British Journal of Pharmacology (2000) 130, 201–204
www.nature.com/bjp

SPECIAL REPORT

Contractile responses to human urotensin-II in rat and human pulmonary arteries: effect of endothelial factors and chronic hypoxia in the rat

*,[1]M.R. MacLean, [1]D. Alexander, [1]A. Stirrat, [1]M. Gallagher, [2]S.A. Douglas, [2]E.H. Ohlstein, [1]I. Morecroft & [1]K. Polland

[1]Division of Neuroscience and Biomedical S ystems, Institute of Biomedical and Life Sciences, Glasgow University, Glasgow G12 8QQ and [2]Department of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, PO Box 1539, King of Prussia, Pennsylvania, PA 19406-0939, U.S.A.

Responses to human urotensin-II (hU-II) were investigated in human and rat pulmonary arteries. Rat pulmonary arteries: hU-II was a potent vasoconstrictor of main pulmonary arteries (2–3 mm i.d.) (pEC_{50}, 8.55 ± 0.08, $n=21$) and was ~4 fold more potent than endothelin-1 [ET-1] ($P<0.01$), although its E_{max} was considerably less (~2.5 fold, $P<0.001$). The potency of hU-II increased 2.5 fold with endothelium removal ($P<0.05$) and after raising vascular tone with ET-1 ($P<0.01$). E_{max} was enhanced ~1.5 fold in the presence of N^{ω}-nitro-L-arginine methylester (L-NAME, 100 μM, $P<0.01$) and ~2 fold in vessels from pulmonary hypertensive rats exposed to 2 weeks chronic hypoxia ($P<0.05$). hU-II did not constrict smaller pulmonary arteries. Human pulmonary arteries (~250 μm i.d.): in the presence of L-NAME, 3 out of 10 vessels contracted to hU-II and this contraction was highly variable. hU-II is, therefore, a potent vasoconstrictor of rat main pulmonary arteries and this response is increased by endothelial factors, vascular tone and onset of pulmonary hypertension. Inhibition of nitric oxide synthase uncovers contractile responses to hU-II in human pulmonary arteries.
British Journal of Pharmacology (2000) **130,** 201–204

Keywords: Human urotensin-II; pulmonary arteries; vasoconstriction; endothelium; nitric oxide; pulmonary hypertension

Abbreviations: CCRCs, cumulative concentration response curves; ET-1, endothelin-1; hU-II, human urotensin-II; i.d., internal diameter; L-NAME, N^{ω}-nitro-L-arginine methylester; LV, left ventricle; NO, nitric oxide; NOS, nitric oxide synthase; PHT, pulmonary hypertension; RV, right ventricle

Introduction Human urotensin-II (hU-II) is a cyclic peptide recently cloned in man and present in human cardiac tissue and human arteries (including coronary atheromatous arteries). Rat, mouse and porcine isoforms of U-II have also been cloned (Coulouarn *et al*., 1999; Mori *et al*., 1999). A receptor for hU-II has recently been described in man and hU-II mediates vasoconstriction in many arteries from non-human primates including coronary, pulmonary and carotid arteries (Ames *et al*., 1999; Douglas *et al*., 2000). It is a magnitude more potent than endothelin-1 (ET-1) and hence the most potent mammalian vasoconstrictor identified so far. To date there has not been any demonstration of hU-II-induced vasoconstriction in human vasculature.

hU-II is a potent vasoconstrictor in monkey pulmonary arteries (Ames *et al*., 1999). The current study is, however, the first investigation of its effects in rat or human pulmonary arteries. Chronic hypoxia, increased pulmonary vascular tone and inhibition of nitric oxide synthase (NOS) have all been shown to enhance responses to endogenous vasoconstrictors in pulmonary arteries (MacLean, 1999). In order to investigate a potential role for hU-II in pulmonary vascular disease, it is of interest to study any changes in contractile responses to hU-II under these conditions. Rat and human U-II have been shown to be equipotent vasoconstrictors in rat aorta (Douglas, unpublished observations).

