Urotensin-II in the lung: A matter for vascular remodelling and pulmonary hypertension?

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Summary
Urotensin-II (U-II) is an evolutionary conserved peptide which has been initially discovered in the urophysis of the fish goby regulating body fluid composition and vascular tone. Mammalian U-II has gained increasing interest since it has been considered as an even more potent vasoconstrictor than endothelin-1, although its efficiency is greatly variable throughout species and vascular beds. More recently, it has been shown that U-II, which mediates its action via binding to the G-protein coupled urotensin-II receptor, is not only involved in the regulation of the vascular tone but can also stimulate a variety of signaling cascades in different cells and organs in the body including generation of reactive oxygen species and nitric oxide, activation of MAP kinases, and modulation of gene expression. Indeed, U-II can stimulate proliferative processes, affect the extracellular matrix and may even add to a prothrombotic state. Such vascular remodelling processes are, in conjunction with enhanced vasoconstriction, involved in the pathogenesis of pulmonary hypertension, suggesting that U-II may play a novel role in the pathogenesis of this disorder.

Keywords
Urotensin-II, oxidative stress, signal transduction, vascular remodelling, pulmonary hypertension

Introduction
Vascular remodelling processes are characterized by structural changes in the vessel wall involving a variety of cellular activities including proliferation, angiogenesis, hypertrophy, apoptosis, rearrangement of vascular wall cells and restructuring of the extracellular matrix. These processes comprise the (mal)adaptive response of vessels to the impact of diverse vascular stress factors including mechanical forces, vasoactive and other hormonal factors, inflammatory and thrombotic events as well as microenvironmental changes such as modulation of oxygen supply leading to disruption of vascular homeostasis. Remodelling of the vascular wall has been associated with many cardiovascular disorders including atherosclerosis and coronary artery disease, metabolic syndrome, diabetes mellitus, hypertension and renal pathologies. Importantly, vascular remodelling processes with progressive narrowing of small pulmonary arteries are in conjunction with enhanced vasoconstriction, endothelial dysfunction and a prothrombotic state hallmarks of pulmonary hypertension (PH) (1). PH which develops as a sporadic disease (idiopathic), as an inherited disorder (familial), or in association with cardiac or pulmonary disorders is characterized by mean pulmonary artery pressure exceeding 25 mm Hg at rest, or 30 mm Hg during exercise (2–5).

Remodelling processes in the pulmonary vasculature are characterized by thickening of all layers of the vessel wall due to hypertrophy (cell growth) and/or hyperplasia (proliferation) of the predominant cell type within each of the layers, as well as by increased deposition of extracellular matrix components and fibrin (Fig. 1) (6–8). Arterial wall thickening can lead to muscularization of normally non-muscularized vessels, stiffening and vasoconstriction resulting in increased vascular resistance. In addition, disordered endothelial cell (EC) proliferation and migration, along with neoangiogenesis as well as proliferation of vascular smooth muscle cells (VSMC) and their transformation into myofibroblasts are the hallmarks of so-called plexiform lesions further resulting in luminal narrowing (7, 9).

In addition to restructuring of the pulmonary vessel wall, endothelial dysfunction, characterized by a relative deficiency of nitric oxide (NO) but enhanced levels of reactive oxygen species...
Urotensin-II and its receptor

UII is a phylogenetically ancient peptide initially isolated in the 1960s from the urophysis, a neurosecretory organ of the caudal spinal cord of *Gillicthys mirabilis*, a fish also known as marine goby, where it is involved in the control of vascular tone and osmoregulation (15, 16). Subsequently, UII has been demonstrated to be evolutionarily conserved from invertebrates to anuran amphibians and ultimately to mammals including humans (17).

UII is composed of 12 amino acids in goby, 14 amino acids in mouse and rat, and 11 amino acids in humans, with a highly conserved C-terminal hexapeptide and disulfide bridge between two cysteine residues (Fig. 2) (13). This peptide is derived from two types of pre-pro UII, which consist of 124 and 139 amino acid residues in humans, and in contrast to the high conservation of carboxy-terminal UII hexapeptide, the amino acid sequence of pre-pro UII shows little similarities between the species (18). Although a specific UII-converting enzyme has not been identified to date, recent studies demonstrated that endogenous proteases such as trypsin or furin may possess UII-converting enzyme activity (18).

Pre-pro-UII mRNA is ubiquitously expressed in human tissues being predominantly located in the kidney and the cardiovascular system (12, 17), but also in the central nervous system and spinal cord (17, 19). Highest plasma UII levels were found in the aortic root, followed by femoral and pulmonary artery indicating the generation of UII in the cardiopulmonary system (20).

