]. In another study, mRNA expression of hUII was also identified in the cerebral cortex, hypothalamus and hypophysis of patients with chronic kidney disease [21].

In the heart, hUII precursor protein was detected in atrial and ventricular cardiomyocytes [22,23]. Immunohistochemical studies also revealed the presence of hUII in endothelial cells of arteries (aorta, coronaries, internal mammary and umbilical artery) and veins (saphenous and umbilical) [24]. Regarding the kidney, high expression of hUII was found in epithelial cells from distal convoluted tubules, as well as from collecting tubules, collecting ducts and proximal convoluted tubules, although with lower expression in these latter tissues. The same study also revealed immunoreactivity in endothelial cells from kidney vasculature, except for the veins [25]. The presence of the peptide precursor mRNA was found in other peripheral tissues, namely in the liver, spleen, thymus, small intestine, stomach, prostate, ovaries, pancreas, adrenal gland and skeletal muscle [16,26].

It is important to highlight that UII distribution seems to be species-specific. As an example, high levels of UII precursors were found in rat testis tissue; however, in the same human tissue samples low levels of UII precursors were found [27]. Moreover, the data regarding hUII distribution are highly variable according to the different studies, even if they use the same methodology. In a study based on northern dot blot of 50 samples of different human tissues (including myocardial tissues), pro-hUII mRNA was only detected in the kidney and, with lower expression, in the spinal cord and medulla oblongata, although its presence in the heart is reported in other studies [26,28]. Another aspect to consider is the fact that hUII detection can vary according to the sample, which can be from a healthy individual or from a patient with multiple comorbidities. An example of this is the study that reported low or even no hUII immunoreactivity in cardiomyocytes, endothelial cells and vascular smooth muscle cells (VSMCs) from healthy individuals, but high immunoreactivity in the same tissues from patients with end-stage congestive heart failure (CHF) [14]. These results demonstrate that the peptide has an important role in cardiovascular pathophysiology.

The ubiquitous presence of hUII in the organism and its low levels detected in human plasma support the hypothesis that it acts as an autocrine and/or paracrine agent. A study that measured its concentration in plasma from patients with CHF reported higher levels in the aortic root compared with the levels found in the pulmonary artery, indicating a putative cardiopulmonary synthesis of the peptide [29]. Another study that used anesthetized sheep also demonstrated an arteriovenous gradient in the heart, liver and kidney, revealing that these organs are responsible for UII production [30].

The UT receptor and its ligands beyond urotensin II

From a reverse pharmacology approach, it was found that UII selectively binds to a rat orphan receptor: GPR14, also named sensory epithelial neuropeptide-like receptor (SENR) [13]. Currently known as the urotensin II (UT) receptor, this receptor is encoded by the *UTS2R* gene, located on human chromosome 17q25.3 [31]. This intronless gene produces a protein formed by 389 residues, sharing 75% of its structure with the rat receptor [13]. UT receptor is a class A G-protein-coupled receptor (GPCR), from the rhodopsin family, and shares high similarity in its amino acid sequence with somatostatin (similarity of 27% with SSTR4 receptor) and opioid receptors (similarity of 25% with κ receptor and 26% with μ and δ) [32]. Like hUII, UT receptor is widely distributed across different human tissues. Receptor mRNA was detected in the CNS (cerebral cortex, hypothalamus, hypophysis, medulla oblongata and motoneurons in spinal cord) and in many peripheral organs, particularly skeletal muscle tissue and renal (renal cortex), endocrine (pancreas and adrenal gland) and cardiovascular (atria and ventricles, endothelial cells and VSMCs from arterial vasculature) systems [13,21,26].

UT receptor endogenous ligands and antagonists

The endogenous selective ligands for human UT receptor are UII and urotensin-related peptide (URP). URP is formed from eight amino acids (Fig. 1), with the same cyclic hexapeptide sequence in the C-terminal region responsible for biological activity, forming the urotensinergic system with UII and UT receptor. The expression of the pre-pro-URP genes, which are located on 3q29 chromosome, follows the same distribution and is comparable to the levels of the pre-pro-UII genes in human tissues, except for the spinal cord, where UII expression is significantly higher. In addition, protein precursors from UII and URP are not similar, sharing only 18.8% of their amino acid sequence [27]. However, owing to the almost identical primary structure of their mature proteins, antibodies used in immunohistochemical studies do not seem to be able to clearly discriminate these two peptides [33]. Through a solid-phase extraction technique based on the more hydrophobic nature of URP compared with UII, one study was able to differentiate and measure their plasma levels in healthy individuals: UII concentration ranged from 0.50 to 3.33 pmol/l and URP ranged from 1.30 to 14.14 pmol/l [34].

The urotensinergic system is a good example of the concept of biased agonism. UII and URP, with similar primary structures, are known as the endogenous ligands of the UT receptor; differences as subtle as their variable N-terminal regions can induce different conformational changes of the receptor that consequently modulate different signaling pathways (Fig. 2a) [35]. Furthermore, the N-terminal domain can also condition the activity of the two ligands as a partial or full agonist, changing their affinity for the receptor [36,37]. Thereby, it becomes important to identify pharmacological tools that discriminate the effects produced by each endogenous ligand upon activation of the receptor [38]. In this context, several compounds have been synthetized to modulate the effects induced by each UII and URP [11,39–41].

The developing of UT receptor antagonists is also essential for a better understanding of UII function in the organism. These antagonists are classified as peptidic [42–46] or non-peptidic [47–52] (Table 1). Palosuran (ACT-058362) was the first non-peptidic antagonist with positive results in animal models but not so effective in humans. Its importance in the study of the urotensinergic system is evident owing to its role not only in cardiovascular diseases (Tables 2 and 3) but also in other condi-