*Author for correspondence; E-mail: M.Maclean@bio.gla.ac.uk

The aims of this study were, therefore, to investigate (i) hU-II-induced vasoconstriction in rat and human pulmonary arteries; (ii) the influence of chronic hypoxic exposure and development of pulmonary hypertension (PHT) on hU-II-induced vasoconstriction in rat pulmonary arteries; (iii) the influence of endothelium removal, NOS inhibition and increased pulmonary vascular tone on hU-II-induced vasoconstriction.

Methods *Chronically hypoxic rats* Male Wistar rats of 28–30 days of age (at start of experiment) were placed in a specially designed perspex hypobaric chamber (Royal Hallamshire Hospital, Sheffield). This was depressurized, over 2 days, to 550 mbar (pO_2 of ~110 mmHg [~10% equivalent]). The temperature of the chamber was maintained at 21–22°C and the chamber was ventilated with air at approximately 45 l min^{-1}. Animals were maintained in these hypoxic/hypobaric conditions for 2 weeks. Aged-matched controls were maintained in room air (20% oxygen). The right ventricle of the heart was carefully dissected free of the septum and left ventricle and these were blotted lightly and weighed. PHT was assessed by measuring the ratio of right ventricular (RV) / total ventricular (TV) weight. This is a well established index of the degree of PHT in rats (Hunter *et al*., 1974).

The main pulmonary artery (2–3 mm i.d.), the pulmonary artery branches (1–2 mm) and the intralobar pulmonary arteries (~1 mm i.d.) were dissected out and set up in 5 ml organ baths under optimal resting tension 1.5, 1.5 and 1.0 g tension respectively. Where indicated, vessels had their endothelium removed by gentle rubbing of the intimal surface.

To assess vascular endothelial function, vessels were pre-constricted with 1 μM phenylephrine and the response to 1 μM acetylcholine determined.

Human pulmonary arteries Macroscopically normal small muscular pulmonary arteries ($\sim$250 μm i.d.) were dissected from lung tissue removed during bronchial carcinoma removal. These were isolated and mounted as ring preparations in isometric wire myographs. Tension was applied to vessels to give a transmural pressure equivalent of approximately 12–16 mmHg, which is similar to *in vivo* pressures of pulmonary arteries.

All vessels were bathed in Krebs-buffer solution (mM: NaCl 18.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 0.6, $CaCl_2$ 2.5, glucose 11 and 23 μM EDTA [pH 7.4]) at 37°C with a constant supply of 16% O_2/5% CO_2 (balance N_2) to mimic *in vivo* pO_2 values (bath pO_2 was $\sim$120 mmHg).

Experimental protocols Following 45 min equilibration, the response to 50 mM KCl was determined. Cumulative concentration-response curves (CCRCs) were constructed to hU-II either in the presence or absence of 100 μM N^{ω}-nitro-L-arginine methylester (L-NAME) or preconstriction with endothelin-1 (ET-1) to give a contraction equivalent to the EC_{10} for ET-1 in this tissue (0.3–1 nM). CCRCs to ET-1 were also constructed in the rat main pulmonary arteries.

Statistical analysis of data All responses to hU-II were calculated both as a percentage of the initial response to 50 mM KCl, to calculate E_{max}, and as a percentage of the maximum response achieved. pEC_{50} values were subsequently calculated from each individual experiment by BBC microcomputer graphical interpolation. Responses to acetylcholine were assessed as a percentage of the preconstriction to phenylephrine. Statistical comparisons of unpaired data sets were carried out using Students' unpaired *t*-test with $P<0.05$ considered significant. Comparisons were made with control data obtained over the same periods of time the experimental procedures were conducted. In those vessels preconstricted with ET-1, responses to hU-II were calculated taking the level of preconstriction with ET-1 as 0%.

