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Novel insights into the role of urotensin II in cardiovascular disease

João Paulo Pereira de Castro

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Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

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Autor:

João Paulo Pereira de Castro, sexto ano do Mestrado Integrado em Medicina no Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP), Porto, Portugal

Endereço eletrónico: chaoppc@gmail.com

Orientadora:

Prof. Doutora Ana Patrícia Nunes Fontes de Sousa, DVM, PhD, Laboratório de Farmacologia e Neurobiologia, Centro de Investigação Farmacológica e Inovação Medicamentosa (MedInUP), Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP), Porto, Portugal

Coorientadora:

Prof. Doutora Carmen Brás Silva, Departamento de Cirurgia e Fisiologia, RD, PhD, UnIC - Unidade de Investigação Cardiovascular, Faculdade de Medicina da Universidade do Porto (FMUP), Porto, Portugal

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Orientadora: Ana Patrícia Fontes do Ó

Coorientadora: Carmen Brás Silva Ribeiro

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Highlights

- Urotensin II is a cyclic undecapeptide involved in cardiovascular regulation.
- Pseudo-irreversible binding to UT receptor may explain urotensin II low efficacy.
- Receptor localization and biased nature support pleiotropic effects of urotensin II.
- Urotensin II antagonists have been developed with promising results in animal models.

Abstract

With actual lifestyle and gradual aging of the population, cardiovascular diseases will become more prevalent. Thereby, it is essential to search and develop new drugs with impact in mortality and control of the disease. The urotensinergic system has not a clearly well-understood function but it has been implicated in cardiovascular regulation, with promising therapeutic applications. This work provides an up-to-date critical and comprehensive overview about urotensin II cardiovascular effects in health and disease induced by its receptor activation. Limited outcomes of the urotensinergic antagonism in humans may be explained by certain properties of this system that should be considered in future investigations to a better comprehension of its role and possible new therapeutic approaches.

Metodologia

As ferramentas utilizadas para a seleção da bibliografia consistiram na base dados MEDLINE-Pubmed. As referências bibliográficas são constituídas, na sua maioria, por artigos originais. Alguns artigos de revisão foram incluídos por formularem hipóteses originais que permitem explicar determinadas características da urotensina II ou por compilarem informações também relevantes. Quanto aos artigos 93 e 94, estes continham figuras e tabelas que serviram de base para a elaboração de outras ilustrações pertinentes para complementar e elucidar a informação apresentada. As figuras 1 e 2 foram elaboradas com recurso ao banco de imagens on-line Servier Medical Art (http://smart.servier.com).

No motor de busca, foram utilizadas as seguintes palavras-chave, isoladas ou em combinação: *urotensin II*, *human urotensin II*, urotensin II *receptor*, *GPR14*, *contractility*, *hypertrophy*, *proliferation*, *heart failure*, *hypertension*, *atherosclerosis*, *diabetes mellitus*, *antagonist e palosuran*. Foram incluídos todos os artigos considerados relevantes para a concretização da revisão bibliográfica proposta. A pesquisa bibliográfica foi realizada entre os meses de Setembro de 2018 e Abril de 2019. Os artigos selecionados, escritos na língua inglesa, foram publicados entre 1980 e 2019.

A organização do trabalho segue a seguinte estrutura: *highlights* (conjunto de frases que pretendem resumir os aspetos essenciais do artigo), *abstract* (texto que pretende atrair a atenção e demonstrar a importância do tema), introdução, desenvolvimento e conclusão. Pretendeu-se abordar, de forma sintética, mas com a devida contextualização, as descobertas recentes sobre um tópico da área das Ciências Cardiovasculares.

Lista de abreviaturas

ACAT-1 – Acetyl-Coenzyme A acetyltransferase 1
ANP – Atrial natriuretic peptide
CAMs – Cellular adhesion molecules
CHF – Congestive heart failure
CNS – Central nervous system
DAG – Diacylglycerol
EDHF – Endothelium-derived hyperpolarizing factor
EGFR – Epidermal growth factor receptor
ET-1 – Endothelin-1
GPCR – G protein-coupled receptor
hUII – Human urotensin II
IP_3 – Inositol trisphosphate (IP_3)
MLC – Myosin light chain
NO – Nitric oxide
PAH – Pulmonary arterial hypertension
PAI-1 – Plasminogen activator inhibitor-1
PKA – Protein kinase A
PKC – Protein kinase C
PLC – Phospholipase C
PLN – Phospholamban
NADPH – Nicotinamide adenosine dinucleotide phosphate
NT-proBNP – Pro-brain natriuretic peptide
NYHA – New York Heart Association
ROS – Reactive oxygen species
SENR – Sensory epithelial neuropeptide-like receptor
UII – Urotensin II
UCE – Urotensin-converting enzyme
URP – Urotensin-related peptide
VSMCs – Vascular smooth muscle cells