By conventional genomics library screening using cDNA encoding rat "orphan" G-protein-coupled receptor-14 (GPR14),
the homologous human receptor was discovered in 1999 by four independent groups (17, 21–23). In order to identify the endogenous ligand for this receptor, a reverse pharmacology approach was performed and HEK293 cells expressing recombinant GPR14 were used as “bait” to screen hundreds of potential ligands. Strong elevation of intracellular Ca\(^{2+}\) was detected only upon stimulation with goby UII, identifying UII as the cognate ligand for the GPR14 receptor, recently renamed as UII receptor.

UII binds to UTR with high-affinity (Kd = 300 pM) and in a pseudo-reversible manner with a slow dissociation rate (24). UTR belongs to the peptide subfamily of the rhodopsin-like family of GPRs (25). Human UTR is comprised of 389 amino acids and exhibits 75% identity with the 386-residue rat homologue (12). UTR has seven transmembrane domains and a cytoplasmic tail which possesses serine/threonine residues as potential primary sites of agonist-dependent receptor phosphorylation (26). Multiple protein kinase A (PKA) and protein kinase C (PKC) phosphorylation sites are present within the intracellular loops 2 and 3 (12). In addition, a specific cluster of serine residues present in the C-terminal tail is involved in β-arrestin-dependent desensitization of UTR, facilitating its slow internalization via clathrin-coated pits (25).

Similar to UII, UTR mRNA can be detected in a wide array of tissues, such as heart, lung, brain, skeletal muscle, bladder, and pancreas. Moreover, UTR mRNA has been found in EC and SMC of different vessels, in particular of aorta and pulmonary artery (17, 24, 27–30).

Urotensin-II – a potent vasoactive peptide

First reports indicated complex vasoactive effects of goby UII on mammalian vessels, demonstrating that UII can possess vasoconstrictive as well as vasodilating effects (31, 32). After the discovery of the human UII homologue, extensive studies have been performed to establish the vasoactive role of this peptide.

Binding of UII to UTR is obtained at picomolar concentrations of UII and strongly sustained (17, 33). It leads to activation of Gq proteins, which then activate PKC, protein tyrosine kinases, calmodulin, and phospholipase C (PLC), resulting in the production of arachidonate, inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (34–36). These messengers stimulate the release of Ca\(^{2+}\) from the sarco-/endoplasmic reticulum and increase extracellular Ca\(^{2+}\) influx (17). The vasoconstrictive effects of UII have been shown to be mediated by the Ca\(^{2+}\)-dependent activation of myosin light chain kinase (MLCK) and also by extracellular signal-regulated kinase 1/2 (ERK1/2), p38 MAP kinase (p38MAPK) and RhoA/Rho kinase (ROCK)–related pathways (Fig. 3) (37, 38).

UII has been described as the most potent vasoconstrictor of isolated blood vessels from a broad range of mammalian species including mice, rats, rabbits, dogs, pigs, monkeys and men (39, 40), being even more potent than ET-1, angiotensin-II (AngII), noradrenalin or serotonin (17, 39). In human pulmonary, but also in coronary, mammary and radial arteries the UII was up to 50 times more potent than ET-1 (17, 29). However, whereas all arteries contracted to ET-1, approximately 30% did not respond to UII (29). Thus, together with the observation that the magnitude of vasoconstrictor responses to UII is often considerably lower than that to ET-1 (17, 29), UII appears to be a less efficient vasoconstrictor than ET-1, but comparable to AngII (17).

Interestingly, exogenous application of UII elicits variable vasoactive responses in different species and/or in different vascular beds within the same species or even in the same organism. These variations have been initially related to differences in the receptor expression profile between vascular beds and species (14). Thus, no response would be initiated by UII when receptor expression would be below the density required to elicit vasoconstrictive responses. Interestingly, by radioligand saturation binding studies the maximum density of UII was found in skeletal muscle, and intermediate densities were observed in human coronary arteries whereas low levels of UTR localized to human pulmonary vessels of varying diameter (29, 33). The so-called “spare receptor reserve hypothesis” postulates that vascular tissue lacks a spare UTR reserve. Since the plasma concentrations of UII are in a nanomolar to picomolar range and therefore several orders of magnitude greater than its binding Kd for UTR,
UTR is highly occupied by endogenous UII (13, 41, 42). Therefore, there is only a low number of unoccupied UTR available to interact with exogenous UII. As a consequence, even subtle changes of receptor expression and availability would have strong effects on responses to UII (24). Moreover, UII binding to rat UTR resulted in rapid internalization followed by a six times slower externalization of the receptor, thus even leading to a transient downregulation of UTR (43). Thus, differential density of unoccupied UTR between various vascular beds or different species may give the explanation for the great variability of vasoconstrictor responses observed when exogenous UII was applied.