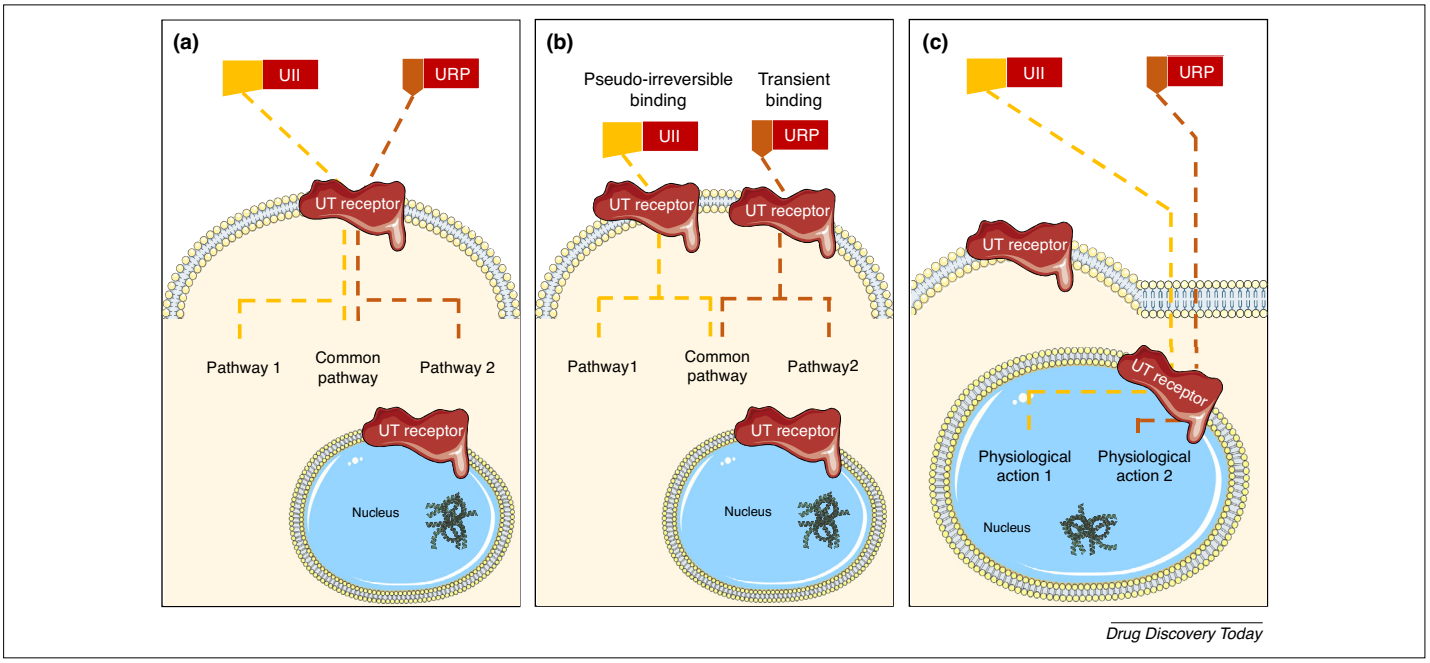

FIGURE 2

Representation of the different ways urotensin II (UII) and urotensin-related peptide (URP) exert their biased agonism. **(a)** Differential activation of the membrane urotensin (UT) receptor, induced by variations in N-terminal domain of the ligands. **(b)** Pseudo-irreversible and transient binding of UII and URP to the receptor, respectively. **(c)** Functional selectivity of the ligands in the nuclear UT receptor. Illustration used elements from Servier Medical Art (http://smart.servier.com).

TABLE 1

List of several peptidic and nonpeptidic antagonists, their structures and respective urotensin (UT) receptor affinities

		Structure	UT affinity	Comments	Refs
Peptidic antagonists	Urantide	H-Asp-c[Pen-Phe-DTrp-Orn-Tyr-Cys]-Val-OH	pK_i = 8.3 (human UT receptor)	Recent studies report that this drug behaves as a partial agonist because it promoted Gi, Gq and Go activation (but blocked β-arrestins) [42]	[43]
	BIM-23127	D-Nal-cyclo-[Cys-Tyr-D-Trp-Orn-Val-Cys]-2-Nal-NH_2	pK_i = 6.7 (human UT receptor)	Initially described as a neuromedin B receptor antagonist [44]	[45]
	SB-710411	Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide	K_i = 903.4 nM (human UT receptor) K_i = 160.0 nM (rat UT receptor)	Agonist in primate vessels and weak antagonist in rat vessels	[46]
Nonpeptidic antagonists	Palosuran (ACT-058362)	1-[2-(4-Benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt	Membrane binding K_i = 5.1 nM (human UT receptor) Whole-cell binding K_i = 276.0 nM (human UT receptor)	Loss of affinity could be explained by a distinct binding site or by high-efficiency catabolism at the intact cell membrane	[47]
	SB-657510	2-Bromo-*N*-[4-chloro-3-((R)-1-methyl-pyrrolidin-3-yloxy)-phenyl]-4,5-dimethoxybenzenesulphonamide hydrochloride	Membrane binding K_i = 61.4 nM (human UT receptor) Whole-cell binding K_i = 46.2 nM (human UT receptor)	Identical affinities in intact cells and membranes	[47]
	SB-611812	2,6-Dichloro-*N*-(4-chloro-3-[[2-(dimethylamino)ethyl]oxy]phenyl-4-(trifluoromethyl)-bezenesulphonamide	K_i = 121 nM (rat UT receptor)		[48]
	SB-706375	2-Bromo-4,5-dimethoxy-*N*-[3-(R)-1-methyl-pyrrolidin-3-yloxy)-4-trifluro-methyl-phenyl]-benzenesulphonamide hydrochloride	K_i = 9.3 nM (human UT receptor)		[49]
	KR-36676	2-(6,7-Dichloro-3-oxo-2H-benzo[b][1,4]oxazin-4(3 H)-yl)-*N*-methyl-*N*-(2-(pyrrolidin-1-yl)-1-(4-(thiophen-3-yl)phenyl)ethyl)acetamide	K_i = 0.7 nM (human UT receptor)		[50]
	KR-36996	*N*-[1-[3-bromo-4-(piperidin-4-yloxy) benzyl]piperidin-4-yl]benzo[b] thiophene3-carboxamide	K_i = 4.44 nM (human UT receptor)		[51]
	DS37001789	Piperazine derivative	–	Its potency (IC_{50} = 0.9 nM) was superior to that of palosuran (IC_{50} = 120 nM)	[52]

This list is not exhaustive, it essentially contains the antagonists mentioned throughout this review.

tions, such as portal hypertension [53,54]. However, its use also reveals that UT receptor activation could have a highly variable response to UII in humans, requiring the developing of more-potent antagonists. In fact, palosuran showed lower affinity in intact cell and functional assays in comparison with its potency in membrane formats (Table 1) [47]. Moreover, its affinity is also variable according to the different species – it acts as a 'primate-selective' ligand in recombinant membrane preparations (K_i ~4 nM, in human UT receptor) but it binds weakly to rat UT receptor (K_i ~1500 nM), potentially explained by different binding sites in the human and rat receptors [55]. The preclinical results must also be interpreted considering the fact that palosuran is a non-selective drug, which means that the beneficial effects attributed to UT receptor antagonism could be caused by the palosuran activation of other receptors, namely sst2 and sst5 somatostatin receptors [47,56]. Other important UT receptor antagonists are included in Table 1.

Biological effects and signaling pathways of urotensin II

Vasoactive effects of urotensin II

One of the first studies about the cardiovascular effects of hUII revealed its strong vasoconstrictive action in isolated rat arteries, with a potency 16-times greater than endothelin-1 (ET-1). The effect of hUII and UII derived from teleost fish was evaluated in rat thoracic aorta, where they induced vasoconstriction; however, both UIIs were not able to constrict other arteries (abdominal, femoral and renal arteries) [13]. In another *in vitro* study, hUII promoted vasoconstriction with a potency 50-times greater than ET-1 in human coronary, mammary and radial arteries, although its efficacy was highly variable; in fact, ET-1 effectively constricted all arteries, whereas hUII failed to induce a response in ~30% of them [26].

The great variability of UII in vascular tone regulation is also clear in its vasodilator action. Vasodilation mediated by UT recep-

TABLE 2

Results of urotensin (UII) receptor antagonism in heart failure and pulmonary hypertension: different antagonists were used to assess its effects on cardiovascular disease

	Treatment	Model and species	Outcome	Comments	Refs
Heart failure	KR-36996	C57BL/6 mice (transverse aortic constriction) and Sprague–Dawley rats (coronary ligation)	↓ Interstitial fibrosis ↓ Left ventricular weight by 40% ↑ Ejection fraction and fractional shortening		[50]
	KR-36676	C57BL/6 mice (transverse aortic constriction) and Sprague–Dawley rats (coronary ligation)	↓ Formation of actin stress fibers ↓ Left ventricle hypertrophy		[51]
	SB-611812	Lewis rats (coronary ligation)	↓ Myocardial and endocardial fibrosis ↓ LVEDP ↓ Collagen type I/III ratio ↓ Proliferation of cardiac fibroblasts	Improvement in myocardial stiffness is due to the significant decrease in type I collagen (more rigid than type III collagen)	[92]
	SB-710411	Sprague–Dawley rats (coronary ligation)	↓ Cardiac I/R-induced infarct size and histological damage Inhibited ST-segment increase in ECG ↓ LDH, CK-MB and cTnI levels	Acts as a vasoconstrictor in monkey arteries	[93]
	SB-706375	Sprague–Dawley rats (coronary ligation)	↓ Cardiac I/R-induced cardiac damage (in diastolic function) ↑ Coronary flow ↓ LDH, CK-MB and cTnI, levels	Inhibition of RhoA/ROCK pathway revealed a cardioprotective effect	[94]
Pulmonary hypertension	KR-36676	Sprague–Dawley rats (MCT-IPHM)	↓ Pulmonary vascular remodeling ↓ Right ventricle remodeling (hypertrophy/myocardial fibrosis)	Antiproliferative and anti-inflammatory actions by inhibiting ERK1/2 and nuclear factor (NF)-κB pathway	[102]
	Palosuran	Wistar albino rats (MCT-IPHM)	↓ ET-1, UII and TGF-β1 levels ↓ mPAP, RVHI and RVMI ↓ Arteriole wall thickness ↓ Perivascular connective tissue thickness	No effects on mean blood pressure	[100]
		Wistar albino rats (MCT-IPHM)	↓ ET-1 and UII levels ↓ mPAP ↓ Arteriole wall thickness ↓ Perivascular connective tissue thickness	The antagonist is at least as effective as bosentan	[103]
	Urantide	Wistar rats (MCT-IPHM)	↓ mPAP and SPAP ↓ Right ventricular diastolic diameter ↑ Time to peak, ejection time and peak flow velocity of pulmonary artery	No effects on pulmonary artery diameter and left ventricular ejection fraction	[104]
		Wistar rats (MCT-IPHM)	↑ Nitric oxide (NO) levels (in early and late treatment groups) ↓ mPAP and right ventricular weight ratio ↓ Pulmonary vascular remodeling	No effects on mean blood pressure	[105]

