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The in vivo effects of human urotensin II in the rabbit and rat pulmonary circulation: Effects of experimental pulmonary hypertension

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Abstract

In vivo haemodynamic responses to human urotensin-II were determined in two models of pulmonary hypertension: rabbits with left ventricular dysfunction following coronary artery ligation and the hypoxic rat. Effects were also examined in the presence of the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME). Human urotensin-II increased pulmonary arterial pressure to a greater extent in ligated rabbits than their controls and L-NAME increased pulmonary pressure without significantly affecting these responses to human urotensin-II. Human urotensin-II raised right ventricular pressure slightly in control rats but not in hypoxic rats. Human urotensin-II did not constrict control rat isolated small pulmonary arteries and only induced a small constriction of these vessels in hypoxic rats. In conclusion, exogenous human urotensin-II exerts pulmonary pressor responses in vivo in rabbits and also induced small pulmonary pressor responses in control rats. Pulmonary pressor responses to urotensin-II were increased by pulmonary hypertension in rabbits but not in rats.

Keywords: Pulmonary circulation; Blood pressure; Urotensin-II; Hypoxia; Heart failure; Pulmonary hypertension

1. Introduction

Human urotensin-II is an agonist for the orphan receptor GPR14 (now classified as the UT receptor) and is a more potent vasoconstrictor of monkey isolated blood vessels than endothelin-1 with i.v. administration of human urotensin-II causing circulatory collapse (Ames et al., 1999). It induces systemic vasoconstriction in a species-dependent fashion and has an anatomically diverse vasoactive profile (Ames et al., 1999). In the rat, vasoconstrictor activity of human urotensin-II is limited to the thoracic aorta and human urotensin-II has no effect on the rat abdominal aorta, femoral or renal arteries (Douglas et al., 2000). In contrast, in the dog, human urotensin-II is a coronary-selective vasoconstrictor (Douglas et al., 2000).

Human urotensin-II constricts the main pulmonary artery in rats (MacLean et al., 2000) and causes constriction of human large pulmonary arteries in the presence of the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME). Human urotensin-II does not, however, cause vasoconstriction in the isolated perfused human lung (Bennett et al., 2004) and actually induces vasodilation in human small muscular pulmonary arteries (Stirrat et al., 2001). There has been much speculation about the role of human urotensin-II in the development of secondary pulmonary arterial hypertension. Human urotensin-II expression is increased in pulmonary artery smooth muscle cells and endothelial cells in rats with pulmonary arterial hypertension secondary to aortocaval shunting (Qi et al., 2004). Human urotensin-II may also promote pulmonary vascular remodelling via pulmonary artery smooth muscle cell proliferation, mediated by NADPH oxidase activation (Djordjevic et al., 2005). It has been shown that elevated plasma U-II immunoreactivity is positively correlated to pulmonary capillary wedge pressure in patients with ischemic heart disease (Heringlake et al., 2004). To date, however, there have been no studies describing the in vivo

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pulmonary haemodynamic response to human urotensin-II and how these may change in models of pulmonary arterial hypertension. An understanding of the in vivo effects of exogenous human urotensin-II on pulmonary arterial haemodynamics in commonly used models of pulmonary arterial hypertension is important in order to assess the potential of future selective human urotensin-II antagonists. The major aim was, therefore, to examine both systemic and pulmonary haemodynamic effects of human urotensin-II and how these change in models of secondary pulmonary arterial hypertension. We studied the effects of human urotensin-II on the pulmonary haemodynamics in animal models of pulmonary arterial hypertension secondary to left ventricular dysfunction or hypoxia. As the in vivo effects of human urotensin-II are likely to involve the small pulmonary resistance arteries, we were also interested in examining these in vitro. We chose to do this in rats as we previously observed a contractile response to h-U-II in isolated rat large pulmonary arteries (MacLean et al., 2000), whereas previous studies (confirmed by ourselves) indicate human urotensin-II has no effect on isolated pulmonary arteries from rabbit (Camarda et al., 2002).

A secondary aim of the study was to investigate the effects of L-NAME on in vivo responses to human urotensin-II in our animal models, given that L-NAME uncovered contractile responses in human large pulmonary arteries (MacLean et al., 2000) and the hypotensive response to human urotensin-II in anaesthetised rats is partly dependent on nitric oxide (Abdelrahman and Pang, 2002).

