Human Urotensin II Is a Novel Activator of NADPH Oxidase in Human Pulmonary Artery Smooth Muscle Cells

Talija Djordjevic, Rachida S. BelAiba, Steve Bonello, Josef Pfeilschifter, John Hess, Agnes Görlach

**Background**—Human urotensin II (hU-II) is a potent vasoactive peptide possibly involved in pulmonary hypertension. Because the signaling mechanisms activated by this peptide in the pulmonary vasculature are largely unknown, we investigated the role of hU-II in the activation of NADPH oxidase and the control of redox-sensitive kinase pathways, expression of plasminogen activator inhibitor-1 (PAI-1), and proliferation in pulmonary artery smooth muscle cells (PASMCs).

**Methods and Results**—hU-II upregulated expression of the NADPH oxidase subunits p22phox and NOX4 and increased the levels of reactive oxygen species (ROS), which were abrogated by transfecting p22phox or NOX4 antisense vectors. p22phox and NOX4 also contributed to hU-II–induced activation of extracellular signal–regulated kinase 1/2, p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, and protein kinase B (Akt). Furthermore, hU-II increased the expression of PAI-1 and enhanced PASMC proliferation in an NADPH oxidase– and kinase-dependent manner.

**Conclusions**—hU-II is a potent activator of ROS generation by NADPH oxidase in PASMCs, leading to redox-sensitive activation of mitogen-activated protein kinases and Akt and subsequently to enhanced PAI-1 expression and increased proliferation. These findings suggest that hU-II may play a novel role in pulmonary hypertension by promoting remodeling processes via activation of NADPH oxidases. (Arterioscler Thromb Vasc Biol. 2005;25:519-525.)

**Key Words:** reactive oxygen species ■ p22phox ■ NOX4 ■ urotensin II ■ plasminogen activator inhibitor-1

Urotensin II (U-II) is a cyclic vasoactive peptide composed of 11 amino acid residues with a structure similar to somatostatin.1 The human form of U-II (hU-II) has been identified as an endogenous ligand for the G-protein–coupled receptor GPR14, now named the U-II receptor. Both hU-II and its receptor are expressed in different tissues, including the heart, brain, kidney, smooth muscle, and endothelium.2 hU-II is among the most potent vasoconstrictor peptides identified, with a potency greater than that of endothelin-1, although regional differences in its effects showing variable vasoconstrictor responses in various vascular beds and blood vessels of some species3 suggest a complex role for this peptide in cardiovascular homeostasis. Elevated levels of hU-II have been found in patients with several cardiovascular diseases such as hypertension, congestive heart failure, cardiac hypertrophy, or atherosclerosis.1,4 In addition, pulmonary arteries from animals with pulmonary hypertension showed significantly increased maximal contractile responses to hU-II compared with controls.5 In human pulmonary arteries, a strong vasoconstrictor response was detectable when nitric oxide synthase was inhibited, suggesting that hU-II may exert its effects particularly in situations of endothelial dysfunction, such as in pulmonary hypertension. Recently, elevated hU-II levels have been found in rats with pulmonary hypertension and have been associated with pulmonary artery hypertrophy.6 Despite the increasing evidence of a role for this peptide in the pathogenesis of pulmonary hypertension and remodeling, the signaling pathways activated by hU-II in (pulmonary) vascular cells are not well resolved.

Reactive oxygen species (ROS) play an important role as signaling molecules in vascular cells, and NADPH oxidases have been described to largely contribute to ROS production within the vasculature.7–9 This multicomponent enzyme with a catalytic cytochrome b558 composed of the subunits p22phox and gp91phox has been best characterized in neutrophils, where it is responsible for the respiratory burst. In recent years, NADPH oxidases have also been identified in vascular and other nonphagocytic cells.7,8 In contrast to neutrophils, nonphagocytic NADPH oxidases generate only moderate levels of ROS. Whereas p22phox appears to be ubiquitously expressed, 4 isoforms of the leukocyte subunit gp91phox (NOX2), termed NOX1, NOX3, NOX4, and NOX5, have been described, and expression of NOX1 and NOX4 has been reported in smooth muscle cells.7,8 NADPH oxidases have been implicated in the pathogenesis of a variety of cardiovascular diseases, including atherosclerosis and hypertension.9 Recently, enhanced ROS formation has...
also been associated with pulmonary hypertension and pulmonary vascular remodeling, a process characterized by increased proliferation of pulmonary artery smooth muscle cells (PASMCs), modified fibrinolysis, and deposition of components of the extracellular matrix in the vascular wall. Fibrinolytic processes within the vasculature are highly regulated by plasma levels of the serine protease plasminogen activator inhibitor-1 (PAI-1), and enhanced PAI-1 levels have been reported under oxidative stress conditions as well as in patients with pulmonary hypertension.