Results *Responses to hU-II in rat main pulmonary arteries* hU-II was a potent vasoconstrictor in rat main pulmonary arteries (Figure 1A) but did not constrict smaller rat vessels or human small pulmonary arteries under control conditions ($n=10$). The pEC_{50} for hU-II was 8.55 ± 0.08 ($n=21$) as shown in Table 1. Control curves were constructed at the same time as all pharmacological manipulations, and neither the pEC_{50} or E_{max} values changed significantly over the period of study. For example, statistical comparisons were made with three control groups: pEC_{50}: 8.65 ± 0.07 ($n=7$), 8.48 ± 0.08 ($n=7$) and 8.54 ± 0.09 ($n=7$) with E_{max} values of 55.0 ± 4.7, 57.1 ± 8.6, 59.0 ± 5.0 respectively. The E_{max} for 50 mM KCl in these vessels was 732 ± 48 mg wt tension ($n=21$). hU-II was $\sim$4 fold more potent ($P<0.01$) than ET-1 although ET-1 induced a maximum response $\sim$2 fold greater ($P<0.001$) than that induced by hU-II (Table 1).

Responses to hU-II in endothelium-denuded rat main pulmonary arteries In endothelium-intact arteries, 1 μM acetylcholine induced a $40\pm5\%$ ($n=5$) relaxation of phenylephrine induced tone (850 ± 45 mg wt) which was absent in endothelium-denuded vessels. Removal of the endothelium resulted in a $\sim$2.5 fold increase in the potency of hU-II ($P<0.05$) with no change in the maximum response induced (Figure 1A, Table 1). No attempt was made to remove the endothelium from the human resistance vessels as experience dictates that this

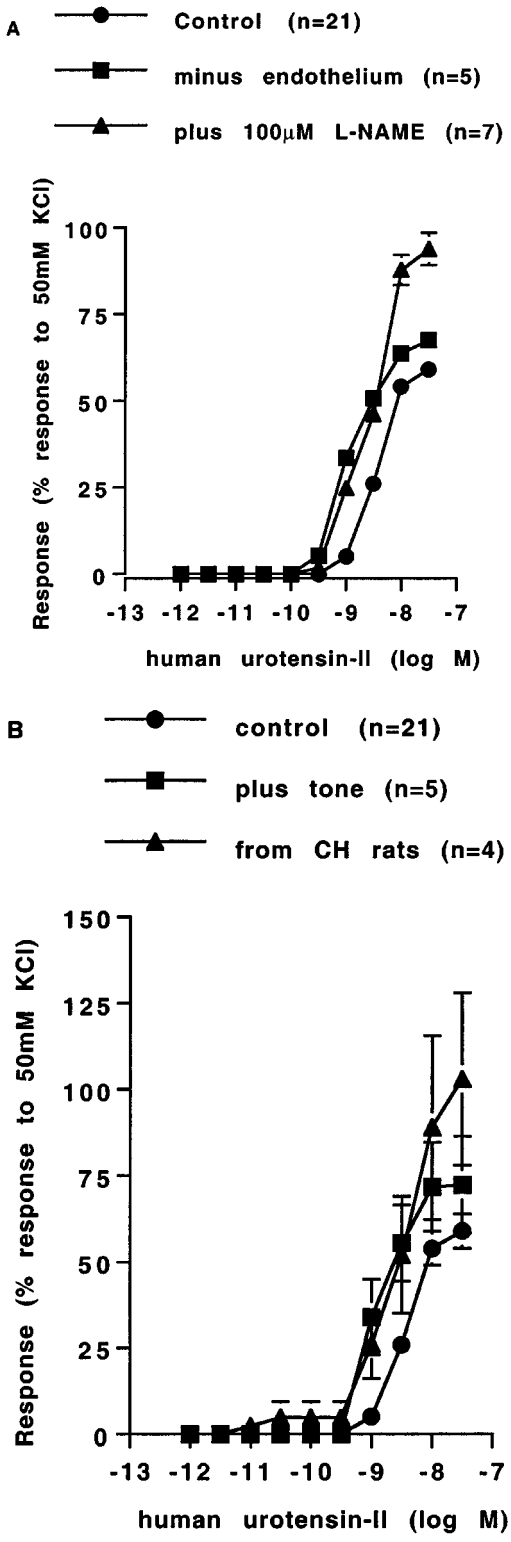

Figure 1 (A) Vasoconstrictor responses to hU-II in rat main pulmonary arteries: effects of mechanical endothelium removal and nitric oxide synthase inhibition with N^{ω}-nitro-L-arginine methylester (L-NAME). (B) Vasoconstrictor responses to hU-II in rat main pulmonary arteries: effects of increased vascular tone induced by 0.3–1 nM endothelin-1 and chronic hypoxia. Chronic hypoxia was induced by exposure of the rats to hypobaric conditions for 2 weeks at an equivalent oxygen tension of 10%. Data is shown as mean$\pm$s.e.mean and calculated as the percentage of a response to 50 mM KCl in the same preparation.