2. Methods

This investigation conforms with the provision of the Animals (Scientific procedures) Act 1986 and was approved by the local ethics committee.

2.1. Rabbit

2.1.1. Coronary artery ligation and haemodynamic measurements

A previously described rabbit model of left ventricular dysfunction following coronary artery ligation (subsequently referred to as 'ligated rabbits') was studied, with sham-operated rabbits acting as controls (Deuchar et al., 1998). Briefly, following pre-medication with an intra-muscular injection of fentanyl (0.315 mg/kg)/fluanizone (10 mg/kg), 0.3–0.4 ml/kg (Hypnorm, Jansen) rabbits (male New Zealand white rabbits, 2.5–3.5 kg) were intubated following the administration of midazolam (0.15–0.3 mg/kg), with anaesthesia being maintained with a mixture of nitrous oxide, oxygen (1 : 1 ratio) and 1–1.5% halothane.

A left thoracotomy was performed through the 4th intercostal space to expose the heart. Quinidine hydrochloride (3–5 mg/kg i.v.) was administered prior to coronary artery ligation to reduce the incidence of ventricular fibrillation. The major branch of the left coronary artery was occluded approximately midway between the base and apex of the left ventricular free wall, giving rise to a large homogeneous, full thickness area of myocardial infarction due to the poor collateral circulation of the rabbit coronary system. When an acceptable area of infarction (approx. 20% of the left ventricle) had been produced and the animal was haemodynamically and electrically stable, the thoracotomy was closed. In sham operated controls, hearts were manipulated as in the coronary artery ligated animals but the artery was left unoccluded. Postoperative analgesia (buprenorphine 0.3 mg/kg) was administered every 8–12 h for the first 24–48 h.

2.1.2. Echocardiography

Left ventricular function was assessed using echocardiograpy, 8 weeks after surgery as previously described (Deuchar et al., 1998). Briefly, under light sedation (Hypnorm 0.3 ml/kg) using a right parasternal transducer position M mode long axis measurements of left ventricular end diastolic dimension at the level just below the mitral valve and left atrial internal diastolic diameter at the level of the aortic root were made. By rotating the transducer 90° a short axis image enabled end-diastolic and end-systolic frames to be captured and traced onto the screen via an on-line cineloop computer analysis facility. The measurement of ejection fraction was taken in a plane with the tip of the papillary muscles. Therefore positioning was such that in the coronary artery ligated animals the short axis view rarely included an area of infarct and if anything is likely to underestimate the severity of left ventricular dysfunction observed in these animals. The ejection fraction was calculated as the (End diastolic area − End systolic area)/End diastolic area.

2.1.3. Haemodynamic measurements

The rabbits were anaesthetised and the pulmonary artery and ascending aorta were catheterised in the closed chest animal as previously described (Deuchar et al., 1998). Briefly, animals were anaesthetised as described above and ventilated via a tracheal cannula. A custom made J-shaped catheter (Portex, 1.65 mm OD) was positioned at the right ventricular outflow tract via the right external jugular vein with the aid of X-ray image intensification and a guide wire. A smaller cannula (Portex, 0.75 mm OD) was then passed through the J-shaped cannula into the pulmonary artery. The catheter position was confirmed by the morphology of the pressure trace and by injection of radio opaque dye. Systemic arterial blood pressure was measured through a catheter in the ascending aorta the tip being positioned just above the aortic valve through the right carotid artery. All pressures were recorded using an Elcomatic E751A pressure transducers connected to an MP100 data acquisition system (BIOPAC Systems Inc., Santa Barbara, CA). Results were analysed off line using the built in software package (Acq*Knowledge* 3.5). Cardiac output was measured using a technique of thermodilution, as previously described in detail (Pye et al., 1996). Briefly, a thermistor was positioned in the thoracic aorta above the level of the diaphragm via the left femoral artery. At least 3 thermodilution curves were obtained by the rapid injection of 1 ml of saline of known temperature into the ascending aorta and detected by the thermistor. Cardiac output values were converted to a cardiac index by dividing by the final body weight in kg.