However, deletion of the UTR in mice resulted in the loss of the UII contractile activity in the aorta (44). Although no other vascular beds have been investigated, and UTR knock-out did not alter basal systemic haemodynamics (44), this study confirms that UII-induced vasoconstriction is directly linked to UTR.

Studies in non-human primates indicated that pulmonary arteries were particularly sensitive to the vasoconstrictor effect of UII (17, 40). Human UII induced a strong constriction of monkey pulmonary artery, being 23–28 times more potent than ET-1 (17, 40) (Table 1). UII also elicited vasoconstrictive responses in 50% of porcine pulmonary arteries (40), and, in common with other vasoconstrictors, contraction to UII was even potentiated in the presence of low concentrations of ET-1 (33). In rat main pulmonary artery UII was approximately four-fold more potent between various agonists. Abbreviations: Akt (protein kinase B); DAG (diacylglycerol); ERK (p42/44 extracellular signal regulated kinase); JNK (c-jun N-terminal kinase); IP3 (inositol triphosphate); MLCK (myosin light chain kinase); MMP-1 (matrix metalloproteinase-1); eNOS (endothelial nitric oxide synthase); NO (nitric oxide); PAI-1 (plasminogen activator inhibitor-1); PI3K (phosphatidylinositol-(3) kinase); PKC (protein kinase C); PLC (phospholipase C); RhoA kinase (ROCK); ROS (reactive oxygen species); Src (Src kinase).

**Table 1: Relative contractile potency of human urotensin-II (UII) compared to ET-1 in non-human primates.** Human UII caused contraction in all non-human primate arterial vessels studied, including renal, mesenteric, left anterior descending coronary and pulmonary artery. EC50 values (peptide concentration at 50% of signal) were sub-nanomolar, making human UII 6–28-fold more potent than ET-1 (in the table presented as: relative potency). Table was modified from Ames et al. (17).

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Human urotensin-II -log (EC)</th>
<th>Endothelin-1 -log (EC)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal artery</td>
<td>9.59 ± 0.36</td>
<td>8.83 ± 0.24</td>
<td>6</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>9.35 ± 0.26</td>
<td>8.24 ± 0.28</td>
<td>13</td>
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<tr>
<td>Left anterior descending coronary artery</td>
<td>9.39 ± 0.40</td>
<td>8.20 ± 0.32</td>
<td>15</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>9.29 ± 0.16</td>
<td>7.84 ± 0.06</td>
<td>28</td>
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than ET-1, although only 50% of the maximal response to ET-1 was achieved (33). However, UII did not constrict small rat pulmonary vessels, suggesting that UII may have specific vasoconstrictive properties not only in particular vascular beds but also selectively in vessels of a particular caliber. In line, UII had no significant vasoconstrictor action in either human pulmonary arteries or veins of small or medium caliber (45).

Initially, it was reported that UII leads to endothelium-independent vasoconstriction, since removal of the endothelium from rat arteries did not influence the constrictor effect of UII, suggesting a direct action of UII on SMC. However, reports in following years led to the recognition that in addition of being an endothelium-independent vasoconstrictor, UII also acts as an endothelium-dependent vasodilator. Similar to ET-1, UII was found to induce endothelium-dependent vasodilatation involving NO, prostacyclin, prostaglandin E2, or endothelium-derived hyperpolarizing factor in rat isolated aorta, mesenteric and coronary arteries (31, 46–50). Indeed, although in rat pulmonary artery UII was a potent and efficient constrictor, endothelial denudation increased the potency of UII by about 2.5 times (33). This is in line with studies in primates, where UII elicited a strong vasoconstrictor response in pulmonary artery when endothelium was removed (17), indicating that also in the pulmonary vasculature the endothelium may prevent maximal vasoconstrictor action by UII. Similarly, no response to UII or even vasodilation was observed in intact small arteries of the lung (49, 51). Interestingly, upon application of the eNOS inhibitor L-nitro-arginine-methyl-ester (L-NAME), constrictor responses to UII in vitro could be observed in human pulmonary resistance arteries with an intact endothelium (33). These findings suggest that UII, by binding to UTR on the endothelium, mediates the release of vasodilating factors which may physiologically antagonize its direct vasoconstrictor activity mediated by binding of UII to SMC UTR (52). The vasoconstrictor activity of UII may thus play a more important role under pathophysiological conditions associated with endothelial dysfunction, such as PH, which may mask its vasoconstrictive effects. Furthermore, upregulation of UTR under disease conditions may result in more continuous and efficient receptor externalization, thereby allowing long lasting UII-mediated responses (43).