Abbreviations: MCT-IPHM, monocrotaline induced pulmonary hypertension model; LVEDP, left ventricular end-diastolic pressure; I/R, ischemia/reperfusion; ECG, electrocardiogram; LDH, lactate dehydrogenase; CK-MB, creatine kinase-muscle/brain; cTnI, troponin I; ET-1, endothelin-1; UII, urotensin II; TGF-β1, transforming growth factor beta 1; mPAP, main pulmonary arterial pressure; RVHI, right ventricular hypertrophy index; RVMI, right ventricular mass index; SPAP, systolic pulmonary arterial pressure.

tors located on the endothelium is endothelium-dependent, whereas vasoconstriction is an endothelium-independent process, mediated by receptors located on VSMCs [57]. A previous study evaluated the effect of hUII on isolated segments of several rat vessels. The peptide caused vasoconstriction of the thoracic aorta and the left anterior descending coronary artery, with a contractile response enhanced by the removal of the endothelium in the latter ones. By contrast, hUII caused potent vasodilation in precontracted mesenteric arteries of a small caliber and a limited vasodilator response in precontracted basilar arteries. This study highlights the role of endothelial cells in vasodilation and the anatomical differences related to the peptide response that might be associated with the variable levels of UT receptor expression [58]. It has been suggested that the vasoactive effects of UII could be related to the blood vessel caliber: in small vessels there is an endothelium-mediated vasodilation, whereas in large vessels the predominant response is a VSMC-mediated vasoconstriction [59].

Besides its activity on the peripheral vascular bed, UII acts through the CNS in cardiovascular regulation. It induces dose-related depressor and bradycardic responses or increases blood pressure and heart rate through the stimulation of different brain areas in rats [60]. Certain studies that evaluated the effects of UII intracerebroventricular injection reported greater blood pressure increases in spontaneously hypertensive rats when compared with normotensive rats [61]. This means that the peptide can act as a neuromodulator that regulates blood pressure with the contribution of the sympathetic nervous system, mainly through the stimulation of β-adrenoreceptors, in contrast to the systemic activity of UII, which is not mediated by those receptors [62].

The mechanisms underlying the great variability and low efficacy of UII are not entirely known but might be explained by the

TABLE 3

Results of urotensin (UII) receptor antagonism in atherosclerosis and diabetes mellitus or diabetic nephropathy. Different antagonists were used to assess its effects on cardiovascular diseases. The gray area corresponds to studies carried out in human patients

	Treatment	Model and species	Outcome	Comments	Refs
Atherosclerosis or vascular dysfunction	Urantide	Wistar rats (on high fat diet)	↓ Progression of aortic atherosclerosis ↓ TG, TC, HDL and LDL levels		[112]
	SB-657510	*Apoe* KO mice	↓ Progression of aortic atherosclerosis	Small decrease in triacylglycerol levels but no effects on other lipid parameters	[113]
	SB657510A	*Apoe* KO mice (on high fat diet)	↓ Bodyweight gain and visceral fat ↓ Blood pressure, serum hyperlipidemia and hyperglycemia ↓ Cytokine formation and aortic atherosclerosis Stabilization of the plaque	Aortic oxidative stress was reduced by ERK1/2 and p44/42-MAPK pathway inhibition	[114]
	KR-36676	C57BL/6 mice (common carotid artery ligation)	↓ VSMC proliferation ↓ Neointima formation	Inhibition of constriction in isolated aortic ring	[115]
	KR-36996	C57BL/6 mice (common carotid artery ligation)	↓ VSMC proliferation ↓ Neointima formation	These inhibitory effects revealed greater potency than GSK-1440115 (another UT receptor antagonist)	[116]
Diabetes mellitus or diabetic nephropathy	Palosuran	Wistar rats (STZ injection and unilateral nephrectomy)	↑ Survival ↑ Insulin secretion ↓ Hyperglycemia and glycosylated hemoglobin ↓ Serum lipids ↓ Proteinuria and renal dysfunction	Little effect on blood pressure or heart rate	[122]
	Palosuran (125 mg b.i.d.)	54 Hypertensive, macroalbuminuric, DM2 patients	No effects on albuminuria, blood pressure, glomerular filtration rate or renal plasma flow	4-Week treatment might have been too short	[123]
	Palosuran (125 mg b.i.d.)	19 Hypertensive, macroalbuminuric, DM2 patients	↓ 24 h urinary albumin excretion rate	No statistical significance reduction in the group with moderately to severely impaired renal function	[124]
	Palosuran (125 mg b.i.d.)	20 Diet-treated DM2 patients	No effects on insulin secretion or sensitivity and daily blood glucose levels		[125]
	Silymarin	Wistar rats (STZ-NIC injection)	↓ Oxidative stress ↓ FBS level and ↑ insulin concentration Improvement of lipid profile Prevented diabetes-induced weight loss	Reduced cardiac urotensin II (UII) and UT receptor expression	[126]

Abbreviations: KO knockout; STZ streptozotocin; DM2 type 2 diabetes mellitus; STZ-NIC streptozotocin and nicotinamide; TG triglycerides; TC total cholesterol; HDL high-density lipoprotein; LDL low-density lipoprotein; VSMCs vascular smooth muscle cells; FBS fasting blood sugar; ERK1/2 extracellular signal-regulated kinase; MAPK mitogen-activated protein kinase.

spare receptor reserve hypothesis. According to Douglas [63], most UT receptors are occupied by endogenous UII, owing to the pseudo-irreversible and high-affinity (K_d = 0.24 nM) [26] binding and slow dissociation of the ligand–receptor complex; this might explain, therefore, why there is low reserve of free receptor compared with the circulating levels of the peptide [63]. This long-lasting activity indicates that UII behaves as a chronic modulator of the vascular tone, in contrast to URP which displays a transient binding to the same receptor [37], suggesting that the N-terminal sequence could have a role in the residence time of the ligands and in their biased signaling (Fig. 2b) [42].

Main signaling pathways

The main intracellular signaling pathway that culminates in vasoconstriction involves the phospholipase C (PLC) pathway, through UT receptor activation which is primarily coupled to $G\alpha_{q/11}$ protein (Fig. 3), although it can also be coupled to $G\alpha_{i/o}$, $G\alpha_{13}$ and β-arrestins 1 and 2 (the latter linked to the receptor internalization process) [42,64,65]. PLC leads to inositol trisphosphate (IP_3) and diacylglycerol (DAG) formation, by hydrolysis of specific components of the cell membrane (phosphatidylinositol-4-5 bisphosphate) [64]. In its turn, IP_3 contributes to increase intracellular calcium levels by binding to its receptor, which acts as a calcium channel on the membrane of the endoplasmic reticulum, opening nonselective cation and voltage-dependent L-type calcium channels. This process results in the vasoconstriction mediated by the Ca^{2+}/calmodulin/myosin light chain system [64,66]. Vasoconstriction can also be mediated by protein kinase C (PKC; activated by DAG and cytoplasmatic calcium mobilization) and the RhoA/ROCK pathway, through a mechanism of calcium sensitization that promotes myosin light chain (MLC) phosphorylation [67,68].