2.1.4. Experimental protocols

All drugs were administered through femoral vein cannulae. Following measurement of baseline pressures and cardiac output, human urotensin-II (0.001–5.0 nmol/kg) was given cumulatively i.v., each dose being given 15 min after the previous concentration. Preliminary experiments involving a single dose of human urotensin-II indicated that any increases in pulmonary arterial pressure would be maximal within 10–12 min and that there was no further increase over the following 1 h. In a few separate animals, bolus injections of 5.0 nmol/kg human urotensin-II were administered and these confirmed the efficacy of the drug and controlled against possible desensitisation of the receptor following cumulative administration. Cardiac output was re-measured following the final dose ($n=8$ for both groups). In other animals, L-NAME (30 μmol/min) was infused throughout the protocol. Following stabilization of the responses to L-NAME, cardiac output was measured and human urotensin-II (0.001–5.0 nmol/kg) was given cumulatively i.v. ($n=12$ for ligated and 13 for controls). Cardiac output was measured following the final dose.

At the end of each experiment the animal was sacrificed and the location of the pulmonary artery catheter confirmed. The ratio of right ventricle weight / final body weight was used as a measure of right ventricular hypertrophy. Right ventricular / total ventricular ratio was not used as an index as, in the ligated animals, the infarct area becomes a thin scar by the end of the 8 week period. This would lead to an overestimate of the extent of right ventricular hypertrophy despite the hypertrophy and remodelling of the surviving left ventricular tissue (Deuchar et al., 2002, 1998).

2.2. Rat

2.2.1. The chronic hypoxic rat

Male Wistar rats (300–400 g) were maintained in normoxic or hypobaric/hypoxic conditions (equivalent to 10% O_2, 0.3% CO_2 balance N_2) for two weeks as described previously (MacLean et al., 2000).

2.2.2. Haemodynamic measurements

Rats were pre-medicated with an intra-peritoneal injection of fentanyl (0.315 mg/kg)/fluanizone (10 mg/kg), 0.9–1.1 ml/kg (Hypnorm, Jansen) mixed with midazolam (0.5 mg/kg). Anaesthesia was maintained using a facemask with a mixture of nitrous oxide, oxygen (1 : 1 ratio) and 0.5–1% halothane.

Systemic blood pressure was measured through a 3F i.v. cannula (Portex Ltd., Hythe, UK) inserted in the ascending aorta via the right carotid artery the tip being positioned just above the aortic valves. This was carried out prior to right heart catheterisation to allow systemic pressure to be monitored throughout this procedure.

The right ventricle was catheterised through the right external jugular vein and right atria using a 3F radio-opaque catheter (William Cook Europe), which had been curved slightly at the tip (approximately 30°). The catheter position within the right ventricle was confirmed by the morphology of the pressure trace and X-ray image intensification. Drugs were administered through the femoral vein using a 2F i.v. cannula (Portex Ltd., Hythe, UK).

Cardiac output was measured by a standard thermodilution technique using a thermistor advanced into the thoracic aorta through the left femoral artery. Briefly, 0.25 ml saline (known, room temperature) was rapidly injected into the ascending aorta and the resulting thermodilution curves detected by the thermistor in the descending thoracic aorta and processed by microcomputer. A minimum of three curves were generated to assure measurement reproducibility. Cardiac index was calculated by dividing cardiac output by the animal weight.

2.2.3. Experimental protocols

Following measurement of baseline pressures, human urotensin-II (0.01–100 nmol/kg) was given cumulatively i.v. ($n=8$ for control and $n=5$ for hypoxic). Each dose was administered 15 min after the preceding dose. In a few separate animals, bolus injections of 100 nmol/kg human urotensin-II were administered and these confirmed the efficacy of the drug and controlled against possible desensitisation of the receptor following cumulative administration. In other control animals, the effect of human urotensin-II was examined in the presence of a continuous venous infusion (30 μmol/min) of the nitric oxide (NO) inhibitor L-NAME which was started at least 45 min prior to the first dose of human urotensin-II ($n=6$). Cardiac output was measured before and after administration of human urotensin-II and L-NAME. At the end of each experiment the animal was sacrificed and the right ventricular / total ventricular ratio was used as an index of right ventricular hypertrophy.