Although hU-II, ROS formation, and PAI-1 expression may play a role in the pathogenesis of pulmonary hypertension and the promotion of vascular remodeling processes, the signaling mechanisms linking hU-II to vascular remodeling are unclear. We therefore investigated the role of hU-II in the formation of ROS by NADPH oxidases, in the stimulation of different intracellular kinase pathways, in the regulation of PAI-1 expression, and in the control of proliferation. We found that hU-II stimulates NADPH oxidase–dependent activation of mitogen-activated protein kinases (MAPKs) and protein kinase B/Akt, PAI-1 expression, and cell proliferation.

**Methods**

**Cell Culture**

Human PASMCs from Clonetics were cultured as recommended. Cells were used only up to passage 13 to maintain the phenotypic characteristics of PASMCs in vivo, as confirmed by monitoring the expression of smooth muscle α-actin in all experiments. Cells were deprived of serum for 24 to 48 hours before stimulation.

**Plasmids**

The cDNA fragment encoding full-length human p22phox was obtained by polymerase chain reaction from pBShp22phox (provided by Dr M.C. Dinauer, Indiana University Medical Center, Indianapolis, Ind) and subcloned into pcDNA3.1, resulting in pcDNA3.1-p22phox sense (p22S) and antisense (p22AS) constructs. Human NOX4 full-length cDNA was cut from pCMV4-SPORT-NOX4 by Avai1, blunt-ended, and inserted into pcDNA3.1, resulting in NOX4 sense (N4S) and antisense (N4AS) constructs.

**Transfections**

PASMCs were plated to a density of 70% and cultured for 24 hours. Transfections were performed with the use of FuGene reagent (Roche) as described.

**Measurements of ROS Levels**

ROS levels were assessed after exposure to hU-II (Polypeptide Laboratories) using the fluoro probe CM-H2DCFDA (MoBiTec) in a microplate reader as described. Alternatively, PASMCs were stimulated, harvested by trypsinization, resuspended in Hanks’ balanced salts solution at a concentration of 10^6 cells per mL, and loaded with 8.5 μmol/L CM-H2DCFDA. Dichlorofluorescein (DCF) fluorescence was monitored by analyzing 10,000 cells in a flow cytometer (Partec).

**Visualization of ROS Levels**

PASMCs were stimulated, washed with Hanks’ balanced salts solution, and loaded with 50 μmol/L dihydroethidium (Molecular Probes) for 10 minutes. Fluorescence was monitored by fluorescence microscopy (Olympus).

**Western Blotting**

Western blot analyses were performed as described with antibodies against p22phox (provided by Dr D. Roos, Academic Medical Center, University of Amsterdam, Netherlands), PAI-1 (American Diagnostics), the phosphorylated forms of p38 MAPK, c-Jun N-terminal kinase (JNK), extracellular signal–regulated kinase (ERK1/2, Cell Signaling), protein kinase B (Akt), and smooth muscle α-actin (Sigma). The antibody against NOX4 was generated by immunizing rabbits with the peptides CAYLRSQKVOSRRT and CSYGTKFEYNKESFS, representing amino acids 81 to 95 and 566 to 578, respectively, of the rat NOX4 protein.

**Northern Blot Analysis**

Total RNA from PASMCs was isolated as described. RNA (10 μg) was subjected to Northern blot analysis. Hybridizations were performed with digoxigenin-labeled antisense RNA for human PAI-1 (provided by Dr T. Kietzmann, University of Kaiserslautern, Kaiserslautern, Germany) as described.

**Proliferation Assays**

The proliferative activity of PASMCs treated with hU-II was evaluated by 5-bromo-2′-deoxyuridine (BrdU) labeling (Roche) as described.

**Statistical Analysis**

Values presented are mean±SD. Results were compared by ANOVA for repeated measures, followed by the Student-Newman-Keuls test. P<0.05 was considered statistically significant.