PKC was also identified as being involved in the positive inotropic effect promoted by UII in isolated human right atrial tissue

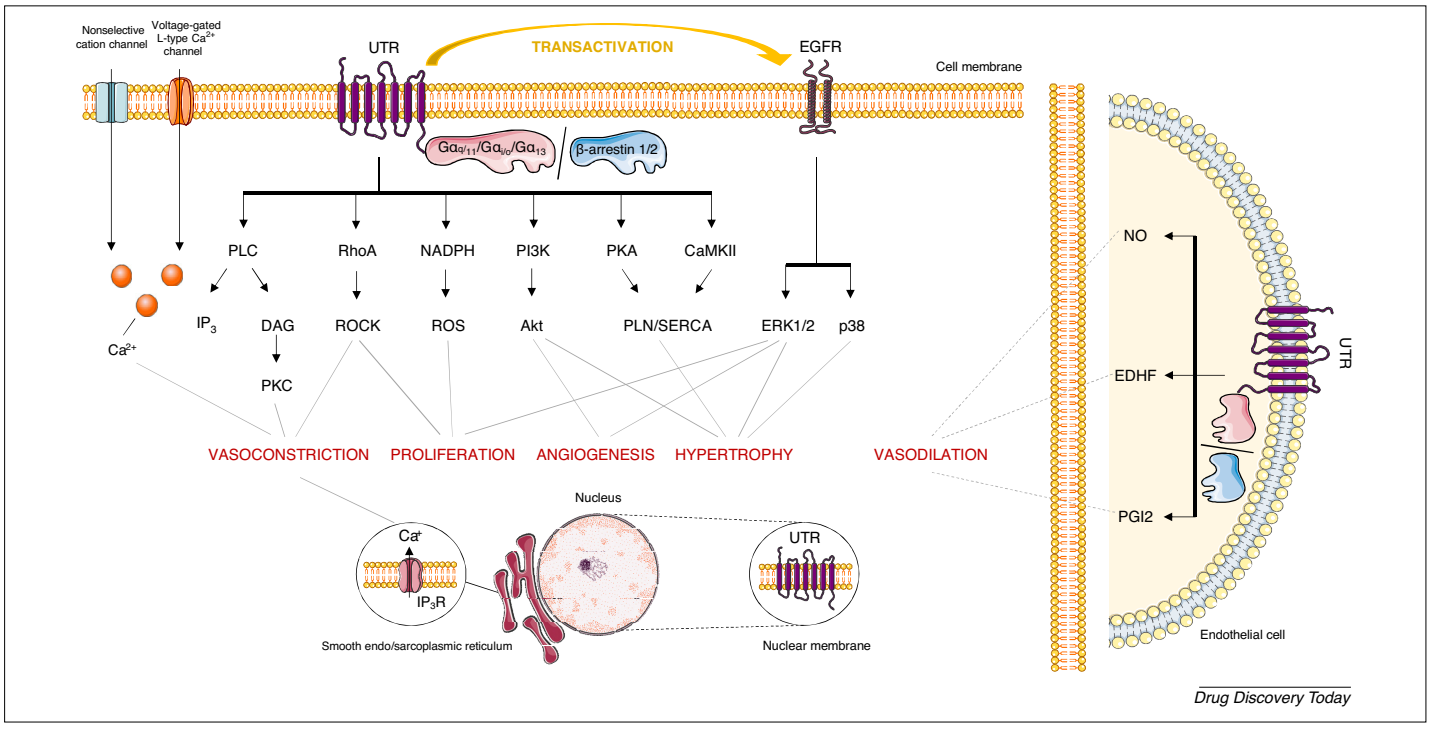

FIGURE 3

Schematic representation of the complex and multiple signaling pathways induced by urotensin (UT) receptor activation. The result of this activation will depend on the cell involved in that process. In endothelial cells, UT receptor activation will induce nitric oxide (NO), EDHF or prostaglandin formation, potentiating vasodilation. This effect can be balanced by vasoconstriction mediated by receptors on vascular smooth muscle cells (VSMCs), where urotensin II (UII) can also induce mitogenic/proliferative actions. Angiogenic activity is also mediated by UT receptors in vascular endothelial cells. In cardiomyocytes, the receptors are associated with hypertrophy and cardiac remodeling. Illustration used elements from Servier Medical Art (http://smart.servier.com). Abbreviations: Akt, protein kinase B; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; DAG, diacylglycerol; EDHF, endothelium-derived hyperpolarizing factor; EFGR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated kinase 1/2; IP3, inositol triphosphate; IP3R, inositol triphosphate receptor; NADPH, nicotinamide adenosine dinucleotide phosphate; MAPK, mitogen-activated protein kinase; PGI2, prostacyclin; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PLN/SERCA, phospholamban/SERCA pathway; RhoA, Ras homolog family member A; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; UTR, urotensin II receptor.

[69], but not in the UII-induced decrease in myocardial stiffness [70]. The peptide was considered to have a potent inotropic activity in the human atrium and ventricle, even higher than ET-1 [71]. Nevertheless, these results are not consistent with those found in other studies in which there was a mild negative inotropic activity (not affected by PKC inhibition) in rabbit papillary muscle, or even severe depression of the myocardial contractility in the heart from non-human primates [13,70]. Some of these variations in UII biological activity can be explained by the different sequence homology in UT receptors of different species. Besides, other factors and signaling cascades could also be involved in UII inotropic activity, which should deserve more investigation.

Vasodilation mediated by UT receptors located on the endothelium seems to promote synthesis and release of NO, endothelium-derived hyperpolarizing factor (EDHF), prostacyclin and other factors derived from the phospholipase A2 pathway [58,72]. UII has angiogenic, hypertrophic and mitogenic/proliferative actions as well. Angiogenesis is mediated by ERK1/2 and PI3K factors (but not by p38/MAPK) [73]. Hypertrophy of the cardiomyocytes seems to depend on epidermal growth factor receptor (EGFR) transactivation, which initiates the signaling pathway that involves ERK1/2 and p38/MAPK [74]. Hypertrophy can also be mediated by Akt/GSK-3β, CaMKII and protein kinase A (PKA) signaling pathways. CaMKII and PKA play an important part in mediating intracellular Ca^{2+} influx, which is regulated by phospholamban (PLN) and the SERCA pump [75–77]. PKA was also found to be involved in the process of myocardial fibrosis, stimulating the synthesis of collagen I and III [78]. Concerning the mitogenic/proliferative action, it requires the activity of factors like the RhoA/ROCK pathway, ERK1/2 and NADPH production. NAPDH promotes reactive oxygen species (ROS) production and potentiates MAPK action (mainly ERK1/2), proangiogenic factors from the PI3K/Akt pathway and vascular remodeling factors (such as plasminogen activator inhibitor-1; PAI-1) [79–81].

It was previously accepted that GPCRs would have a localization restricted to the cell membrane, nevertheless these receptors could also be found in the nuclear membrane, as happens with the UT receptor. The nuclear localization of this receptor, as occurs in the heart and CNS of rat and monkey tissues, implies ligand internalization through a receptor-independent mediated endocytosis, which could partially explain the pseudo-irreversible binding of UII [82,83]. Because higher levels of hUII were found in the cytoplasm compared with those of URP, it seems that variations in the N-terminal region could also explain the higher propensity of hUII to cross the plasma membrane. Moreover, both ligands can also differentially modulate the nuclear receptors and lead to divergent physiological actions (Fig. 2c). This intracrine mechanism could originate new intracellular signaling cascades, but

additional investigation is required to assess whether these two systems (intracrine and autocrine/paracrine) work independently or in synergy [83].

Role of urotensin II in cardiovascular disease

Heart failure

Multiple neurohormonal factors are known to be implicated in heart failure, namely the renin-angiotensin-aldosterone and adrenergic systems, which currently represent the main therapeutic targets. UII has been shown to interact with these systems, especially with angiotensin II and ET-1. In fact, some of its cardiovascular actions might be the result of the interaction of different neurohormonal systems and crosstalk of intracellular signaling pathways [84]. Some studies revealed elevated plasma levels of the peptide and higher UT receptor expression in cardiomyocytes, endothelial cells and VSMCs from patients with end-stage CHF [14,29,85,86]. Moreover, the peptide was also found to be related to the New York Heart Association (NYHA) functional class and inversely correlated with left ventricular ejection fraction [85].