damages the underlying smooth muscle due to the sparcity of the medial layer. In these vessels acetylcholine induced a $50 \pm 5\%$ reduction in phenylephrine induced tone ($n = 10$).

Responses to hU-II in the presence of L-NAME in rat main pulmonary arteries and human pulmonary arteries Rat vessels: Whilst L-NAME did not significantly increase the potency of hU-II, it significantly increased the maximum response ($\sim 64\%$, $P < 0.01$) induced by hU-II (Figure 1A, Table 1). The response to 50 mM KCl in these groups was not significantly different: 726 ± 48 (control group) vs 739 ± 50 mg wt tension (L-NAME treated group).

Human vessels: Out of 10 vessels tested, three contracted to hU-II (in the same concentration range as observed in the rat) in the presence of L-NAME and these responses varied enormously. Adjacent ring sections from the same human vessels and not treated with L-NAME did not respond to hU-II. The maximum responses were 14, 38 and 220% of the response to 50 mM KCl. The response to 50 μM KCl in these vessels was 135 ± 32 mg wt tension. The contraction to hU-II was slowly developing as illustrated in Figure 2.

Responses to hU-II in main pulmonary arteries from chronic hypoxic vs control rats RV/TV was 0.199 ± 0.08 in control rats and 0.303 ± 0.005 in chronic hypoxic rats ($n = 4$, $P < 0.001$) indicating the development of PHT. Whilst the potency of hU-II was not altered in the chronic hypoxic rat vessels, the maximum response to hU-II was increased [$\sim 80\%$, $P < 0.05$] (Figure 1B, Table 1).

Table 1 Vasoconstriction to urotensin-II in the rat main pulmonary artery: effect of endothelium removal, nitric oxide synthase inhibition, vascular tone and chronic hypoxia

	pEC_{50}	E_{max}	n
hU-II control	8.55 ± 0.08	57.0 ± 6.1	21
hU-II minus endothelium	8.94 ± 0.10*	67.4 ± 8.9	5
hU-II plus L-NAME	8.50 ± 0.07	93.7 ± 9.0**	7
hU-II plus tone	8.93 ± 0.07**	72.4 ± 14	5
hU-II chronic hypoxia	8.71 ± 0.11	103 ± 25*	4
Endothelin-1	7.97 ± 0.16**	153 ± 20***	6

Data is shown as mean $\pm$ s.e.mean. Tone was induced by preconstriction with 0.3–1.0 nM endothelin-1. Chronic hypoxia was induced by exposure of the rats to hypobaric conditions for 2 weeks at an equivalent oxygen tension of 10%. Nitric oxide synthase inhibition was induced with 100 μM L-NAME. Statistical comparisons were by Students paired *t*-test. Statistically significant differences vs hU-II controls carried out over the same time period as the experimental protocols ($n = 7$): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. L-NAME: N^{ω}-nitro-L-arginine methylester; hU-II: urotensin-II.

Responses to hU-II in the presence of ET-1-induced tone in rat main pulmonary arteries and human pulmonary arteries Rat vessels: The preconstriction with ET-1 was $24.7 \pm 2.8\%$ of the response to 50 mM KCl ($n = 5$) and equivalent to the pEC_{10} for ET-1 itself in this tissue. hU-II was 2.5 fold ($P < 0.01$) more potent in vessels preconstricted with ET-1 (Figure 1B, Table 1).

Human vessels: hU-II failed to constrict vessels preconstricted with ET-1 ($n = 10$).