2.2.4. In vitro studies

Rats were killed by overdose of sodium pentobarbitone (60 mg/kg) and the lungs removed and placed in cold Krebs–buffer solution. Small pulmonary arteries (2 mm long, 150–300 μm i.d.) were dissected out and set up for wire myography as previously described in detail (Keegan et al., 2001; MacLean et al., 2000). All vessels were bathed in Krebs–buffer solution at 37 °C with a constant supply of 16%O_2/5%CO_2 (balance N_2) (bath pO_2 was ~120 mm Hg). Tension was then applied to all vessels to give a transmural pressure equivalent of approximately 12–16 mm Hg (controls) and 30–35 mm Hg (hypoxic rats). The response to human urotensin-II in the main pulmonary artery was also examined as a positive control to confirm efficacy of the human urotensin-II.

Following a 45 min equilibration period, the response to 50 mM KCl was determined twice, followed by wash-out and further equilibration. This is the concentration at which KCl produces the greatest contraction in these vessels. Endothelial function was tested by pre-constricting the arteries with 1 μM phenylephrine and then checking for the presence of a vasodilator response to 1 μM acetylcholine. All vessels were then subjected to one of the following experiments. Cumulative concentration–response curves to human urotensin-II (1 pM–1 μM) were constructed either in the presence or absence of 100 μM L-NAME (20 min preincubation). To examine if human urotensin-II could induce vasodilation, vessel vascular tone was raised using 10 nM endothelin-1 then cumulative concentration response curves to human urotensin-II (1 fM–1 μM) were constructed.

2.3. Statistical analysis of data

A repeated measures one way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to compare responses to human urotensin-II vs. basal pressure readings in anaesthetised animals. Statistical comparisons of all other unpaired data sets were carried out by one way ANOVA followed by Tukey's multi-comparisons test. $P<0.05$ was considered significant.

2.4. Drugs

The following drugs were used for anaesthesia, analgesia and euthanasia: Hypnorm (Jansen animal health), hypnovel (Roche), Fluothane (Zeneca Ltd., UK), buprenorphine (Alstoe animal health) and pentobarbitone sodium B.P (Rhone Merieux). The following drugs were prepared in distilled water on experimental days: N^{ω}-nitro-L-arginine methyl ester (L-NAME) and Quinidine hydrochloride monohydrate (Sigma Chemical Co. Ltd., Poole, Dorset, UK). Human urotensin-II, was a kind gift from GlaxoSmithKline.

3. Results

3.1. In vivo experiments

3.1.1. Rabbit

Ligated rabbits had a depressed left ventricular ejection fraction when compared to sham operated controls (0.45±0.01, $n=21$ cf. 0.73±0.01, $n=20$, $P<0.001$). In addition, both left atrial (13.7±0.2 mm cf. 10.7±0.2 mm, $P<0.001$) and left ventricular end diastolic (22.3±0.3 mm cf. 17.9±0.3 mm, $P<0.001$) diameters were significantly increased in the ligated rabbits. These findings are consistent with previous data in this model (Deuchar et al., 2002; Deuchar et al., 1998) and provide evidence of left ventricular dysfunction. Right ventricular hypertrophy was evident with right ventricular weight corrected for final body weight being greater in the ligated animals (0.69±0.03 g/kg cf. 0.48±0.01 g/kg in controls, $n=8$, $P<0.001$). There was also evidence of lung congestion with greater lung weight corrected for final body weight in coronary ligated rabbits (4.56±0.21 g/kg cf. 3.76±0.09 g/kg in controls, $P<0.01$).

Pulmonary arterial pressure was significantly elevated in the ligated rabbits (Fig. 1A, $P<0.001$). Human urotensin-II induced a 20–27% increase in pulmonary pressure in both groups of rabbits (Fig. 1A) with the magnitude of the maximum response being 2–3 fold greater in rabbits with pulmonary arterial hypertension (increase in pulmonary arterial pressure: 2.6±0.5 mm Hg in pulmonary arterial hypertension rabbits cf. 1.0±0.3 mm Hg in shams, $P<0.05$).