UII induces myocardial fibrosis by increasing fibronectin, type I and III procollagen gene expression in neonatal cardiac fibroblast cultures from rats, as well as myocardial hypertrophy by increasing cardiomyocyte growth and myofibril organization [87,88]. The peptide also has positive inotropic activity, although it induced negative inotropic responses in patients with advanced heart failure, indicating that UII might have opposite contractility effects in failing and nonfailing hearts [89]. UII was proposed as a marker for the diagnosis of heart failure, especially in combination with N-terminal pro-brain natriuretic peptide (NT-proBNP). Whereas NT-proBNP is elevated with age and female gender, high levels of UII in patients with CHF seem to be unaffected by these factors, favoring its use as a biomarker. UII levels were also not correlated with NYHA class, in contrast to the studies previously mentioned [86]. UII could also be used as a biomarker in patients with rheumatic valvular diseases, complementary to echocardiographic evaluation, with an important prognostic role [90]. Additionally, serum hUII levels were markedly elevated in human patients with left ventricular hypertrophy secondary to hypertrophic cardiomyopathy [91]. These studies used specific enzyme-linked immunoassay (EIA) and ELISA tests to discriminate UII plasma levels from its precursors or other similar peptides.

UII involvement in cardiac remodeling motivated the development of UT antagonists with potential therapeutic properties. Some of the antagonists are reviewed in Table 2, regarding its beneficial effects on hypertrophy, fibrosis and myocardial injury in heart failure [50,51,92–94]. In summary, the urotensinergic system seems to have the potential to be used as a biomarker of diagnosis and/or prognosis and as a therapeutic target in heart failure.

Systemic arterial hypertension

In hypertension, which is characterized by endothelial dysfunction, the endothelium-independent vasoconstriction induced by UII could be enhanced. This aspect was demonstrated in a study where the effects of exogenous UII on patients with essential hypertension were compared to healthy subjects. Hypertensive patients revealed a dose-dependent vasoconstriction in the forearm skin microcirculation contrary to the dose-dependent vasodilation observed in healthy subjects [95]. Previous studies have shown that elevated UII plasma levels were positively correlated with hypertension, systolic and diastolic blood pressure, although there was no association between UII and nitric oxide (NO) metabolite levels, which were measured to evaluate endothelial dysfunction [96]. Echocardiographic parameters of systemic hypertension severity, namely interventricular septal thickness, left ventricular posterior wall thickness and left ventricular mass index, were positively correlated with plasma UII [97].

UTS2 gene polymorphisms responsible for hypertension and left ventricular posterior wall thickness were also identified in subjects with hypertension and cardiac hypertrophy in a Chinese female population [98]. Other studies claimed a possible role for UII in preeclampsia, because peptide expression was positively correlated with systolic blood pressure and urinary protein level and upregulated in the placenta of patients with this hypertensive disorder of pregnancy [99]. The fact that the UII system preserves its vasoconstrictive effects during the progression of systemic arterial hypertension suggests that the pharmacological modulation of the UT receptor might have potential therapeutic advantages in this condition.

Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) has a complex pathophysiology and it is associated with vascular remodeling of the small pulmonary arteries, which involves inflammation, fibrosis, vasoconstriction, medial hypertrophy and intimal hyperplasia mediated by cytokines, such as transforming growth factor (TGF)-β1, and other neurohumoral mediator like ET-1 [100]. In addition to its association with systemic hypertension and vascular dysfunction, the role of UII in PAH is related to vasoconstriction of the pulmonary artery and inhibition of atrial natriuretic peptide (ANP) secretion, which is a vasodilator of the pulmonary circulation [101].

Some UT receptor antagonists, such as KR-36676 [102], palosuran [100,103] and urantide [104,105], reviewed in Table 2, revealed positive outcomes in PAH treatment. Specifically, palosuran was at least as effective as bosentan, an antagonist of ET-1 receptors, which is considered a standard therapy for PAH. Taking these findings into account, the blockade of the urotensinergic system also seems to be a promising therapeutic target in pulmonary hypertension.

Atherosclerosis

UII was detected by immunoreactivity in endothelial cells and VSMCs of carotid and aortic plaques, particularly in the intima. Lymphocytes were identified as the largest producers of UII mRNA, whereas monocytes and macrophages were the cells with the most UT receptor expression [106]. In monocytes, UII functions as a chemotactic factor, mediated by the RhoA/Rho kinase pathway, with implications in the pathogenesis of atherosclerotic plaques [68]. Furthermore, based on elevated levels and expression of the receptor in the atheroma of coronary arteries, UII can also be indicated as a potential factor for the development of coronary atherosclerosis [107].

Atherosclerosis is a disease potentiated by UII that upregulates the expression of cellular adhesion molecules (CAMs) in endothelial cells, namely ICAM-1 and VCAM-1, enabling leukocyte adhesion and infiltration into the vascular wall [108]. The peptide also

promotes atherosclerosis by inducing VSMC proliferation [acting synergistically with mildly oxidized low-density lipoprotein (LDL)], activating fibroblasts and accelerating macrophage-derived foam cell formation owing to the upregulation of acetyl-coenzyme A acetyltransferase 1 (ACAT-1) expression [109,110]. Another study also revealed that UII and URP stimulate osteogenic differentiation and calcium deposition in VSMCs, explaining the higher expression of UII, URP and UT receptor in unstable plaques compared with stable plaques [111]. In Table 3 the outcomes of UT receptor antagonism in delaying atherosclerosis progression are reported. These preclinical studies point to a potential antiatherosclerotic role of urotensinergic system inhibition [112–116].

Important risk factors for cardiovascular disease

Diabetes mellitus and diabetic nephropathy

UII can contribute to the development and progression of type 2 diabetes mellitus because of its participation in metabolic syndrome and direct influence on pancreatic β cells, by inhibiting insulin response to glucose [117,118]. Some studies demonstrated elevated plasma levels of the peptide and higher UT receptor expression in diabetic patients with normal renal function or with overt proteinuria, but there was no correlation between fasting blood sugar and UII levels [22]. Single nucleotide polymorphisms in the *UTS2* gene were associated with greater susceptibility to type 2 diabetes mellitus and diabetic retinopathy [119,120]. Yet, the opposite was also found as one polymorphism in the same gene was associated with reduced risk of diabetes mellitus [121].

Long-term blockage of UT receptor with palosuran was effective at increasing insulin secretion and improving glycemic and lipid profile in diabetic mice, as well as improving renal function [122] (Table 3). However, the same antagonist failed to promote these positive outcomes in clinical studies carried out in hypertensive and diabetic patients [123–125]. The antidiabetic effects of silymarin, a flavonoid mixture with antioxidant properties that reduces the expression of UII and UT receptor in the heart, are also reviewed in Table 3 [126].

Concluding remarks

Since the isolation of UII from the teleost fish and its discovery in mammals, this cyclic undecapeptide has been implicated in the regulation of multiple physiological systems and pathological conditions. Given the potential of new therapeutic approaches to cardiovascular diseases, several antagonists have been developed with promising results in animal models. However, their limited efficacy in some human diseases might be attributed, to some extent, to the biased agonism of UII and URP as they interact with UT receptor or their inability to reach and block the receptors with nuclear localization [35,83]. These properties highlight the importance of searching new pharmacological tools to discriminate the biological effects of each endogenous UT receptor ligand and to understand the effects of nuclear UT receptor activation. In particular, new allosteric compounds should be developed to allow a better discrimination and an effective blockade of the UII-system-induced signaling pathways involved in cardiovascular disease pathophysiology [38].