**Urotensin-II: a potential mediator in pulmonary hypertension and vascular remodelling**

First evidence that UII may contribute to PH came from a study in rats exposed to chronic hypoxia which developed PH, pulmonary vascular remodelling and right ventricular hypertrophy. Application of UII further enhanced the contractile responses of pulmonary arteries isolated from rats with PH compared to those from normal rats (33). In line, it has been shown that in chronically hypoxic rats exhibiting pulmonary vascular remodelling and PH, UII protein levels were elevated in EC and SMC in pulmonary arteries (53). In addition, compared to controls, UII protein levels were increased in the right ventricle and to a lesser extent also in the left ventricle in chronically hypoxic rats, but remained unchanged in lung tissue (54). Furthermore, it has been shown that elevated plasma UII immunoreactivity is positively correlated to pulmonary capillary wedge pressure in patients with ischemic heart disease (55). In line, a recent study demonstrated elevated levels of UII in patients with cyanotic congenital heart disease (56). These results indicate that UII is upregulated in hypoxia-induced PH and may act as an autocrine and/or paracrine hormone rather than as a circulating hormone in the myocardium as well as in the pulmonary vasculature leading to ventricular hypertrophy and pulmonary vascular remodelling. However, UII exerted pulmonary pressor responses in rabbits, and these responses were potentiated by PH due to left ventricular dysfunction following coronary artery ligation without changes in cardiac output or heart rate, suggesting that UII is likely to directly affect pulmonary vascular resistance (57).

In addition to the clear experimental link of UII to hypoxia-induced PH, an implication for UII in the pathogenesis of PH due to high pulmonary blood flow has been suggested by studies in rats with aorto-caval shunting, where enhanced levels of UII were observed in the pulmonary artery (58). Although in-vitro experiments in cultured pulmonary artery EC subjected to shear stress showed decreased UII levels in response to additionally elevated pressure (59), UII protein was upregulated in EC and SMC in median and small pulmonary arteries of shunt rats with clear signs of pulmonary vascular disease (60), indicating that UII may also contribute to pulmonary vascular remodelling associated with high pulmonary blood flow.

Taken together, these findings show that levels of UII and UTR are elevated in different models of PH. However, there is a striking lack of studies in patients with primary or secondary pulmonary arterial hypertension. A recent study determined the levels of UII in patients with congenital heart disease and left-to-right-shunt and different levels of pulmonary arterial pressure (PAP) (61). Congenital heart disease has been reported to be associated with enhanced levels of UII (56). Thus, probably due to the already elevated levels of UII in these patients, no further increase could be observed in patients with high PAP. Thus, upcoming studies need to be designed to determine UII levels in patients with different forms of PH and the mechanisms underlying the complex regulatory networks determining the levels of UII and its receptor in various forms of PH. In addition, use of UII and UTR antagonists will help to elucidate the role of the UII / UTR system in the pulmonary vasculature.

**Urotensin-II: an activator of proliferative responses**

On the cellular level, pulmonary vascular remodelling is associated with increased proliferation, migration and hypertrophy of SMC and EC as well as activation of angiogenic responses (Fig. 1).

Supporting a role for UII in regulating remodelling processes in the pulmonary vasculature, UII has been shown to stimulate proliferation of human pulmonary artery smooth muscle cells (PASMC) as was determined by 5-bromo-2’-deoxyuridine (BrdU) incorporation. This response involved the activation of ERK1/2, p38MAPK, c-Jun N-terminal kinase (JNK), PI3 kinase (PI3K) and protein kinase B (Akt) (62). UII also showed a mitogenic effect in rabbit SMC, which was mediated by G proteins,
c-Src tyrosine kinase, PKC, ERK1/2 or Rho kinase (37). Interestingly, UII-induced proliferation of VSMC was synergistically potentiated by serotonin, oxLDL, lysophosphatidylcholine, or hydrogen peroxide, suggesting that interaction and interplay of UII with different agonists could even result in the acceleration of remodelling processes (63–65). Furthermore, UII increased migration of human SMC, cell motility and stress fiber formation in an ERK-dependent pathway (66).

In addition to the profound effects on SMC migration and proliferation, UII also promoted proliferation and inhibited apoptosis of human umbilical vein EC in an ERK1/2-dependent manner (67). Interestingly, in cultured rat neuromicrovascular EC the UTR antagonist palosuran abolished the angiogenic response by UII, but did not affect cell proliferation, indicating that not only vasoactive properties, but also the cellular response to UII could be different in various vascular beds or species (68).