L-NAME resulted in an increase in pulmonary pressure in both groups of rabbits with this effect being ~ 2 fold greater in rabbits with pulmonary arterial hypertension (an increase of 7.9±1.8 mm Hg cf. 3.8±0.8 mm Hg in sham operated controls, $P<0.05$). In the presence of L-NAME, human urotensin-II increased mean pulmonary pressure in all animals (Fig. 1A). In comparison to pulmonary effects seen to human urotensin-II alone there was a trend towards an increase in the magnitude of the response to each dose of urotensin-II in both groups of rabbits following L-NAME (e.g. for the maximum dose used, there was an increase of 8.5±2.6 mm Hg c.f. 2.6±0.5 mm Hg with human urotensin-II alone in ligated rabbits, $P=0.08$, and an increase of 4.8±1.5 mm Hg c.f. 1.0±0.3 mm Hg with human urotensin-II alone in sham operated controls, $P=0.06$). There was no difference in baseline systemic arterial pressure and no effect on mean systemic arterial pressure in either of the experimental groups following cumulative administration of human urotensin-II alone (Fig. 1B). L-NAME induced a ~ 25% elevation in mean

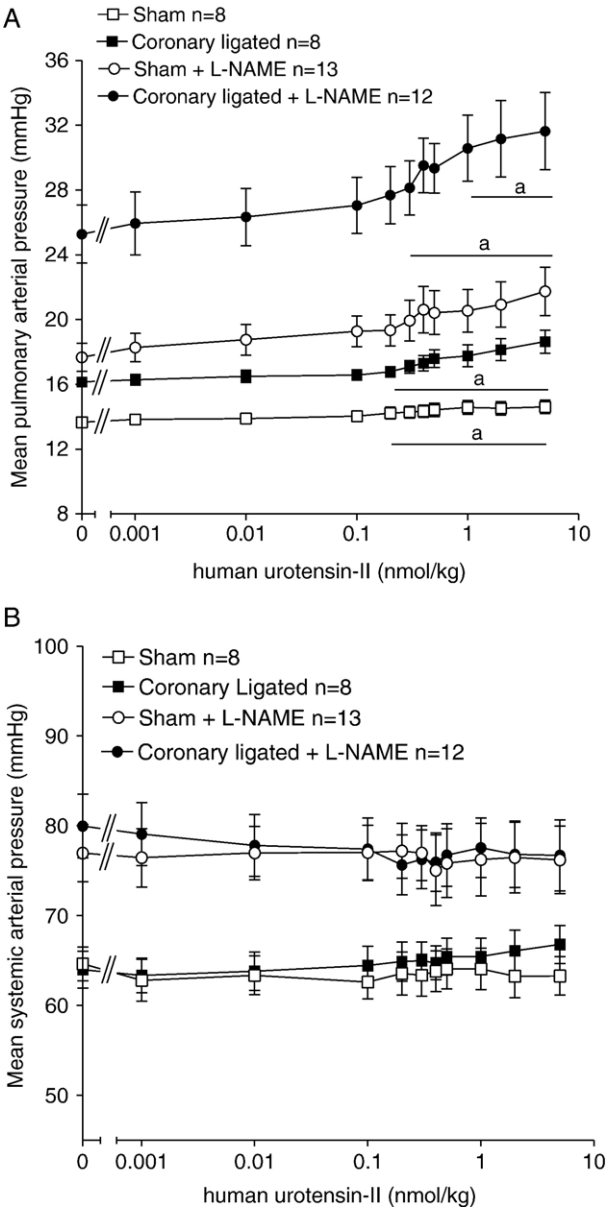

Fig. 1. Changes in (a) mean pulmonary arterial pressure and (b) mean systemic arterial pressure in response to human urotensin-II in rabbits with left ventricular dysfunction secondary to coronary ligation and sham-operated controls. Effect of L-NAME (30 μmol/min). Data is shown as mean±S.E.M. 0 on x-axis: Pressures immediately prior to human urotensin-II administration (basal values). Statistical analysis was by repeated measures one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. $^{a}P<0.05$ vs. 0 nmol/kg. n=number of animals.