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

1 Pearson, D. *et al.* (1980) Urotensin II: a somatostatin-like peptide in the caudal neurosecretory system of fishes. *Proc. Natl. Acad. Sci. U. S. A* 77, 5021–5024
2 Le Mevel, J.C. *et al.* (1996) Cardiovascular actions of trout urotensin II in the conscious trout, *Oncorhynchus mykiss*. *Am. J. Physiol.* 271, R1335–R1343
3 Lancien, F. *et al.* (2004) Central effects of native urotensin II on motor activity, ventilatory movements, and heart rate in the trout *Oncorhynchus mykiss*. *Brain Res.* 1023, 167–174
4 Le Mevel, J.C. *et al.* (2008) Central and peripheral cardiovascular, ventilatory, and motor effects of trout urotensin-II in the trout. *Peptides* 29, 830–837
5 Nobata, S. *et al.* (2011) Potent cardiovascular effects of homologous urotensin II (UII)-related peptide and UII in unanesthetized eels after peripheral and central injections. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R437–R446
6 Yano, K. *et al.* (1995) Cardiovascular actions of frog urotensin II in the frog, *Rana catesbeiana*. *Gen. Comp. Endocrinol.* 97, 103–110
7 Douglas, S.A. and Ohlstein, E.H. (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
8 Douglas, S.A. *et al.* (2004) From 'gills to pills': urotensin-II as a regulator of mammalian cardiorenal function. *Trends Pharmacol. Sci.* 25, 76–85
9 McDonald, J. *et al.* (2007) Role of urotensin II and its receptor in health and disease. *J. Anesth.* 21, 378–389
10 Vaudry, H. *et al.* (2010) Urotensin II, from fish to human. *Ann. N. Y. Acad. Sci.* 1200, 53–66
11 Vaudry, H. *et al.* (2015) International Union of Basic and Clinical Pharmacology. XCII. Urotensin II, urotensin II-related peptide, and their receptor: from structure to function. *Pharmacol. Rev.* 67, 214–258
12 Zhu, Y.C. *et al.* (2006) The role of urotensin II in cardiovascular and renal physiology and diseases. *Br. J. Pharmacol.* 148, 884–901
13 Ames, R.S. *et al.* (1999) Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature* 401, 282–286
14 Douglas, S.A. *et al.* (2002) Congestive heart failure and expression of myocardial urotensin II. *Lancet* 359, 1990–1997
15 Tostivint, H. *et al.* (2006) Comparative genomics provides evidence for close evolutionary relationships between the urotensin II and somatostatin gene families. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2237–2242
16 Coulouarn, Y. *et al.* (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. U. S. A.* 95, 15803–15808
17 Schluter, H. *et al.* (2003) Detection of protease activities with the mass-spectrometry-assisted enzyme-screening (MES) system. *Anal. Bioanal. Chem.* 377, 1102–1107
18 Russell, F.D. *et al.* (2004) Urotensin-II-converting enzyme activity of furin and trypsin in human cells *in vitro*. *J. Pharmacol. Exp. Ther.* 310, 209–214
19 Prosser, H.C. *et al.* (2006) Cardiovascular effects of native and non-native urotensin II and urotensin II-related peptide on rat and salmon hearts. *Peptides* 27, 3261–3268
20 Chartrel, N. *et al.* (2004) Biochemical characterization and immunohistochemical localization of urotensin II in the human brainstem and spinal cord. *J. Neurochem.* 91, 110–118
21 Totsune, K. *et al.* (2001) Role of urotensin II in patients on dialysis. *Lancet* 358, 810–811
22 Totsune, K. *et al.* (2003) Increased plasma urotensin II levels in patients with diabetes mellitus. *Clin. Sci.* 104, 1–5