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1. Introduction

Urotensin II (UII) is a peptide originally found in caudal neurosecretory system of the teleost fish *Gillichthys mirabilis*, in a structure named urophysis, in which other peptides with vasoactive properties coexist [1]. As this organ is exclusive of teleost fish, despite some structural and functional similarity with hypothalamus-hypophysis system, it was initially thought that UII was exclusive of fish and non-mammalian vertebrates. Nevertheless, the presence of homologue peptides in vertebrate species, including in humans, was later proven [2]. Nowadays, we know that UII has multiple biological effects on the organism not totally well-understood, consistent with its wide distribution in the body. Because of the vasoactive properties and significant expression of the peptide and its receptors in the cardiomyocytes and arterial vasculature, particularly in patients with cardiovascular diseases, its cardiovascular effects are of special interest [3].

2. Molecular structure and biosynthesis of urotensin II

Human urotensin II (hUII) is derived from a protein precursor, coded by UTS2 gene located on chromosome 1p36 [4]. In humans, two of those proteins were identified, formed by 124 and 139 residues that result from alternative splicing [2, 5]. 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 mass-spectrometry-assisted enzyme-screening system, in porcine renal tissue [6]. 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 [7].

The mature peptide (Figure 1) is formed by 11 amino acids, with a C-terminal region that includes a cyclic hexapeptide sequence (Cys-Phe-Trp-Lys-Tyr-Cys) due to 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 [2, 5]. The conserved sequence, responsible for its biological activity, explains the fact why the non-native UII (species non-specific form) is active in different species. Nonetheless, it was also found that non-native UII may produce different biological responses in comparison with its native form, suggesting that N-terminal region is essential to receptor activation [8].

3. Distribution and origin of urotensin II

In the central nervous system (CNS), hUII pre-pro-hormone mRNA was found by northern dot blot in spinal cord, more exactly in motoneurons, and in medulla oblongata, although with a lower expression compared to the signal found in spinal cord [2]. In another study that used RT-PCR (reverse transcription polymerase chain reaction) to identify protein precursors mRNA in patients with chronic kidney disease, hUII expression was also identified in cerebral cortex, hypothalamus and hypophysis [9].

In the heart, hUII precursor protein was detected in both atrial and ventricular cardiomyocytes [10, 11]. 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) [12]. Regarding the kidney, it was found high expression of hUII 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 last tissues. The same study also revealed immunoreactivity in endothelial cells from kidney vasculature, except for the veins [13]. The presence of the peptide precursor mRNA was found in other peripheral tissues, namely in liver, spleen, thymus, small intestine, stomach, prostate, ovaries, pancreas, adrenal gland and skeletal muscle tissue [2, 14].

It is important to highlight that the data about hUII distribution is highly variable according to the different studies, even if they use the same methodology. As an example, 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 spinal cord and medulla oblongata, even though its presence in heart is reported in other studies [14, 15]. Another aspect to consider is the fact that hUII detection may 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). These results demonstrate that the peptide may have an important role in cardiovascular pathophysiology [3].

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/paracrine agent. A study that measured its concentration in plasma from patients with CHF reported higher levels in aortic root compared to the levels found in pulmonary artery, indicating a putative cardiopulmonary synthesis of the peptide [16]. Another study that used anesthetized sheep also demonstrated an

arteriovenous gradient in heart, liver and kidney, revealing that these organs may be responsible for UII production [17].

4. The UT receptor and its ligands beyond UII

From a reverse pharmacology approach, it was found that UII selectively binds to a rat orphan receptor: GPR14, also named by SENR (sensory epithelial neuropeptide-like receptor) [5]. Currently known as UT receptor, this receptor is coded by UTS2R gene, located on 17q25.3 human chromosome [18]. This intronless gene produces a protein formed by 389 residues, sharing 75% of its structure with rat's receptor [5]. UT receptor is a class A G protein-coupled receptor (GPCR), from rhodopsin family, and shares high similarity in its amino acid sequence with somatostatin (similarity of 27% with SSTR4 receptor) and opioids receptors (similarity of 25% with κ receptor and 26% with μ and δ) [19].