**Results**

**hU-II Elevates ROS Levels**

To investigate whether the vasoactive peptide hU-II can increase ROS levels, PASMCs were exposed to 100 nmol/L hU-II for 2 hours and stained with dihydroethidium. A strong increase in fluorescence in stimulated PASMCs compared with controls indicated increased ROS levels (Figure 1A). Alternatively, PASMCs were treated with hU-II (100 or 200 nmol/L) for 2 hours, and ROS levels were measured by DCF fluorescence in a flow cytometer. Significantly increased levels of ROS compared with control cells were observed after challenge with both concentrations (Figure 1B). Pretreatment with the flavin inhibitor diphenyleneiodonium (DPI, 10 μmol/L) for 30 minutes significantly decreased hU-II–induced ROS elevation, as measured by DCF fluorescence in a microplate reader (Figure 1C).

**hU-II Elevates p22phox and NOX4 Protein Levels**

Because DPI is known to (nonspecifically) inhibit NADPH oxidases, we further evaluated the role of this enzyme in response to hU-II. PASMCs were stimulated with 100 nmol/L hU-II for increasing time periods (from 1 to 16 hours), and protein levels of the NADPH oxidase subunits p22phox and NOX4 were determined by Western blot analysis (Figure 2A). Application of hU-II resulted in strong upregulation of p22phox and NOX4 protein levels, peaking at 2 hours after stimulation. To confirm the role of these proteins in ROS generation in response to hU-II, PASMCs were transfected with sense or antisense vectors for p22phox or NOX4 and exposed to hU-II for 2 hours. Depletion of p22phox or NOX4 abolished the hU-II–stimulated increase in ROS, whereas ROS levels were slightly enhanced in p22phox- and NOX4-overexpressing PASMCs (Figure 2B). Transfection of p22phox or NOX4
antisense constructs prevented the increase in both subunits in response to hU-II (Figure 2B and 2C).

hU-II Increases Protein Kinase Activity Involving NADPH Oxidase

To investigate the signaling pathways activated by hU-II, PASMCs were treated with hU-II (100 nmol/L) for increasing time periods. Phosphorylation of ERK1/2, p38MAPK, JNK, and protein kinase B (Akt) was determined by Western blot analysis. hU-II increased the phosphorylation of all kinases, peaking at 5 minutes (Figure 3A). Phosphorylation of all kinases was observed with 10 nmol/L and stayed elevated up to 100 or 500 nmol/L of hU-II, whereas higher concentrations of this peptide decreased kinase phosphorylation (Figure 3B).

Pretreatment with DPI (10 μmol/L) for 30 minutes or transfection of the p22phox or NOX4 antisense vectors abrogated the hU-II–induced phosphorylation of ERK1/2, p38MAPK, JNK, and Akt (Figure 4A and 4B), indicating that hU-II can stimulate MAPKs and Akt in PASMCs via a redox-sensitive pathway involving NADPH oxidases.

hU-II Induces PAI-1 Expression

PAI-1, a component of the extracellular matrix and an inhibitor of fibrinolysis, has been implicated as a contributor to vascular remodeling.11 We therefore investigated whether hU-II affects the expression of PAI-1. Exposure to 100 nmol/L hU-II increased PAI-1 mRNA levels, peaking at 4 hours, and PAI-1 protein levels, determined in culture supernatants, after 4 and 8 hours (Figure 5A). Furthermore, application of inhibitors of p38MAPK (SB220025, 20 μmol/L), MAPK/ERK kinase (MEK) 1 (PD98059 or U0126, each 20 μmol/L), phosphatidylinositol-3-kinase (PI3K; LY294002, 10 μmol/L), Akt (SH-5, 10 μmol/L),19 or JNK (SP600125, 25 μmol/L)20 or pretreatment with DPI decreased hU-II–induced PAI-1 protein levels (Figure 5B). Because all inhibitors prevented the phosphorylation of their respective target kinases (data not shown), these data suggest that hU-II regulates PAI-1 expression...
by stimulating NADPH oxidase–dependent ROS generation and subsequent activation of MAPKs and the PI3K/Akt pathway. In support of these findings, transfection of p22phox or NOX4 antisense vectors prevented the increase in PAI-1 protein by hU-II (Figure 5C).

hU-II Stimulates the Proliferation of PASMCs

To evaluate whether hU-II can regulate proliferation of PASMCs, cells were stimulated with 100 or 1000 nmol/L hU-II, and the proliferative activity of PASMCs was determined by BrdU incorporation. Stimulation with hU-II for 48 hours significantly increased the proliferative activity of PASMCs (Figure 6A). Depletion of p22phox or NOX4 specifically abrogated hU-II–stimulated proliferation of PASMCs (Figure 6B), because the proliferation in response to sphingosine 1-phosphate (S1P, 1 μmol/L), known to promote proliferation of smooth muscle cells,21 was not significantly reduced by depletion of p22phox or NOX4 (Figure 6C). In addition, specific inhibition of MAPKs and the PI3K/Akt pathway completely blocked proliferation of PASMCs in response to hU-II (Figure 6D).