Although no data exist about the role of UII in the function of pulmonary fibroblasts, a contribution of UII to fibroblast cell proliferation has been shown in a study using cardiac fibroblasts (69) or adventitial fibroblasts from spontaneously hypertensive rats (70), suggesting that UII is also a strong mitogen of these cells. Thus, the strong effect of UII on proliferative responses described in PASM, EC and fibroblasts clearly suggests that UII may activate cells of all layers of the pulmonary vessel wall. In addition, UII also enhanced proliferation of epithelial cells and even tumor cells (71, 72) confirming an important role of this peptide in controlling proliferative responses in a wide spectrum of cells.

Furthermore, in cultured neonatal cardiomyocytes over-expressing UTR, UII promoted hypertrophic growth and phenotypic changes, including cell enlargement and sarcomere reorganization involving ERK1/2 and p38MAPK, and trans-activation of the epidermal growth factor receptor. Interestingly, these responses were not affected by JNK, PKC, calcium or PI3K (73, 74). However, future in-vitro studies in pulmonary vascular cells and fibroblasts are needed in order to highlight if any preferential pathways relevant to pulmonary remodelling are specifically activated.

In summary, these findings clearly illustrate that UII activates multiple signaling cascades which stimulate proliferation and migration of SMC and EC, hypertrophic growth of cardiomyocytes as well as angiogenesis. Given the importance of these cellular processes in pulmonary vascular remodelling, UII may play a significant role in promoting PH via these mechanisms.

**Urotensin-II-mediated signaling: changes in the redox balance**

ROS have been shown to act as vascular signaling molecules modulating migration, cell growth, proliferation, apoptosis, inflammation, extracellular matrix composition and vascular tone. Therefore, ROS have been suggested to play an important role in pulmonary vascular remodelling in PH (75–79).

Indeed, several reports indicated enhanced levels of ROS in pulmonary arteries in different models of PH including ovine models with increased pulmonary blood flow due to aorto-pulmonary vascular graft (80) or PH due to ligation of the ductus arteriosus (81). Interestingly, enhanced levels of ROS were also observed in a mouse model of PH due to chronic hypoxia (82, 83).

NADPH oxidases which catalyze the 1-electron reduction of oxygen using NADH or NADPH as electron donor resulting in the formation of superoxide anion radicals (O2−) and subsequently further ROS, have now been recognized as the most prominent sources of ROS generation in vascular cells (78, 84–87).

Interestingly, mice deficient of the NADPH oxidase subunit gp91phox (now also termed NOX2) did not show enhanced right ventricular pressure, medial wall thickening of small pulmonary arteries, and right heart hypertrophy when exposed to chronic hypoxia, indicating that NADPH oxidases may promote pulmonary vascular remodelling in this model of PH (82). Furthermore, in lambs with PH due to an aorto-pulmonary shunt or ligation of the ductus arteriosus levels of the NADPH oxidase subunits p47phox and Rac1 were elevated (80, 88).

UII has been identified as an important activator of NADPH oxidases in PASMC leading to enhanced levels of ROS accompanied by elevated protein levels of the NADPH oxidase subunits p22phox and NOX4 (62). The importance of ROS and NADPH oxidase activation by UII for pulmonary vascular remodelling is further emphasized by findings that UII-stimulated proliferation of PASMC was abrogated by antioxidants or by inhibition of the NADPH oxidase (62). Similarly, inhibition of the NADPH oxidase also prevented EC proliferation and the angiogenic response by UII (unpublished data).

Furthermore, activation of ERK1/2, p38MAPK, JNK and Akt by UII was dependent on ROS and was abrogated by antioxidants and by depletion of p22phox or NOX4 (62), confirming the involvement of NADPH oxidase-derived ROS in UII signaling in pulmonary cells.

In addition to ROS, UII has been shown to enhance the levels of NO in the endothelium of renal arteries (89). The radical NO is produced in the endothelium in a Ca2+-dependent manner by endothelial NOS. Since activation of the UTR by UII has been shown to increase intracellular [Ca2+] levels (35, 38, 90), and UTR is present in EC (27), UII may directly activate eNOS through a calcium-dependent mechanism. In line, UII enhanced eNOS expression and activity and increased NO levels in human umbilical vein endothelial cells (HUVEC) (unpublished data). Furthermore, functional studies using the eNOS inhibitor L-NAME indicated that the endothelium-dependent vasodilator effect of UII may be at least in part mediated by NO (33, 46, 57, 91, 92). In addition, UII increased L-arginine uptake, iNOS mRNA levels and NO production in rat aortic adventitia (50).