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 systems (atria and ventricles, endothelial cells and VSMCs from arterial vasculature) [5, 9, 14].

a. UII endogenous ligands and antagonists

The endogenous selective ligands for human UT receptor are UII and urotensin-related peptide (URP). URP is a peptide formed by 8 amino acids, with the same cyclic hexapeptide sequence in C-terminal region responsible for its biological activity, forming the urotensinergic system with UII and UT receptor. Protein precursors from UII and URP are not similar, sharing only 18.8% of their amino acid sequence [20]. However, due to the almost identical primary structure of their mature proteins, antibodies used in immunohistochemical studies are not able to clearly discriminate these two peptides, making it difficult to measure their concentration in body fluids [21]. Through a solid phase extraction technique based on the more hydrophobic nature of URP compared to 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 [22].

The urotensinergic system is a good example of the concept of biased agonism. Although UII and URP, with similar primary structures, are known as endogenous ligands of the same

shared receptor, differences as subtle as their variable N-terminal regions may activate UT receptor and induce different conformational changes which will consequently modulate a divergent subset of signalling pathways [23]. Thereby, it becomes important to identify pharmacological tools that discriminate the effects produced by each endogenous ligand upon activation of the receptor.

The UT receptor antagonists can be classified as peptidic or non-peptidic. The non-peptidic (for example, palosuran, SB-611812, SB-657510, KR-36676, KR-36996 or DS37001789) have more favourable pharmacokinetic properties because of their higher bioavailability and tissue distribution, whereas peptidic drugs (such as urantide, BIM-23127 or SB-710411) tend to reveal lower toxicity but greater selectivity for the receptor [24-27].

Palosuran (ACT-058362) was the first non-peptidic antagonist of the UT receptor with positive results in animal models but not so effective in humans. It has proven to be orally active and safe to humans at a dose up to 500 mg b.i.d., without serious adverse effects [28]. However, its use also revealed that UT receptor activation may have different functions between species and a highly variable response to UII in humans, requiring the developing of more potent antagonists. DS37001789, a newly developed antagonist and piperazine derivative, showed to be more potent than palosuran, without having variable efficacy between species [27].

5. Biological effects and signaling pathways of urotensin II

a. Vasoconstriction and vasodilation

One of the first studies about the cardiovascular effects of hUII revealed its strong vasoconstriction action in rat isolated 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 UII were not able to constrict other arteries (abdominal, femoral and renal arteries) [5]. In another *in vitro* study, hUII promoted vasoconstriction with a potency 50 times greater than ET-1 in human coronary, mammary and radial arteries, though its efficacy was highly variable; in fact, ET-1 effectively constricted all arteries, whereas hUII failed to induce a response in approximately 30% of them [14].

The great variability of UII in vascular tone regulation is also patent in its vasodilator action. Vasodilation mediated by UT receptors located on endothelium is endothelium-dependent, whereas vasoconstriction is an endothelium-independent process, mediated by receptors located on VSMCs [29]. A previous study evaluated the effect of hUII on isolated segments of several rat vessels. The peptide caused vasoconstriction of the thoracic aorta and left

anterior descending coronary arteries, with a contractile response enhanced by the removal of the endothelium in the latter arteries. In contrast, hUII caused potent vasodilation in precontracted mesenteric arteries of 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 may be associated with the variable levels of UT receptor expression [30]. It has been suggested that vasoactive effects of UII may be related to the blood vessel caliber: in small vessels occurs an endothelium mediated vasodilation, whereas in large vessels the predominant response is a VSMCs mediated vasoconstriction [31].

The mechanisms underlying the great variability and low efficacy of UII are not entirely known but may be explained by the spare receptor reserve hypothesis. According to Douglas et al. [32], most UT receptors are occupied by endogenous UII, due to the pseudo-irreversible binding and slow dissociation of the ligand-receptor complex, explaining, therefore, why there is low reserve of free receptor compared to the circulant levels of the peptide [32].

b. Main signaling pathways and other properties of urotensin II

The main intracellular signaling pathway that culminates in vasoconstriction involves phospholipase C (PLC) pathway, through UT receptor activation which is primarily coupled to $G\alpha_{q/11}$ protein (Figure 2), although it can be also coupled to $G\alpha_{i/0}$ protein [33, 34]. PLC leads to inositol trisphosphate (IP_3) and diacylglycerol (DAG) formation, by hydrolysis of specific components of cell membrane (phosphatidylinositol-4-5 bisphosphate) [33]. 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, occurring, at the same time, opening of non-selective cation and voltage-dependent L-type calcium channels. This process results in the vasoconstriction mediated by Ca^{2+}/calmodulin/myosin light chain system [33, 35]. Vasoconstriction may also be mediated by protein kinase C (PKC) factor (activated by DAG and cytoplasmatic calcium mobilization) and RhoA/ROCK pathway, through a mechanism of calcium sensitization that promotes myosin light chain (MLC) phosphorylation [36].