Discussion

hU-II has been described as a potent vasoactive peptide and has been suggested to contribute to several cardiovascular diseases, including pulmonary hypertension.1,3,6 Herein we showed that hU-II increases the levels of NADPH oxidase–derived ROS, leading to the activation of MAPKs and Akt, followed by enhanced PAI-1 expression and increased proliferation of PASMCs. This conclusion is supported by the findings that: (1) hU-II strongly enhanced ROS levels that were blocked by DPI or depletion of the NADPH oxidase subunits p22phox or NOX4; (2) hU-II elevated protein levels of p22phox and NOX4; (3) hU-II increased the phosphorylation of ERK1/2, p38MAPK, JNK, and Akt, which was prevented by treatment with DPI and by depletion of p22phox or NOX4; (4) hU-II upregulated the expression of PAI-1 in a redox-sensitive, NADPH oxidase–and kinase-dependent manner; and (5) hU-II increased the proliferative activity of PASMCs, which was abrogated by depletion of p22phox or NOX4.

hU-II Stimulates NADPH Oxidase

In this study, we demonstrated that hU-II significantly increased ROS levels in PASMCs. This response was accompanied by elevated protein levels of the NADPH oxidase subunits p22phox and NOX4 but was abrogated by the flavin inhibitor DPI and by depletion of p22phox or NOX4. These findings show for the first time that hU-II is able to activate ROS generation and identify NADPH oxidases as the source in PASMCs. Interestingly, depletion of either p22phox or NOX4 also downregulated the expression of the other subunit (NOX4 or p22phox, respectively) but did not affect expression levels of the p47phox subunit (data not shown). These findings suggest that, similar to the neutrophil NADPH oxidase, protein expression of p22phox or NOX4 requires concomitant expression of NOX4 or p22phox, respectively.

Although a role for ROS in pulmonary hypertension and vascular remodeling has been recently suggested,10 so far only limited data are available supporting this assumption. In fetal PASMCs, endothelin-1 increased ROS production, which was sensitive to treatment with the nonspecific NADPH oxidase inhibitors DPI and apocynin.22 Similarly, serotonin enhanced ROS production in murine pulmonary arteries, which was inhibited by apocynin,23 suggesting that both peptides, similar to hU-II, may induce ROS production via activation of NADPH oxidases. A role for NADPH oxidases in pulmonary hypertension was further supported by a recent study demonstrating increased ROS production and elevated levels of the NADPH oxidase subunit p67phox in...
pulmonary arteries from newborn lambs with pulmonary hypertension and vascular remodeling.\textsuperscript{24} Whereas p22phox has been described to contribute to ROS production in pulmonary endothelial and airway smooth muscle cells,\textsuperscript{10} the involvement of NOX4 in the ROS production of pulmonary vascular cells has not been reported so far. In support of our study, it was recently shown that NOX4 and p22phox colocalize in smooth muscle cells.\textsuperscript{25} However, in contrast to our study, wherein hU-II increased NOX4 protein, stimulation with angiotensin II decreased NOX4 levels in rat aortic smooth muscle cells.\textsuperscript{26} Although the reasons for these differences are not clear to date, they may relate to stimulus- and/or cell type–specific signaling mechanisms and/or intracellular localization, as has been suggested recently.\textsuperscript{25} However, in favor of our data, NOX4 was upregulated in vessels from hypertensive rats.\textsuperscript{27} Together with the findings that in animals with pulmonary hypertension hU-II levels are elevated,\textsuperscript{6} the data that hU-II increases ROS levels via activation of NADPH oxidase in PASMCs suggest that enhanced NADPH oxidase expression and activity in response to hU-II may contribute to the development of pulmonary hypertension and vascular remodeling processes.

hU-II Activates Redox-Sensitive Signaling Cascades and PAI-1 Expression

We further demonstrated that hU-II rapidly increased the phosphorylation of ERK1/2, p38MAPK, JNK, and Akt in PASMCs. In vascular smooth muscle cells, hU-II has been reported to activate ERK1/2.\textsuperscript{28} Thus, hU-II appears to be an effective activator of PASMCs, because these kinases have been shown to be involved in the regulation of cell proliferation, migration, and survival.