In essence, these findings demonstrated that UII can not only increase ROS levels, e.g. by activating and inducing NADPH oxidases, but can also enhance NO levels via activation and induction of eNOS and iNOS. O2− rapidly interacts with NO, thus reducing the bioavailability of NO by forming peroxynitrite. Peroxynitrite itself is very reactive and has been shown to activate several redox-sensitive inflammatory mediators, apoptosis, and growth factors, all of which can induce injury and remodelling in the pulmonary circulation (93–96). Although there are no studies to date providing direct evidence for the formation of peroxynitrite by UII, the importance of NO bioavailability in the pathogen-
esis and maintenance of PH has been acknowledged by many authors (94, 97–100). However, conflicting data exist with regard to the levels of NO and eNOS mRNA or protein in PH. For example, reduced levels of eNOS have been reported in patients with PH (101) and in some animal models (102). In contrast, several studies performed in different animal models as well as in patients reported elevated levels of eNOS associated with PH (60, 103–105), indicating a complex role of the redox balance in PH.

The complicated interplay between NO and ROS in PH is further exemplified by studies in newborn lambs with increased pulmonary blood flow due to aorto-pulmonary grafts. These lambs exhibited not only increased expression of eNOS in the lung but also enhanced levels of NADPH oxidase subunits and ROS (80, 105).

Although the link between NADPH oxidases and eNOS is not clear yet and under intense investigation, several reports have suggested that NADPH oxidase-derived superoxide interacts with eNOS-derived NO to form peroxynitrite (ONOO·), which can oxidize the essential NOS cofactor (6R-)5,6,7,8-tetrahydrobiopterin (BH4) to biologically inactive products such as BH3-radical or 6,7-[8H]-H-biopterin (BH3). This leads to the uncoupling of O2 reduction by eNOS from NO-formation and to the generation of O2·− by eNOS (106).

Indeed, the importance of BH4 for the pathogenesis of PH has been appreciated in animal models with different defects in BH4 synthesis which developed PH even spontaneously (107, 108). In the newborn lamb model with aorto-pulmonary shunt, BH4 levels were unchanged to controls, but levels of oxidized BH4 were increased suggesting that at least part of eNOS is uncoupled and thus may contribute to enhanced ROS levels in this model (80). Thus, increased levels of UII may lead to activation and induction of NADPH oxidases and eNOS. Subsequent formation of peroxynitrite could result in uncoupling of eNOS and may aggravate PH and pulmonary vascular remodelling (Fig. 4). Interestingly, a link between the NO pathway and UII has been recently described. This study showed that inhaled nitroglycerin reduced pulmonary artery UII levels and PASMC proliferation and ameliorated PH in rats with aorto-pulmonary shunt (58), suggesting that enhanced NO levels may induce a negative feed back loop by reducing UII levels which may lead to decreased ROS production and diminished vascular remodelling.

Taken together, these findings suggest that UII could be importantly involved in the development of PH and pulmonary vascular remodelling by affecting the pulmonary redox balance at multiple sites. The complex effects of UII on the redox balance may also explain at least in part the multiple, sometimes even contradictory effects of UII on the vascular tone in different vascular beds. However, much remains to be learned about the effects and consequences of UII-mediated redox-signaling. Thus, future extensive in-vitro studies in pulmonary vascular cells need to further confirm present findings and identify new pathways activated by this peptide. Extensive efforts are presently devoted to developing specific inhibitors of NADPH oxidases (109). Their use may provide experimental tools in investigating the role of the redox-balance in the pathogenesis of diseases associated with UII, such as PH, and together with UII antagonists might have therapeutic potential in the treatment of this pathology.

Urotensin-II-mediated signaling: modulation of gene expression

Structural remodelling of the pulmonary vascular bed requires modulation of the expression of various genes involved in proliferation, apoptosis and extracellular matrix restructuring. In recent years, extensive studies have been performed in order to determine the genes regulated by UII.

In PASMC UII strongly elevated the expression of plasminogen activator inhibitor-1 (PAI-1), by an NADPH oxidase-dependent increase in ROS levels and the subsequent activation of MAP kinases and Akt (62). PAI-1 is known as the major inhibitor of fibrinolysis, thus promoting a prothrombotic state (see below) but also exerts major activities in extracellular matrix remodelling (110), and has been shown to be elevated in patients with PH (111, 112).