Table 1
Cardiac index and heart rate in the rat and rabbit: effect of human urotensin-II and L-NAME alone or human urotensin-II in the presence of L-NAME

	Cardiac index, ml/min/kg			Heart rate, beats/min		
	Baseline	+ L-NAME	+ Urotensin-II	Baseline	+ L-NAME	+ Urotensin-II
Rat						
Control	574±37	n/a	607±25	410±6	n/a	391±6
+ L-NAME	434±56	229±33[a]	262±18[a]	372±20	377±12	386±7
Rabbit						
Sham-operated control	146±12	n/a	188±27[a]	245±9	n/a	243±13
L-NAME treated, sham-operated	131±7	110±7[a]	110±6[a]	253±7	233±10	244±9
Coronary-ligated control	155±8	n/a	172±11	229±6	n/a	23±16
L-NAME treated, coronary-ligated	136±9	103±7[a]	102±10[a]	238±9	222±11	227±9

Values are means±S.E.M.; L-NAME, N^{ω}-nitro-L-arginine methyl ester; statistical analysis was by one way analysis of variance followed by Dunnett's post hoc test. [a]$P<0.05$ vs. respective baseline value; $n=3$–4 rats and 8–12 rabbits; n/a no L-NAME applied.

systemic arterial pressure but human urotensin-II did not have any further effect over the concentrations studied. With regards to cardiac index and heart rate the only effect seen was an increase in cardiac index following human urotensin-II in sham control rabbits. Human urotensin-II had no effect on either cardiac index or heart rate in the ligated rabbits (Table 1). L-NAME alone reduced cardiac index in all rabbits without affecting heart rate (Table 1) but the subsequent administration of human urotensin-II had no further effects.

3.1.2. Rat

Right ventricular / total ventricular ratios in the control rats were 0.211±0.002 ($n=8$) and in the hypoxic rats were 0.308±0.008 ($n=10$, $P<0.001$ vs. controls). Mean right ventricular pressures were markedly elevated in rats exposed to chronic hypoxia (Fig. 2A). Human urotensin-II, at high doses, induced a small rise in right ventricular pressures in control rats but had no significant effect on right ventricular pressures in the hypoxic rats (Fig. 2A). L-NAME had no significant effect on baseline right ventricular pressures or responses to human urotensin-II, other than to inhibit the small pressor responses observed in control rats (Fig. 2A). In these control rats human urotensin-II, at concentrations between 3 and 50 nmol/kg, resulted in a ~14% decrease in systemic arterial pressure (Fig. 2B) but had no effect on cardiac index or heart rate (Table 1). Infusion of L-NAME caused a profound increase in systemic arterial pressure in control rats (Fig. 2). As L-NAME consistently resulted in the appearance of arrhythmias in the hypoxic rats even at the very lowest concentrations, pulmonary and systemic pressures became uninterpretable and were, hence, not recorded. The administration of human urotensin-II in the presence of L-NAME had no effect on systemic pressures in the control rats (Fig. 2B). L-NAME itself did induce a decrease in cardiac index but no change in heart rate in control rats (Table 1) but there were no additional effects of human urotensin-II observed during the subsequent cumulative dose response experiments in the presence or absence of L-NAME.

3.2. In vitro experiments

Human urotensin-II induced no contractile effect in small pulmonary resistance arteries from control rats and only induced very small (~ 4% of the response to KCl) responses in hypoxic rat small pulmonary resistance arteries. In these vessels (from both control rats and hypoxic rats), responses to human urotensin-II were unaffected by L-NAME. Human urotensin-II did induce constriction of the main pulmonary artery, however, confirming activity (Fig. 3). Human urotensin-II had no vasodilator effect in small pulmonary resistance arteries from either control rats or hypoxic rats. Acetylcholine induced a vasodilation of 23±5% ($n=4$) of the pre-constriction