Discussion hU-II was a potent vasoconstrictor in the rat main pulmonary artery being some 4 fold more potent than ET-1. hU-II did not, however, constrict smaller pulmonary arteries of the rat nor did it contract human small pulmonary arteries under normal conditions. ET-1 is a potent vasoconstrictor of these vessels (MacLean & McCulloch, 1998; McCulloch *et al*., 1998). This 'anatomically diverse' contractile profile of hU-II is consistent with previous observations made in the rat and dog (Ames *et al*., 1999; Douglas *et al*., 2000). In the rat, the vasoconstrictor activity of hU-II is limited to the thoracic aorta and hU-II has no effect on the rat abdominal aorta or femoral and renal arteries. In contrast, in the dog, hU-II is a coronary-selective vasoconstrictor (Douglas *et al*., 2000). The pEC_{50} for hU-II in the rat aorta was $\sim 8.9-9.3$ and hence similar to the potency observed in the rat main pulmonary artery. The high sensitivity of rat aorta for U-II has been previously reported for fish U-II (Gibson, 1987; Itoh *et al*., 1987; 1988). hU-II has, however been shown to constrict all non-human primate arteries tested to date (Ames *et al*., 1999).

We wished to investigate the possibility that responses to hU-II could be enhanced by conditions which prevail in the rat pulmonary arteries after the development of pulmonary hypertension. These include endothelial dysfunction and elevated vascular tone (for review see MacLean, 1999). The results show that the potency of hU-II was increased by removal of the endothelium and by raising the vascular tone. E_{max} was enhanced by inhibition of NOS and in vessels removed from rats exposed to 2 weeks chronic hypoxia. The observation that E_{max} was increased by L-NAME but not endothelium removal suggests that endothelium-derived vasoactive agents other that NO may interact with hU-II. It has previously been shown that fish U-II can cause endothelium-dependent vasodilations in rat aorta (Gibson,

Figure 2 Cumulative concentration-dependent response to hU-II in a human small muscular pulmonary artery (~ 250 μM i.d.) in the presence of 100 μM L-NAME. hU-II was added in 0.5 logM steps. In this preparation, hU-II induced a maximum contraction 220% that of the response to 50 mM KCl in the same tissue. L-NAME: N^{ω}-nitro-L-arginine methylester; hU-II: urotensin-II.

1987). Therefore it is possible that the effect of L-NAME on contractile responses to hU-II observed here may be due to hU-II-induced release of NO antagonizing its direct vasoconstrictor effect but this requires clarification.

These results are the first to indicate that vasoconstrictor responses to hU-II can be enhanced by removal of the vascular endothelium, NOS inhibition, increased vascular tone and PHT.

Due to the positive nature of our results in the rat pulmonary arteries, we investigated if NO inhibition or raised vascular tone could 'uncover' responses to hU-II in human small pulmonary arteries. Increased vascular tone failed to uncover responses to hU-II in these vessels but 30% of the vessels tested contracted to hU-II in the presence of L-NAME. These responses were slowly developing and highly variable which is likely to be due to the anatomically diverse nature of hU-II receptor density. We cannot, however, determine the precise region of human lung from which the vessels are dissected due to the random nature of the surgical procedures.

This is the first demonstration of a vasoconstrictor effect of hU-II in human tissue however and the results are of great interest as they suggest that deficiencies in NOS or cyclic GMP accumulation could have a profound influence on hU-II-induced vasoconstriction in the human lung which could have implications in PHT. NOS activity can decrease in lungs from patients with severe PHT and guanosine 3′ 5′ cyclic monophosphate accumulation can be compromised in pulmonary arteries removed from chronic hypoxic rats (Giaid & Saleh, 1995; MacLean *et al*., 1996). The observation that pulmonary responses to hU-II are enhanced in the chronic-hypoxic, pulmonary hypertensive rat also lend support to this hypothesis.

In summary, we have shown that hU-II is a more potent vasoconstrictor than ET-1 in rat main pulmonary arteries. It demonstrates anatomical diversity in the rat pulmonary circulation in that it does not constrict smaller pulmonary arteries. In addition, responses to hU-II are enhanced by endothelium removal, NOS inhibition, increased vascular tone and in pulmonary hypertension. Vasoconstriction to hU-II in human small pulmonary arteries can be uncovered by NOS inhibition. Further investigations into the role of hU-II in the pathobiology of PHT are warranted.