In line, in EC, UII increased mRNA and protein expression of collagen-1 in a concentration- and time-dependent manner, but decreased the expression and activity of matrix metalloproteinase-1 (MMP-1) indicating a role of UII in remodelling of the extracellular matrix (113). This is further supported by findings in cardiac fibroblasts where UII increased the synthesis of procollagen and fibronectin and induced TGF-β1 mRNA and protein expression (114, 115). Since increased deposition of extracellular matrix components and fibrin is one of the main features of pulmonary vascular remodelling, these data further suggest that UII can be relevant in promoting these processes.
In cardiomyocytes UII markedly induced the expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (73). BNP levels have been also shown to correlate with mean pulmonary arterial pressure (mPAP) and pulmonary vascular resistance (PVR) and are prognostic markers for the severity of PH (73, 116). Thus, one may speculate that elevated UII levels in PH may contribute to increase BNP levels.

Interestingly, in vascular EC UII also increased the secretion of adrenomedullin in an ERK1/2-, p38MAPK-, and Ca²⁺-dependent manner (117). Adrenomedullin plays an important role in the regulation of pulmonary vascular tone and can decrease pulmonary arterial pressure and pulmonary vascular resistance in patients with PH (118). However, adrenomedullin appears to also have pro-angiogenic and anti-apoptotic properties, contributing to remodelling processes (119). Since enhanced adrenomedullin levels have been described in patients with PH and pulmonary vascular remodelling (120), upregulation of adrenomedullin by UII may represent, similar to the situation with BNP, a modulating or even counterbalancing response elicited by UII in EC to limit further progression of disease.

Finally, upregulation of c-fos mRNA and inflammatory cytokines, such as interleukin-6 and interleukin-1β, by UII has been reported in cardiomyocytes (121, 122), suggesting that UII may also be involved in inflammatory responses which can be associated with cardio-pulmonary remodelling. Indeed, UII levels have been found to be highly expressed in lymphocytes whereas UTR is mainly found in monocytes and macrophages (123). Interestingly, levels of UTR mRNA and functional UIR high-affinity binding sites have been shown to be up-regulated by interferon gamma, suggesting that UTR could also be up-regulated as a consequence of an inflammatory response, leading to progression of pulmonary disease (27).

Taken together, UII appears to have profound effects on gene expression profiles associated with pulmonary vascular remodelling. However, to date it is not clear whether UII modulates gene expression in a cell-specific manner. Studies demonstrating that UII elicits the expression of vasodilatory and vasoprotective factors in EC suggest that, similar to the differences in the vasoconstrictive responses to UII in VSMC and EC, differences in the UII-mediated gene expression profiles exist between VSMC and EC. Future studies using e.g. the microarray technology in UII-stimulated EC, SMC or fibroblasts, or in cells derived from UTR knock out mice would further help to increase our understanding of the impact of UII on cellular functions important for pulmonary vascular physiology and pathophysiology.

**Urotensin-II: a positive effector of a prothrombotic state?**

In addition to vasoconstriction and remodelling processes, activation of the coagulation cascade plays a major role in mediating functional and structural changes in the pulmonary vasculature (4, 124). Endothelial injury allows the leakage of various bloodborne factors and their contact with the underlying SMC resulting in increased coagulability and thrombogenicity within the vessel wall (125).

Thrombin is a multifunctional serine protease that regulates activation of platelets and the blood coagulation cascade by affecting a series of factors, cofactors and proteins inside the plasmatic hemostatic system leading to the conversion of fibrinogen to fibrin (126–128). Under normal conditions, fibrin deposition is counterbalanced by fibrinolysis due to the plasmin/plasminogen system. However, fibrinolysis is inhibited by the action of PAI-1, thus further promoting a prothrombotic state (110). Indeed, as indicated above, PAI-1 levels have been found elevated in patients with PH (111, 112), and UII is able to increase PAI-1 levels in PASM (62). In a similar way, thrombin, which is also enhanced in patients with PH (129), can upregulate PAI-1 levels in an ROS-dependent manner in PASM (130). Interestingly, ROS production and MAP kinase activation can be potentiated by costimulation of UII with thrombin in PASMC, further indicating that there is a cross-talk between UII and the coagulation cascade (unpublished observation). This is further supported by studies in UTR overexpressing cardiomyocytes, where heparin, an inhibitor of thrombin, decreased UII-mediated activation of ERK1/2 and p38MAPK (74). Interestingly, evidence has been provided for expression of small, although detectable amounts of UTR in platelets in atherosclerotic vessels (123). Since thrombin is the major activator of platelets, these findings may further point to a cross-talk between UII and the coagulation system in disorders associated with a prothrombotic state.