PKC was also identified as being involved in the positive inotropic effect promoted by UII in isolated human right atrial tissue [37], but not in UII-induced decrease in myocardial stiffness [38]. The peptide was considered to have a potent inotropic activity in human atrium and ventricle, even higher than ET-1 [39]. 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 nonhuman primates [5, 38].

Vasodilation mediated by UT receptors located on endothelium seems to promote synthesis and release of NO, endothelium-derived hyperpolarizing factor (EDHF), prostacyclin and other factors derived from phospholipase A2 pathway [30, 40]. UII has angiogenic, hypertrophic and mitogenic/proliferative actions as well. Angiogenesis is mediated by ERK1/2 and PI3K factors (but not by p38-MAPK) [41]. 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 [42]. Hypertrophy may also be mediated by Akt/GSK-3β, CaMKII and protein kinase A (PKA) signaling pathways. Both CaMKII and PKA play an important role in mediating intracellular Ca^{2+} influx, which is regulated by phospholamban (PLN) and SERCA pump [43-45]. PKA was also found to be involved in the process of myocardial fibrosis, stimulating the synthesis of collagen I and III [46]. Concerning mitogenic/proliferative action, it requires the activity of factors like RhoA/ROCK pathway, ERK1/2 and NADPH (nicotinamide adenosine dinucleotide phosphate) production. NAPDH promotes reactive oxygen species (ROS) production and potentiates MAPK action (mainly ERK1/2), pro-angiogenic factors from PI3K-Akt pathway and vascular remodeling factors (such as plasminogen activator inhibitor-1, PAI-1) [47-49].

It was previously accepted that GPCRs would have a localization restricted to cell membrane, nevertheless it was found that those receptors could also take place in the nucleus, as happens with UT receptor. The nuclear localization of this receptor implies ligand internalization, through a receptor-independent mediated endocytosis, which could partially explain the pseudo-irreversible binding. This intracrine mechanism, complementary to autocrine/paracrine signaling, could originate new intracellular signaling cascades, but some more investigation is required in order to assess if these two systems (intracrine and autocrine/paracrine) work independently or in synergy [50]. The distribution of nuclear UT receptors, assessed in rat and monkey tissues, is apparently restricted to the heart and CNS [51].

6. Role of urotensin II in cardiovascular disease

a. Heart failure

Multiple neurohormonal factors are known to be implicated in heart failure, namely renin-angiotensin-aldosterone and adrenergic systems, which represent nowadays the main therapeutic targets. UII has 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 [52]. 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 [3, 16, 53, 54]. Moreover, the peptide was also found to be related to the NYHA (New York Heart Association) functional class and inversely correlated with left ventricular ejection fraction [53].

The neurohormonal mechanisms that compensate the loss of function in dysfunctional hearts, through positive inotropic and peripheral vasoconstriction effects, are beneficial in short term. However, chronic activation of these mechanisms promotes cardiac remodeling, an increase in oxygen consumption and an energetic deficit state (which characterizes heart failure), contributing to the deterioration of the contractile function. UII induces myocardial fibrosis by increasing fibronectin, type I and III procollagen gene expression in neonatal cardiac fibroblasts cultures from rats, as well as myocardial hypertrophy by increasing cardiomyocyte growth and myofibril organization [55, 56]. The peptide also has positive inotropic activity, although it induced negative inotropic responses in patients with advanced heart failure, indicating that UII may have opposite contractility effects in failing and nonfailing hearts [57].

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, favouring its use as a biomarker [54]. UII may also be used as a biomarker in patients with rheumatic valvular diseases, complementary to echocardiographic evaluation, with an important prognostic role [58]. Another positive correlation was found as serum hUII levels were markedly elevated in human patients with left ventricular hypertrophy secondary to hypertrophic cardiomyopathy [59].