23 Dschietzig, T. *et al.* (2002) Plasma levels and cardiovascular gene expression of urotensin-II in human heart failure. *Regul. Pept.* 110, 33–38
24 Maguire, J.J. *et al.* (2004) Cellular distribution of immunoreactive urotensin-II in human tissues with evidence of increased expression in atherosclerosis and a greater constrictor response of small compared to large coronary arteries. *Peptides* 25, 1767–1774
25 Shenouda, A. *et al.* (2002) Localization of urotensin-II immunoreactivity in normal human kidneys and renal carcinoma. *J. Histochem. Cytochem.* 50, 885–889
26 Maguire, J.J. *et al.* (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
27 Sugo, T. *et al.* (2003) Identification of urotensin II-related peptide as the urotensin II-immunoreactive molecule in the rat brain. *Biochem. Biophys. Res. Commun* 310, 860–868
28 Nothacker, H.P. *et al.* (1999) Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. *Nat. Cell Biol.* 1, 383–385
29 Russell, F.D. *et al.* (2003) Elevated plasma levels of human urotensin-II immunoreactivity in congestive heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 285, H1576–H1581
30 Charles, C.J. *et al.* (2005) Urotensin II: evidence for cardiac, hepatic and renal production. *Peptides* 26, 2211–2214
31 Protopopov, A. *et al.* (2000) Assignment of the GPR14 gene coding for the G-protein-coupled receptor 14 to human chromosome 17q25.3 by fluorescent *in situ* hybridization. *Cytogenet. Cell Genet.* 88, 312–313
32 Marchese, A. *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
33 Aiyar, N. *et al.* (2004) Differential levels of "urotensin-II-like" activity determined by radio-receptor and radioimmuno-assays. *Peptides* 25, 1339–1347
34 Jani, P.P. *et al.* (2013) The differential extraction and immunoluminometric assay of urotensin II and urotensin-related peptide in heart failure. *Peptides* 40, 72–76
35 Brancaccio, D. *et al.* (2015) An investigation into the origin of the biased agonism associated with the urotensin II receptor activation. *J. Pept. Sci* 21, 392–399
36 Billard, E. *et al.* (2018) Discovery of new allosteric modulators of the urotensinergic system through substitution of the urotensin II-related peptide (URP) phenylalanine residue. *J. Med. Chem.* 61, 8707–8716
37 Jarry, M. *et al.* (2010) The vasoactive peptides urotensin II and urotensin II-related peptide regulate astrocyte activity through common and distinct mechanisms: involvement in cell proliferation. *Biochem. J.* 428, 113–124
38 Douchez, A. *et al.* (2017) Design, synthesis, and biological assessment of biased allosteric modulation of the urotensin II receptor using achiral 1,3,4-benzotriazepin-2-one turn mimics. *J. Med. Chem.* 60, 9838–9859
39 Leprince, J. *et al.* (2008) Structure–activity relationships of urotensin II and URP. *Peptides* 29, 658–673
40 Chatenet, D. *et al.* (2013) Discovery of new antagonists aimed at discriminating UII and URP-mediated biological activities: insight into UII and URP receptor activation. *Br. J. Pharmacol.* 168, 807–821
41 Chatenet, D. *et al.* (2013) Development and pharmacological characterization of conformationally constrained urotensin II-related peptide agonists. *J. Med. Chem.* 56, 9612–9622
42 Brule, C. *et al.* (2014) Biased signaling regulates the pleiotropic effects of the urotensin II receptor to modulate its cellular behaviors. *FASEB J.* 28, 5148–5162
43 Patacchini, R. *et al.* (2003) Urantide: an ultrapotent urotensin II antagonist peptide in the rat aorta. *Br. J. Pharmacol.* 140, 1155–1158
44 Orbuch, M. *et al.* (1993) Discovery of a novel class of neuromedin B receptor antagonists, substituted somatostatin analogues. *Mol. Pharmacol.* 44, 841–850
45 Herold, C.L. *et al.* (2003) The neuromedin B receptor antagonist, BIM-23127, is a potent antagonist at human and rat urotensin-II receptors. *Br. J. Pharmacol.* 139, 203–207
46 Behm, D.J. *et al.* (2006) The peptidic urotensin-II receptor ligand GSK248451 possesses less intrinsic activity than the low-efficacy partial agonists SB-710411 and urantide in native mammalian tissues and recombinant cell systems. *Br. J. Pharmacol.* 148, 173–190
47 Behm, D.J. *et al.* (2008) Palosuran inhibits binding to primate UT receptors in cell membranes but demonstrates differential activity in intact cells and vascular tissues. *Br. J. Pharmacol.* 155, 374–386
48 Rakowski, E. *et al.* (2005) A role for urotensin II in restenosis following balloon angioplasty: use of a selective UT receptor blocker. *J. Mol. Cell. Cardiol.* 39, 785–791
49 Douglas, S.A. *et al.* (2005) Nonpeptidic urotensin-II receptor antagonists I: *in vitro* pharmacological characterization of SB-706375. *Br. J. Pharmacol.* 145, 620–635
50 Oh, K.S. *et al.* (2015) The orally active urotensin receptor antagonist, KR36676, attenuates cellular and cardiac hypertrophy. *Br. J. Pharmacol.* 172, 2618–2633
51 Oh, K.S. *et al.* (2017) A novel urotensin II receptor antagonist, KR-36996, improved cardiac function and attenuated cardiac hypertrophy in experimental heart failure. *Eur. J. Pharmacol.* 799, 94–102
52 Nishi, M. *et al.* (2019) A novel and highly potent urotensin II receptor antagonist inhibits urotensin II-induced pressure response in mice. *J. Cardiovasc. Pharmacol.* 73, 15–21
53 Zhang, R. *et al.* (2019) Urotensin II receptor antagonist reduces hepatic resistance and portal pressure through enhanced eNOS-dependent HSC vasodilatation in CCl4-induced cirrhotic rats. *Front. Med.* 13, 398–408
54 Trebicka, J. *et al.* (2008) Hemodynamic effects of urotensin II and its specific receptor antagonist palosuran in cirrhotic rats. *Hepatology* 47, 1264–1276
55 Kim, S.K. *et al.* (2010) Prediction of the three-dimensional structure for the rat urotensin II receptor, and comparison of the antagonist binding sites and binding selectivity between human and rat receptors from atomistic simulations. *ChemMedChem* 5, 1594–1608
56 Malagon, M.M. *et al.* (2008) Urotensin II and urotensin II-related peptide activate somatostatin receptor subtypes 2 and 5. *Peptides* 29, 711–720
57 Gibson, A. (1987) Complex effects of Gillichthys urotensin II on rat aortic strips. *Br. J. Pharmacol.* 91, 205–212
58 Bottrill, F.E. *et al.* (2000) Human urotensin-II is an endothelium-dependent vasodilator in rat small arteries. *Br. J. Pharmacol* 130, 1865–1870
59 Desai, N. *et al.* (2008) Urotensin II: a new pharmacologic target in the treatment of cardiovascular disease. *Cardiol. Rev.* 16, 142–153
60 Lu, Y. *et al.* (2002) Cardiovascular effects of urotensin II in different brain areas. *Peptides* 23, 1631–1635
61 Lin, Y. *et al.* (2003) Central cardiovascular action of urotensin II in spontaneously hypertensive rats. *Hypertens. Res.* 26, 839–845
62 Hood, S.G. *et al.* (2005) Cardiac actions of central but not peripheral urotensin II are prevented by beta-adrenoceptor blockade. *Peptides* 26, 1248–1256
63 Douglas, S.A. (2003) Human urotensin-II as a novel cardiovascular target: 'heart' of the matter or simply a fishy 'tail'? *Curr. Opin. Pharmacol.* 3, 159–167
64 Saetrum Opgaard, O. *et al.* (2000) Human urotensin II mediates vasoconstriction via an increase in inositol phosphates. *Eur. J. Pharmacol.* 406, 265–271
65 Ziltener, P. *et al.* (2002) Urotensin II mediates ERK1/2 phosphorylation and proliferation in GPR14-transfected cell lines. *J. Recept. Signal Transduct. Res.* 22, 155–168
66 Tasaki, K. *et al.* (2004) Mechanism of human urotensin II-induced contraction in rat aorta. *J. Pharmacol. Sci.* 94, 376–383
67 Rossowski, W.J. *et al.* (2002) Human urotensin II-induced aorta ring contractions are mediated by protein kinase C, tyrosine kinases and Rho-kinase: inhibition by somatostatin receptor antagonists. *Eur. J. Pharmacol.* 438, 159–170
68 Segain, J.P. *et al.* (2007) Urotensin II is a new chemotactic factor for UT receptor-expressing monocytes. *J. Immunol.* 179, 901–909
69 Russell, F.D. and Molenaar, P. (2004) Investigation of signaling pathways that mediate the inotropic effect of urotensin-II in human heart. *Cardiovasc. Res.* 63, 673–681
70 Fontes-Sousa, A.P. *et al.* (2007) Urotensin II acutely increases myocardial length and distensibility: potential implications for diastolic function and ventricular remodeling. *Naunyn. Schmiedebergs. Arch. Pharmacol.* 376, 107–115
71 Russell, F.D. *et al.* (2001) Cardiostimulant effects of urotensin-II in human heart *in vitro*. *Br. J. Pharmacol.* 132, 5–9
72 Gray, G.A. *et al.* (2001) Human urotensin II increases coronary perfusion pressure in the isolated rat heart: potentiation by nitric oxide synthase and cyclooxygenase inhibition. *Life Sci.* 69, 175–180
73 Guidolin, D. *et al.* (2010) The pro-angiogenic activity of urotensin-II on human vascular endothelial cells involves ERK1/2 and PI3K signaling pathways. *Regul. Pept.* 162, 26–32
74 Onan, D. *et al.* (2004) Urotensin II promotes hypertrophy of cardiac myocytes via mitogen-activated protein kinases. *Mol. Endocrinol.* 18, 2344–2354
75 Gruson, D. *et al.* (2010) Urotensin II induction of adult cardiomyocytes hypertrophy involves the Akt/GSK-3beta signaling pathway. *Peptides* 31, 1326–1333
76 Shi, H. *et al.* (2016) Urotensin II induction of neonatal cardiomyocyte hypertrophy involves the CaMKII/PLN/SERCA 2a signaling pathway. *Gene* 583, 8–14
77 Xu, J. *et al.* (2017) Role of PKA in the process of neonatal cardiomyocyte hypertrophy induced by urotensin II. *Int. J. Mol. Med.* 40, 499–504
78 Liu, W. *et al.* (2015) An investigation into the expression and mechanism of action of urotensin II in chronic pressure-overloaded rat hearts. *Mol. Med. Rep.* 12, 6626–6634
79 Sauzeau, V. *et al.* (2001) Human urotensin II-induced contraction and arterial smooth muscle cell proliferation are mediated by RhoA and Rho-kinase. *Circ. Res.* 88, 1102–1104

80 Djordjevic, T. *et al.* (2005) Human urotensin II is a novel activator of NADPH oxidase in human pulmonary artery smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 25, 519–525

81 Tamura, K. *et al.* (2003) Urotensin II-induced activation of extracellular signal-regulated kinase in cultured vascular smooth muscle cells: involvement of cell adhesion-mediated integrin signaling. *Life Sci.* 72, 1049–1060

82 Nguyen, T.T. *et al.* (2012) Presence of urotensin-II receptors at the cell nucleus: specific tissue distribution and hypoxia-induced modulation. *Int. J. Biochem. Cell. Biol* 44, 639–647

83 Doan, N.D. *et al.* (2012) Biochemical and pharmacological characterization of nuclear urotensin-II binding sites in rat heart. *Br. J. Pharmacol.* 166, 243–257

84 Fontes-Sousa, A.P. *et al.* (2009) Urotensin II-induced increase in myocardial distensibility is modulated by angiotensin II and endothelin-1. *Physiol. Res* 58, 653–660

85 Gruson, D. *et al.* (2006) Circulating urotensin II levels in moderate to severe congestive heart failure: its relations with myocardial function and well established neurohormonal markers. *Peptides* 27, 1527–1531

86 Ng, L.L. *et al.* (2002) Plasma urotensin in human systolic heart failure. *Circulation* 106, 2877–2880

87 Tzanidis, A. *et al.* (2003) Direct actions of urotensin II on the heart: implications for cardiac fibrosis and hypertrophy. *Circ. Res.* 93, 246–253