References

AMES, R.S., SARAU, H.M., CHAMBERS, J.K., WILLETTE, R.N., AIYAR, N.V., ROMANIC, A.M., LOUDEN, C.S., FOLEY, J.J., SAUERMELCH, C.F., COATNEY, R.W., AO, Z., DISA, J., HOLMES, S.D., STADEL, J.M., MARTIN, J.D., LIU, W.-S., GLOVER, G.I., WILSON, S., MCNULTY, D.E., ELLIS, C.E., ELSHOURBAGY, N.A., SHABON, U., TRILL, J.J., HAY, D.W.P., OHLSTEIN, E.H., BERGSMAN, D.J. & DOUGLAS, S.A. (1999). Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature,* **401,** 282 – 286.

COULOUARN, Y., JEGOU, S., TOSTIVINT, H., VAUDRY, H. & LIHRMANN, I. (1999). Cloning, sequence analysis and tissue distribution of the mouse and rat urotensin II precursors. *FEBS Lett.,* **457,** 28 – 32.

DOUGLAS, S.A., ASHTON, D.J., SAUERMELCH, C.F., COATNEY, R.W., OHLSTEIN, D.H., RUFFOLO, M.R., OHLSTEIN, E.H., AIYAR, N.V. & WILLETTE, R.N. (2000). Human U-II is a potent vasoactive peptide: pharmacological characterization in the rat, mouse, dog and primate. *J. Cardiovasc. Pharmacol.,* in press.

GIAID, A. & SALEH, D. (1995). Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.,* **333,** 214 – 221.

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

HUNTER, C., BARER, G.R., SHAW, J.W. & CLEGG, E.J. (1974). Growth of the heart and lungs in hypoxic rodents: a model of human hypoxic disease. *Clin. Sci. Mol. Med.,* **46,** 375 – 391.

ITOH, H., ITOH, Y., RIVIER, J. & LEDERIS, K. (1987). Contraction of major artery segments of rat by fish neuropeptide urotensin II. *Am. J. Physiol.,* **252,** R361 – R366.

ITOH, H., MCMASTER, D. & LEDERIS, K. (1988). Functional receptors for fish neuropeptide urotensin II in major rat arteries. *Eur. J. Pharmacol.,* **149,** 61 – 66.

MACLEAN, M.R. (1999). Pulmonary hypertension, anorexigens and 5-hydroxytryptamine: pharmacological synergism in action? *Trends Pharmacol. Sci.,* **20,** 490 – 495.

MACLEAN, M.R. & MCCULLOCH, K.M. (1998). Influence of applied tension and nitric oxide on responses to endothelins in rat pulmonary resistance arteries: effect of chronic hypoxia. *Br. J. Pharmacol.,* **123,** 991 – 999.

MACLEAN, M.R., SWEENEY, G., BAIRD, M., MCCULLOCH, K.M., HOUSLAY, M. & MORECROFT, I. (1996). 5-hydroxytryptamine receptors mediating vasoconstriction in pulmonary arteries from control and pulmonary hypertensive rats. *Br. J. Pharmacol.,* **119,** 917 – 930.

MCCULLOCH, K.M., DOCHERTY, C.C. & MACLEAN, M.R. (1998). Endothelin receptors mediating contraction of rat and human pulmonary resistance arteries: effect of chronic hypoxia in the rat. *Br. J. Pharmacol.,* **123,** 1621 – 1630.

MORI, M., SUGO, T., ABE, M., SHIMOMURA, Y., KURIHARA, M., KITADA, C., KIKUCHI, K., SHINTANI, Y., KUROKAWA, T., ONDA, H., NISHIMURA, O. & FUJINO, M. (1999). Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14). *Biochem. Biophys. Res. Commun.,* **265,** 123 – 129.

(Received January 26, 2000
Revised February 16, 2000
Accepted February 22, 2000)