**Figure 5.** hU-II stimulates the expression of PAI-1. PASMCs were stimulated with hU-II (100 nmol/L) for the indicated times. A, PAI-1 mRNA levels were evaluated by Northern blot analysis, and PAI-1 protein levels were determined by Western blot analysis. B, PASMCs were pre-treated with the p38MAPK inhibitor SB202020 (SB, 20 μmol/L), the MEK1 inhibitor PD98059 (PD, 20 μmol/L) or U0126 (U, 20 μmol/L), the JNK inhibitor SP600125 (SP, 25 μmol/L), the PI3K inhibitor LY294002 (LY, 10 μmol/L), or the Akt inhibitor SH-5 (SH, 10 μmol/L) or exposed to DPI (10 μmol/L) for 30 minutes before stimulation with hU-II for 4 hours or (C) transfected with control vector (Ctrl), p22S, and p22AS, or N4S and N4AS vectors. PAI-1 protein levels from the culture supernatants were determined by Western blot analysis. Equal loading of proteins was confirmed by Ponceau S staining. In each experiment, the mRNA or protein levels under nonstimulated, untreated conditions were set equal to 100% (n=4, *P<0.05 vs nonstimulated cells [Ctrl]; #P<0.05 vs hU-II–stimulated cells). All other abbreviations are as defined in the text.

**Figure 6.** hU-II stimulates the proliferative activity of PASMCs. A, PASMCs were exposed to hU-II (100 or 1000 nmol/L) for 48 hours. Proliferative activity was assessed by labeling with BrdU (10 μmol/L; n=3, *P<0.05 vs unstimulated cells [0 nmol/L]). B, PASMCs were transfected with control vector (Ctrl), p22S and p22AS, or N4S and N4AS vectors. After stimulation with hU-II for 48 hours, proliferative activity was assessed by BrdU incorporation (n=3, *P<0.05 vs unstimulated control cells [Ctrl]; #P<0.05 vs hU-II–stimulated control cells [Ctrl]). C, PASMCs were transfected with control vector (Ctrl), p22AS, or N4AS vectors and stimulated with hU-II or S1P (1 μmol/L) for 48 hours. Proliferative activity was assessed by BrdU incorporation. Stimulated proliferative activity in control cells was set equal to 100% (n=3, *P<0.05 vs stimulated control cells [Ctrl]). D, PASMCs were pretreated with the inhibitors of p38MAPK (SB202020 [SB], 20 μmol/L), MEK1 (PD98059 [PD], 20 μmol/L), or U0126 [U], 20 μmol/L), JNK (SP600125 [SP], 25 μmol/L), PI3K (LY294002 [LY], 10 μmol/L), or Akt (SH-5 [SH], 10 μmol/L) 30 minutes before stimulation with hU-II for 48 hours. Proliferative activity was assessed by labeling with BrdU (10 μmol/L; n=3, *P<0.05 vs stimulated control cells [Ctrl]; #P<0.05 vs hU-II–stimulated control cells [Ctrl]). All other abbreviations are as defined in the text.
been linked to various cellular processes, including proliferation, differentiation, cell growth, and apoptosis. Activation of the kinases by hU-II was redox-sensitive and was inhibited by depletion of p22phox or NOX4, suggesting the involvement of NADPH oxidase–derived ROS. Whereas p38MAPK, JNK, and Akt have been shown to be activated by thrombin or angiotensin II in a redox-sensitive, NADPH oxidase–dependent manner in vascular smooth muscle cells from different beds and species, ERK1/2 has been found to be rather insensitive to ROS in response to thrombin.15,16,29 However, conflicting data exist regarding the role of ROS in ERK1/2 activation by angiotensin II, indicating a stimulus–dependent involvement of NADPH oxidase and ROS in the activation of ERK1/2.9,29 Interestingly, hU-II–stimulated, NADPH oxidase–dependent kinase activation was faster than the increase in ROS levels measured, indicating that in addition to increased ROS levels due to enhanced NADPH oxidase expression, hU-II may also activate NADPH oxidase. Similar observations have been made with thrombin and angiotensin II in smooth muscle cells.