**Urotensin-II and remodelling in other cardiovascular diseases**

Although remodelling processes are of particular importance in the pathogenesis of PH, structural changes of the vascular wall or the heart have been implicated in many other cardiovascular disorders. Indeed, UII has been suggested to play an important role in the pathogenesis of different cardiovascular diseases associated with remodelling processes, such as congestive heart failure, atherosclerosis and coronary artery disease, metabolic syndrome, diabetes mellitus, hypertension and renal pathologies (131).

A number of studies demonstrated the importance of UII in regulating heart physiology. UII has been reported to be a potent cardiac stimulant resulting in a concentration-dependent increase in contractile forces (132), and enhanced UII levels have been associated with diastolic myocardial dysfunction in ischemic heart failure (55). On the cellular level, UII induced hypertrophy of rat cardiomyocytes, protein synthesis and expression of cellular markers of hypertrophy (73, 115, 122, 133, 134). In line, myocardial expression of UII and its receptor were found increased in congestive cardiac failure, and their expression levels were proportional to the degree of left ventricular dysfunction (27). Elevated concentrations of UII have also been reported in patients with severe heart failure (134, 135) and in patients with acute myocardial infarction (136). Interestingly, increased levels of UII were associated with better prognosis, suggesting a possible cardioprotective role for this peptide (136).

Recent reports also suggested that UII contributes to the pathogenesis of atherosclerosis and coronary artery disease. UII was reported to upregulate the expression of acyl-coenzyme A cholesterol acyltransferase-1 (ACAT-1) via the UTR/G-protein/c-Src/PKC/
MEK and ROCK pathways (137). Since ACAT-1 plays a crucial role in the formation of macrophage-derived foam cells, these findings suggest that UII significantly contributes to the development of atherosclerosis in hypertension. These data are substantiated by other studies showing increased expression of UII and UTR in atherosclerosis and atherosclerotic lesions of the human aorta (123, 138, 139). In addition, UII levels correlated with the severity of coronary artery disease and were higher in patients with triple-vessel disease than in patients with single- or double-vessel disease and healthy volunteers (55). Furthermore, plasma UII levels have been associated with a high risk of carotid plaque formation (140).

In addition, various studies have demonstrated that UII is closely associated with the metabolic syndrome, and increased levels of UII have been reported in patients with type 2 diabetes (28, 141) or essential hypertension (28, 140, 142). Moreover, enhanced levels of UII and its receptor in renal biopsy tissue samples from patients with diabetic nephropathy implicated this vasoactive peptide in the pathogenesis of renal disease (143).

Interestingly, the first clinical trial in which the UTR antagonist palosuran has been administered to hypertensive diabetic patients who are prone to cardiovascular disease, showed that UTR blockade has beneficial effects on diabetic nephropathy (144). In line, the UII antagonist SB-611812 attenuated cardiac remodelling in experimental ischemic heart disease (145) and improved cardiac function in a rat model of myocardial infarction (146). Therefore, further development and widespread use of UII and UTR antagonists will help to elucidate the role of the UII / UTR system in the cardiovascular and pulmonary system.

Conclusion

In summary, the current knowledge about UII strongly implies that this vasoactive peptide could play a central role in the pathogenesis of pulmonary vascular remodelling and hypertension. In addition to its vasoconstrictive potency especially in conditions of disturbed vascular homeostasis and endothelial dysfunction, UII may be essentially involved in activating signaling cascades leading to cell proliferation, migration, hypertrophy and angiogenesis, mechanisms that are highly relevant in promoting pulmonary vascular remodelling processes.

However, there is a clear need for extensive in-vitro and in-vivo studies to further substantiate the association of UII with remodelling processes in the lung. Subsequent clinical studies will have to further confirm these findings and may provide the basis for the development of novel strategies targeting UII signalling for the treatment of pulmonary vascular remodelling and hypertension as well as other disorders associated with remodelling processes.

Abbreviations

EC, endothelial cells; eNOS, endothelial nitric oxide synthetase; ET-1, endothelin-1; GPR14, G-protein-coupled receptor-14; NO, nitric oxide; PASM/C, pulmonary artery smooth muscle cells; PH, pulmonary hypertension; ROCK, RhoA/Rho kinase; ROS, reactive oxygen species; SMC, smooth muscle cells; UII, urotensin-II; UTR, UII receptor; VSM/C, vascular smooth muscle cells.

References

Djordjevic, Gorlach: Urotensin-II in pulmonary vascular remodelling

69. Gorlach A, Brandes RP, Nguyen K, et al. A gp91phox containing NADPH oxidase selectively ex-
pressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res 2000; 87: 26–32.
100. Michelakis ED. The role of the NO axis and its therapeutic implications in pulmonary arterial hypertension. Heart Fail Rev 2003; 8: 5–21.