UII involvement in cardiac remodeling motivated the development of UT antagonists with potential therapeutic properties (Table I). KR-36996, an UT receptor antagonist, improved cardiac functional parameters (ejection fraction and fractional shortening) and decreased interstitial fibrosis and cardiomyocyte hypertrophy in rats with chronic heart failure [26]. KR-36676 also revealed cardiac anti-hypertrophic properties in rats [25]. Additionally, the UT receptor blockade with SB-611812 reduced myocardial fibrosis and collagen deposition *in vivo* and inhibited myocardial fibroblast proliferation *in vitro* [60]. Another UT antagonist, SB-710411, decreased myocardial ischemia-reperfusion injury in rat myocardium and modulated collagen synthesis and accumulation in rat aortic VSMCs [61].

b. 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 [62]. 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 NO metabolite levels, which were measured to evaluate endothelial dysfunction [63]. Regarding echocardiographic parameters of systemic hypertension severity, namely interventricular septal thickness, left ventricular posterior wall thickness and left ventricular mass index, they were positively correlated with plasma UII [64].

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 [65]. Other studies claimed a possible role for UII in preeclampsia, since the peptide expression was positively correlated with systolic blood pressure and urinary protein level and up-regulated in placenta of patients with this hypertensive disorder of pregnancy [66].

c. 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 TGF-β1, and ET-1 [67]. 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 ANP (atrial natriuretic peptide) secretion, which is a vasodilator of the pulmonary circulation [68].

KR-36676, a novel UT antagonist, prevented pulmonary hypertension progression and pulmonary vascular remodeling in rats with PAH induced by monocrotaline (Table I) [69]. Palosuran effectively improved hemodynamic, histological and biochemical parameters in PAH rats [67]. In another study, the same antagonist was at least as effective as bosentan, an antagonist of ET-1 receptors and standard therapy for PAH [70]. Urantide, a peptide UT antagonist, also improved PAH-related echocardiographic parameters in both early and late treatment group animals [71]. In a previous study, urantide already revealed to be effective in improving pulmonary arterial vascular remodeling and vasodilating the intralobar pulmonary arteries (with possible association with NO pathway) in both control group and monocrotaline induced PAH rats [72].

d. 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 [73]. 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 [74].

Atherosclerosis is a disease involving endothelial injury and recruitment of inflammatory cells, cytokines and growth factors to the arterial tunica intima, leading to the formation of an atheromatous plaque with risk of rupture. This process is potentiated by UII which up-regulates 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 [75]. The peptide also promotes atherosclerosis by inducing VSMCs proliferation (acting synergistically with mildly oxidized LDL), activating fibroblasts and accelerating macrophage-derived foam cells formation due to the upregulation of ACAT-1 (Acetyl-Coenzyme A acetyltransferase 1) expression [76, 77]. Another study also revealed that UII and URP stimulate osteogenic differentiation and calcium deposition in VSMCs which explains the higher expression of UII, URP and UT receptor in unstable plaques compared to stable plaques [78]. Moreover, urantide was effective to protect against aortic atherosclerosis progression in rats, which is consistent with the involvement of urotensinergic system in atherosclerosis (Table II) [79].

Diabetes is one of the most important factors that contributes to endothelial dysfunction and atherosclerosis. UII is also upregulated in diabetes-associated atherosclerosis as it was demonstrated in a study that revealed high UII staining in carotid endarterectomies from diabetic patients in comparison to non-diabetic individuals. In the same study, SB-657510, a non-peptide UT antagonist, was able to delay diabetes-associated plaque development in mice [80]. In another study, the same antagonist improved lipid and glycaemic profile, reduced weight gain and visceral fat and prevented oxidative stress and cytokines formation, corroborating its effects on delaying atherosclerosis progression [81]. Two other UT receptor antagonists, KR-36676 and KR-36996, were also studied to assess their effects on vascular dysfunction. Both antagonists inhibited VSMCs proliferation *in vitro* and neointima formation *in vivo* [82, 83].

7. Important risk factors for cardiovascular disease

a. Diabetes mellitus and diabetic nephropathy

UII may 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 [84]. 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 [10]. Single nucleotide polymorphisms in UTS2 gene were associated with greater susceptibility to type 2 diabetes mellitus and diabetic retinopathy [85, 86]. Yet, the opposite was also found as one polymorphism in the same gene was associated with reduced risk of diabetes mellitus [87].