The matrix protein and inhibitor of fibrinolysis PAI-1 plays a central role in tissue remodeling by degrading extracellular matrix and activating growth factors.11 Increased PAI-1 expression has been implicated to contribute to vascular remodeling and pulmonary hypertension.11,13,32 In this study, we showed that hU-II strongly elevates PAI-1 expression in PASMCs, demonstrating that hU-II is able to modulate gene expression. Depletion of NADPH oxidase subunits or inhibition of MAPKs and PI3K/Akt prevented this response, indicating that hU-II–induced PAI-1 expression was mediated by an NADPH oxidase–dependent increase in ROS levels and the subsequent activation of MAPKs and Akt. PAI-1 has been found to be sensitive to oxidative stress in several cell types,12 and a role for NADPH oxidase has been shown in thrombin-induced PAI-1 expression in aortic smooth muscle cells.13 Moreover, in vascular smooth muscle cells, MAPKs and PI3K/Akt were involved in the regulation of PAI-1 by thrombin, platelet-derived growth factor, and angiotensin II.13,33,34 Because enhanced levels of PAI-1 contribute not only to matrix deposition and remodeling of the vascular wall but also to enhanced thrombosis,13 which is frequently observed in pulmonary hypertension, our findings that hU-II upregulates PAI-1 expression via NADPH oxidase and MAPKs in PASMCs implicate a novel pathway contributing to this disease.

hU-II Increases Proliferation of PASMCs

On the cellular level, vascular remodeling is associated with increased proliferation of smooth muscle cells. We demonstrated that the proliferation of PASMCs was stimulated by hU-II, consistent with previous reports demonstrating increased proliferation by hU-II in adrenal tumors, epithelial cells, and vascular smooth muscle cells and elevated hU-II levels in carotid and aortic atherosclerotic plaques.35 Depletion of p22phox or NOX4 completely abrogated the proliferative activity of PASMCs by hU-II, but not by S1P, indicating that hU-II specifically involves NADPH oxidase activation and supporting previous findings that S1P increases the proliferation of smooth muscle cells but does not act via NADPH oxidase.38 In addition, inhibition of MAPKs or the PI3K/Akt pathway blocked proliferation in response to hU-II, indicating that activation of NADPH oxidase–dependent signaling cascades is crucially involved in hU-II–stimulated proliferation of PASMCs. ROS and NADPH oxidases, as well as redox-sensitive activation of MAPKs and the PI3K/Akt pathway, have been suggested to play an important role in vascular proliferation associated with many diseases, including pulmonary hypertension.9,10,39 p22phox has been described to be required for the proliferation of human airway smooth muscle cells and for diabetic smooth muscle cell growth.40 However, the role of NOX4 in proliferation is less clear. Consistent with our study, depletion of NOX4 suppressed the proliferation of melanoma cells and enhanced apoptosis of pancreatic cancer cells, whereas reduced levels of NOX4 were associated with increased proliferation of NIH3T3 cells.43 In a rat carotid artery injury model, delayed NOX4 upregulation has been related to differentiation and matrix deposition, whereas enhanced NOX1 levels have been associated with proliferation.44 Although hU-II did not increase NOX1 levels in PASMCs (data not shown), we cannot exclude a role for NOX1 in the control of PASMC proliferation at that point. However, the concomitant increase in p22phox and NOX4 protein by hU-II may allow the formation of an active NADPH oxidase, explaining why depletion of each of these subunits not only affected the protein levels of the other subunit but also prevented hU-II–induced increases in ROS levels, MAPK activation, PAI-1 expression, and proliferation.

In summary, our findings identify hU-II as a novel agonist of NADPH oxidases in PASMCs and demonstrate that enhanced levels of ROS and the subsequent redox-sensitive activation of MAPKs and Akt increase PAI-1 expression and proliferation. Because PAI-1 is essentially involved in vascular remodeling processes, possibly by facilitating migration and/or proliferation of PASMCs, such a mechanism may be highly relevant in promoting pulmonary vascular remodeling processes. Subsequent in vivo studies will be needed to confirm these findings and may provide a basis for the development of novel treatment strategies for this disease.

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References

5. MacLean MR, Alexander D, Stirrat A, Gallagher M, Douglas SA, Ohlstein EH, Morecroft I, Polland K. Contractile responses to human urotensin-II in rat and human pulmonary arteries: effect of endothelial...


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