88 Zou, Y. *et al.* (2001) Urotensin II induces hypertrophic responses in cultured cardiomyocytes from neonatal rats. *FEBS Lett.* 508, 57–60

89 Quaile, M.P. *et al.* (2009) Direct inotropic effects of exogenous and endogenous urotensin-II: divergent actions in failing and nonfailing human myocardium. *Circ. Heart Fail.* 2, 39–46

90 Elmadbouh, I. *et al.* (2017) The value of urotensin II in patients with left-sided rheumatic valvular regurgitation. *Egypt Heart J.* 69, 133–138

91 Jumaah, S. *et al.* (2018) The role of human urotensin-II in patients with hypertrophic cardiomyopathy. *J. Immunoassay Immunochem* 39, 150–162

92 Bousette, N. *et al.* (2006) Urotensin-II receptor blockade with SB-611812 attenuates cardiac remodeling in experimental ischemic heart disease. *Peptides* 27, 2919–2926

93 Luo, S.Y. *et al.* (2016) Urotensin-receptor antagonist SB-710411 protects rat heart against ischemia-reperfusion injury via RhoA/ROCK pathway. *PLoS One* 11 e0146094

94 Duan, J.S. *et al.* (2019) Urotensin-II receptor antagonist SB-706375 protected isolated rat heart from ischaemia-reperfusion injury by attenuating myocardial necrosis via RhoA/ROCK/RIP3 signalling pathway. *Inflammopharmacology* . http://dx.doi.org/10.1007/s10787-019-00598-1 [Epub ahead of print]

95 Sondermeijer, B. *et al.* (2005) Effect of exogenous urotensin-II on vascular tone in skin microcirculation of patients with essential hypertension. *Am. J. Hypertens* 18, 1195–1199

96 Peng, H. *et al.* (2013) Association between human urotensin II and essential hypertension–a 1:1 matched case-control study. *PLoS One* 8, e81764

97 Zhu, L. *et al.* (2015) Association between essential hypertension and three vasoactive peptides, urotensin II, endothelin and adrenomedullin. *Clin. Exp. Hypertens.* 37, 604–608

98 Zhao, J. *et al.* (2017) Genetic polymorphisms of UTS2 rs2890565 Ser89Asn in cardiac hypertrophy in Chinese Han population. *Postgrad. Med. J.* 93, 406–413

99 Zhang, L.J. *et al.* (2017) Expressions of irisin and urotensin II and their relationships with blood pressure in patients with preeclampsia. *Clin. Exp. Hypertens.* 39, 460–467

100 Onat, A.M. *et al.* (2013) Urotensin inhibition with palosuran could be a promising alternative in pulmonary arterial hypertension. *Inflammation* 36, 405–412

101 Gao, S. *et al.* (2010) Urotensin II receptor antagonist attenuates monocrotaline-induced cardiac hypertrophy in rats. *Am. J. Physiol. Heart Circ. Physiol.* 299, H1782–H1789

102 Lee, J.H. *et al.* (2016) A urotensin II receptor antagonist, KR36676, decreases vascular remodeling and inflammation in experimental pulmonary hypertension. *Int. Immunopharmacol.* 40, 196–202

103 Pehlivan, Y. *et al.* (2014) Palosuran treatment effective as bosentan in the treatment model of pulmonary arterial hypertension. *Inflammation* 37, 1280–1288

104 Wang, Y. *et al.* (2019) Urantide improves the structure and function of right ventricle as determined by echocardiography in monocrotaline-induced pulmonary hypertension rat model. *Clin. Rheumatol.* 38, 29–35

105 Mei, Y. *et al.* (2011) Urantide alleviates monocrotaline induced pulmonary arterial hypertension in Wistar rats. *Pulm. Pharmacol. Ther.* 24, 386–393

106 Bousette, N. *et al.* (2004) Increased expression of urotensin II and its cognate receptor GPR14 in atherosclerotic lesions of the human aorta. *Atherosclerosis* 176, 117–123

107 Hassan, G.S. *et al.* (2005) Expression of urotensin-II in human coronary atherosclerosis. *Peptides* 26, 2464–2472

108 Cirillo, P. *et al.* (2008) Human urotensin II induces tissue factor and cellular adhesion molecules expression in human coronary endothelial cells: an emerging role for urotensin II in cardiovascular disease. *J. Thromb. Haemost.* 6, 726–736

109 Watanabe, T. *et al.* (2006) Human urotensin-II potentiates the mitogenic effect of mildly oxidized low-density lipoprotein on vascular smooth muscle cells: comparison with other vasoactive agents and hydrogen peroxide. *Hypertens. Res* 29, 821–831

110 Watanabe, T. *et al.* (2005) Human urotensin II accelerates foam cell formation in human monocyte-derived macrophages. *Hypertension* 46, 738–744

111 Albanese, I. *et al.* (2016) The urotensin II system and carotid atherosclerosis: a role in vascular calcification. *Front. Pharmacol.* 7, 149

112 Zhao, J. *et al.* (2013) The urotensin II receptor antagonist, urantide, protects against atherosclerosis in rats. *Exp. Ther. Med.* 5, 1765–1769

113 Watson, A.M. *et al.* (2013) Urotensin II receptor antagonism confers vasoprotective effects in diabetes associated atherosclerosis: studies in humans and in a mouse model of diabetes. *Diabetologia* 56, 1155–1165

114 You, Z. *et al.* (2012) Genetic and pharmacological manipulation of urotensin II ameliorate the metabolic and atherosclerosis sequalae in mice. *Arterioscler. Thromb. Vasc. Biol.* 32, 1809–1816

115 Kim, Y.A. *et al.* (2016) Blockade of urotensin II receptor prevents vascular dysfunction. *Biomol. Ther.* 24, 523–528

116 Kim, T.H. *et al.* (2017) A novel urotensin II receptor antagonist, KR-36996 inhibits smooth muscle proliferation through ERK/ROS pathway. *Biomol. Ther.* 25, 308–314

117 Silvestre, R.A. *et al.* (2009) Characterization of the insulinostatic effect of urotensin II: a study in the perfused rat pancreas. *Regul. Pept.* 153, 37–42

118 Silvestre, R.A. *et al.* (2004) Urotensin-II is present in pancreatic extracts and inhibits insulin release in the perfused rat pancreas. *Eur. J. Endocrinol.* 151, 803–809

119 Wenyi, Z. *et al.* (2003) Role of urotensin II gene in genetic susceptibility to type 2 diabetes mellitus in Japanese subjects. *Diabetologia* 46, 972–976

120 Okumus, S. *et al.* (2012) Association between Thr21Met and Ser89Asn polymorphisms of the urotensin-II (UTS2) gene, diabetes mellitus, and diabetic retinopathy. *Curr. Eye Res.* 37, 921–929

121 Zhao, Y. *et al.* (2018) Urotensin-II gene rs228648 polymorphism associated with the risk of diabetes mellitus. *Biosci. Rep.* 38 BSR20181275

122 Clozel, M. *et al.* (2006) The urotensin-II receptor antagonist palosuran improves pancreatic and renal function in diabetic rats. *J. Pharmacol. Exp. Ther.* 316, 1115–1121

123 Vogt, L. *et al.* (2010) Effect of the urotensin receptor antagonist palosuran in hypertensive patients with type 2 diabetic nephropathy. *Hypertension* 55, 1206–1209

124 Sidharta, P.N. *et al.* (2006) Pharmacodynamics and pharmacokinetics of the urotensin II receptor antagonist palosuran in macroalbuminuric, diabetic patients. *Clin. Pharmacol. Ther.* 80, 246–256

125 Sidharta, P.N. *et al.* (2009) Effect of the urotensin-II receptor antagonist palosuran on secretion of and sensitivity to insulin in patients with Type 2 diabetes mellitus. *Br. J. Clin. Pharmacol* 68, 502–510

126 Rahimi, R. *et al.* (2018) Silymarin ameliorates expression of urotensin II (U-II) and its receptor (UTR) and attenuates toxic oxidative stress in the heart of rats with type 2 diabetes. *Biomed. Pharmacother.* 101, 244–250