Long-term blockage of UT receptor with palosuran was effective to improve survival, increase insulin secretion and improve glycaemic and lipid profile in diabetic mice, which were previously subjected to streptozotocin injection to induce pancreatic β cell destruction and unilateral nephrectomy to accelerate renal dysfunction (Table II). Regarding its effects on diabetic nephropathy, it managed to increase renal blood flow and delay the development of proteinuria [88]. However, in a randomized placebo-controlled study, the same antagonist did not affect albuminuria, blood pressure or renal blood flow in hypertensive patients with type 2 diabetic nephropathy [89]. Other studies that investigated the effect of palosuran on macroalbuminuric and diabetic patients revealed that it was effective to decrease 24-hour urinary albumin excretion rate, although the decrease in the group with moderately to severely impaired renal function did not reach statistical significance. In the same study, the antagonist did not improve other renal function parameters (glomerular filtration rate or renal blood flow) [90]. The antagonism of the urotensinergic system with the same drug also did not show any effects on insulin sensitivity and glucose regulation in diet-treated patients with type 2 diabetes [91].

Silymarin, a flavonoid mixture with antioxidant properties, reduced the expression of UII and its receptor in the heart tissues of diabetic rats, improved glycaemic parameters and almost completely normalized serum lipid profile. This antidiabetic effect may be explained by the inhibition of ROS formation that potentiate heart disease development and pancreatic β-cell destruction [92].

8. Conclusion

Since the isolation of UII from the teleost fish and its discovery in mammals, the cyclic undecapeptide has been implicated in the regulation of multiple physiological systems and

pathological conditions. Given the potential of new therapeutic approaches to cardiovascular disease, several antagonists have been developed with promising results in animal models. However, the limited efficacy of UT receptor antagonists in some human diseases, such as diabetic nephropathy [89], may be attributed to biased agonism of U-II and URP as they interact with UT receptor or their inability to reach and block the action of the receptors with nuclear localization [23, 50]. These properties highlight the importance of searching new pharmacological tools to discriminate the biological effects of each endogenous UT receptor ligand and understand the effects of nuclear UT receptor activation.

9. Conflicts of interest

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

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Figures 1 and 2 use templates from Servier Medical Art.

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TABLE I: Results of UT antagonism in heart failure and pulmonary hypertension. Different antagonists were used to assess its effects on cardiovascular diseases.

	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		[26]
	KR-36676	C57BL/6 mice (transverse aortic constriction) and Sprague-Dawley rats (coronary ligation)	↓ Formation of actin stress fibres ↓ Left ventricle hypertrophy		[25]
	SB-611812	Lewis rats (coronary ligation)	↓ Myocardial and endocardial fibrosis ↓ LVEDP[b] ↓ 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).	[60]
	SB-710411	Sprague-Dawley rats (coronary ligation)	↓ Cardiac I/R[c]-induced infarct size and histological damage Inhibited ST-segment increase in ECG[d] ↓ LDH[e], CK-MB[f] and cTnI[g] levels	Acts as a vasoconstrictor in monkey arteries.	[61]
Pulmonary hypertension	KR-36676	Sprague-Dawley rats (MCT-IPHM[a])	↓ Pulmonary vascular remodeling ↓ Right ventricle remodeling (hypertrophy/myocardial fibrosis)	Anti-proliferative and anti-inflammatory actions by inhibiting ERK1/2 and NF-κB pathway.	[69]
	Palosuran	Wistar albino rats (MCT-IPHM)	↓ ET-1[h], UII[i] and TGF-β1[j] levels ↓ mPAP[k], RVHI[l] and RVMI[m] ↓ Arteriole wall thickness ↓ Perivascular connective tissue thickness	No effects on mean arterial pressure.	[67]
	Urantide	Wistar rats (MCT-IPHM)	↓ mPAP and SPAP[n] ↓ 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.	[71]

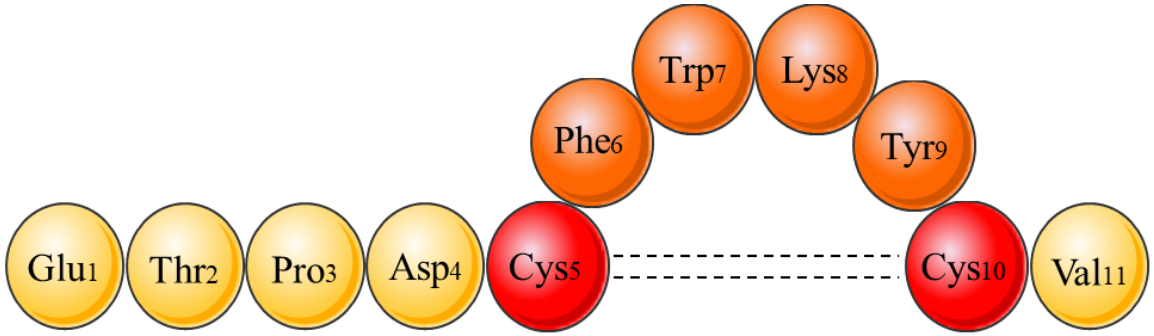
MCT-IPHM[a] – Monocrotaline induced pulmonary hypertension model; LVEDP[b] – Left ventricular end-diastolic pressure; I/R[c] – Ischaemia/reperfusion; ECG[d] – electrocardiogram; LDH[e] – Lactate dehydrogenase; CK-MB[f] – Creatine kinase-muscle/brain; cTnI[g] – Troponin I; ET-1[h] – Endothelin-1; UII[i] – urotensin II; TGF-β1[j] – Transforming growth factor beta 1; mPAP[k] – Main pulmonary arterial pressure; RVHI[l] – Right ventricular hypertrophy index; RVMI[m] – Right ventricular mass index; SPAP[n] – Systolic pulmonary arterial pressure.

TABLE II: Results of UT antagonism in atherosclerosis and diabetes mellitus/diabetic nephropathy. Different antagonists were used to assess its effects on cardiovascular diseases. The grey area corresponds to studies carried out in human patients.

	Treatment	Model and species	Outcome	Comments	Refs
Atherosclerosis/Vascular dysfunction	Urantide	Wistar rats (on high fat diet)	↓ Progression of atherosclerosis ↓ TG[e], TC[f], HDL[g] and LDL[h] levels		[79]
	SB-657510	*Apoe* KO[a] mice	↓ Progression of aortic atherosclerosis	Small decrease in triacylglycerol levels but no effects on other lipid parameters.	[80]
	SB657510A	*Apoe* KO mice (on high fat diet)	↓ Body weight gain ↓ Blood pressure, serum hyperlipidaemia and hyperglycaemia ↓ Cytokines and aortic atherosclerosis Stabilization of the plaque	Aortic oxidative stress was reduced by ERK1/2[k] and p44/42-MAPK[l] pathway inhibition.	[81]
	KR-36676	C57BL/6 mice (common carotid artery ligation)	↓ VSMCs[i] proliferation ↓ Neointima formation	Inhibition of constriction in isolated aortic ring.	[82]
	KR-36996	C57BL/6 mice (common carotid artery ligation)	↓ VSMCs proliferation ↓ Neointima formation	These inhibitory effects revealed greater potency than GSK-1440115 (another UT antagonist).	[83]
Diabetes mellitus/Diabetic nephropathy	Palosuran	Wistar rats (STZ[b] injection and unilateral nephrectomy)	↑ Survival ↑ Insulin concentration ↓ Hyperglycaemia and glycosylated haemoglobin ↓ Serum Lipids ↓ Proteinuria and renal dysfunction	Little effect on blood pressure or heart rate.	[88]
	Palosuran (125 mg b.i.d.)	54 hypertensive, macroalbuminuric, DM2[c] patients	No effects on albuminuria, blood pressure, glomerular filtration rate or renal plasma flow	4-week treatment may have been too short.	[89]
	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.	[90]
	Palosuran (125 mg b.i.d.)	20 diet-treated DM2 patients	No effects on insulin secretion or sensitivity and daily blood glucose levels		[91]
	Silymarin	Wistar rats (STZ-NIC[d] injection)	↓ Oxidative stress ↓ FBS[j] level and ↑ insulin concentration Improvement of lipid profile Prevented diabetes-induced weight loss	Reduced cardiac UII and UT receptor expression.	[92]

KO[a] – knockout; STZ[b] – Streptozotocin; DM2[c] – Type 2 diabetes mellitus; STZ-NIC[d] – Streptozotocin and nicotinamide; TG[e] – Triglycerides; TC[f] – Total cholesterol; HDL[g] – High-density lipoprotein; LDL[h] – Low-density lipoprotein; VSMCs[i] – Vascular smooth muscle cells; FBS[j] – Fasting blood sugar; ERK1/2[k] – Extracellular signal-regulated kinase; MAPK[l] – mitogen-activated protein kinase phosphorylation.

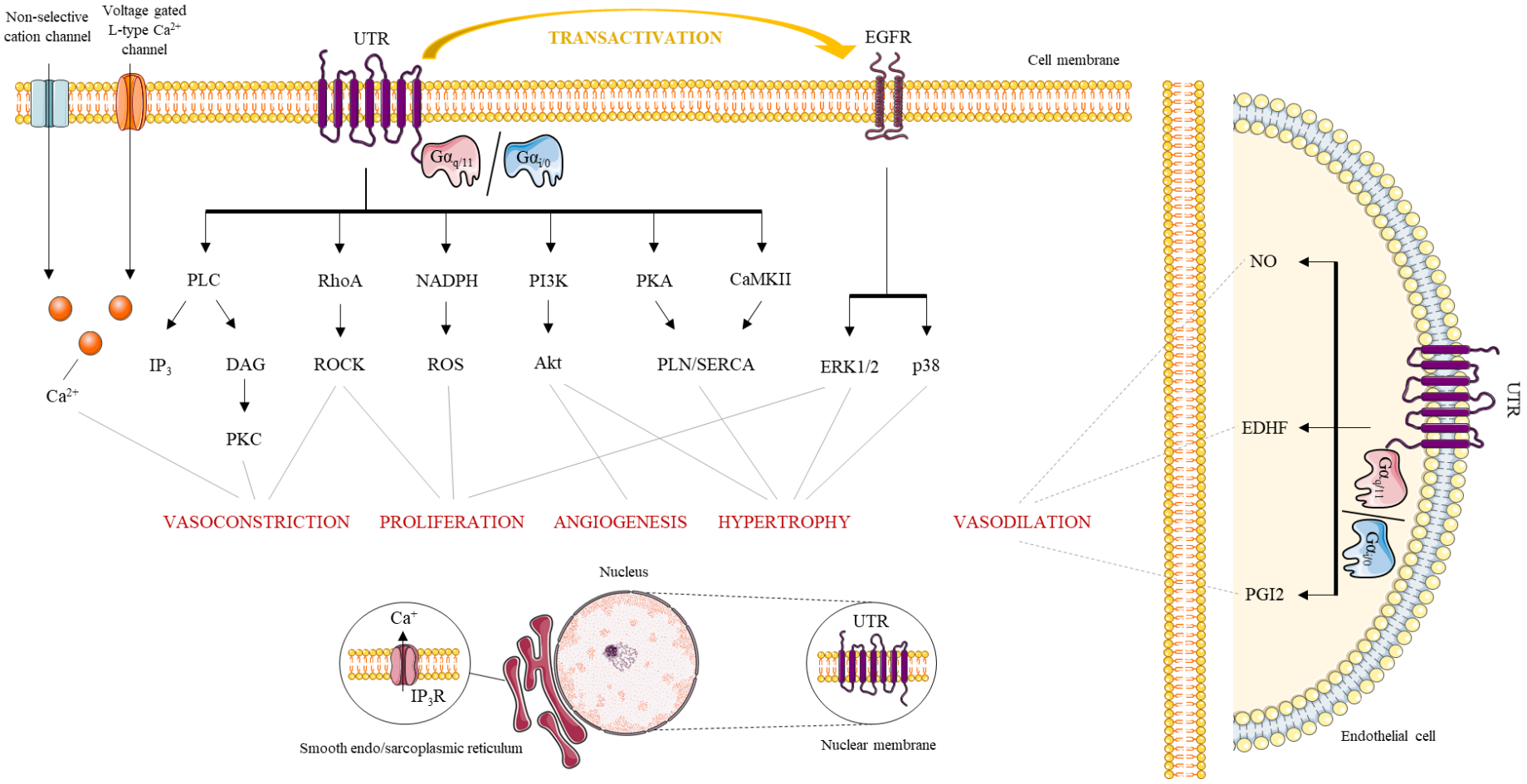
FIGURE 1:



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

Representation of amino acid sequence for mature human UII and comparison of primary structures of UII and URP from various species. The conserved cyclic hexapeptide is highlighted in red. Modified from Vaudry et al. (2015) [93]. Illustration used elements from Servier Medical Art (http://smart.servier.com).

FIGURE 2:



Schematic representation of the complex and multiple signaling pathways induced by 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 NO, EDHF or prostaglandins formation that potentiate vasodilation. This effect can be balanced by vasoconstriction mediated by receptors on VSMCs, where 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. Adapted from Zhu et al. (2006) [94]. Illustration used elements from Servier Medical Art (http://smart.servier.com).

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; NO – Nitric oxide; p38 – p38 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.