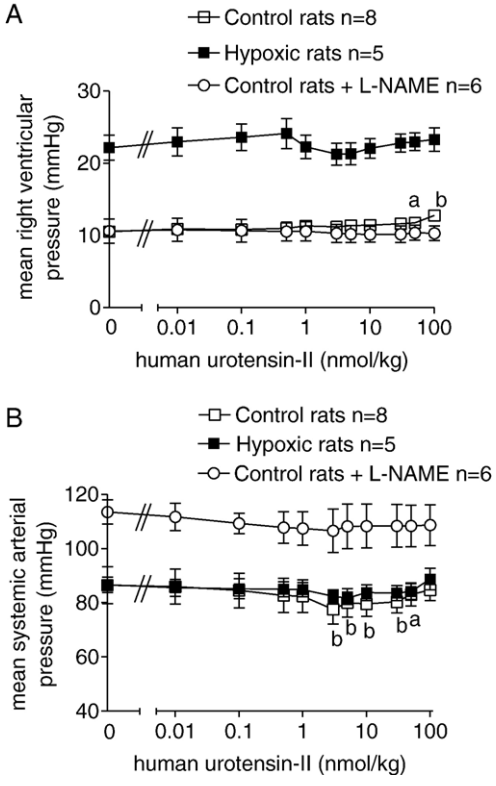

Fig. 2. Changes in (a) mean right ventricular pressure (b) mean systemic arterial pressure in response to human urotensin-II in control and chronic hypoxic rats. Effect of L-NAME (30 μmol/min) in control rats. 0 on *x* axis: pressures immediately prior to human urotensin-II administration. Statistical analysis (differences from baseline mean pressures in control rats) was by repeated measures one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. [a]$P<0.05$, [b]$P<0.01$ vs. 0 nmol/kg. Data is shown as mean±S.E.M.

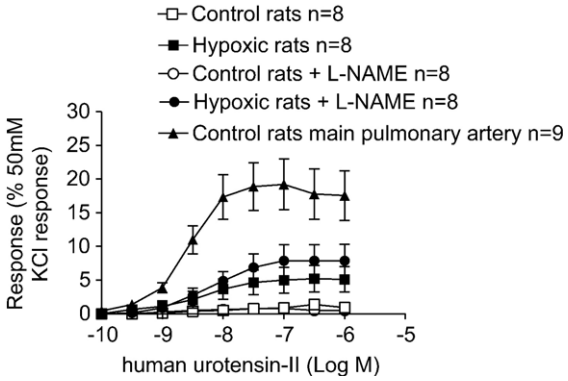

Fig. 3. Contractile responses to human urotensin-II in rat small pulmonary resistance arteries in control rats and rats exposed to two weeks of hypoxia. The effect of L-NAME (100 μM) is also shown. The contractile response is expressed as a % of the response to 50 mM KCl in each vessel. Data is shown as mean ± S.E.M. *n* = number of animals. The response to human urotensin-II in main pulmonary arteries from control rats is shown as a positive control.

to phenylephrine in control rat small pulmonary resistance arteries.

4. Discussion

It has been suggested that the human urotensin-II receptor system may be a target for the treatment of pulmonary arterial hypertension (Russell, 2005). Yet, prior to this study, in vivo effects of human urotensin-II on the pulmonary circulation, or possible changes in human urotensin-II pulmonary responses to human urotensin-II in animal models of pulmonary arterial hypertension, had not been investigated. This study addressed these issues by studying in vivo pulmonary responses to human urotensin-II in models of pulmonary arterial hypertension secondary to left ventricular dysfunction and chronic hypoxia. The ligated model was chosen as: (i) these rabbits develop early onset pulmonary arterial hypertension (Deuchar et al., 2002, 1998), (ii) there is a positive relationship between plasma human urotensin-II and diastolic myocardial dysfunction in ischemic heart disease (Heringlake et al., 2004), and (iii) there is evidence of increased plasma and myocardial human urotensin-II in heart failure patients (Richards et al., 2002; Douglas et al., 2002). Hence evidence indicates that human urotensin-II may be involved in the pathophysiology of heart disease, which is often accompanied by pulmonary arterial hypertension. We chose the hypoxic rat model of pulmonary hypertension as we have previously shown that contractile responses to human urotensin-II, were increased in isolated main pulmonary arteries from hypoxic rats (MacLean et al., 2000). In addition, hypoxic rats demonstrate right ventricular remodelling and an increase in right ventricular pressure, and this is accompanied by increased human urotensin-II levels in the right ventricle (Keegan et al., 2001; MacLean et al., 2000; Zhang et al., 2002).

In this study we have, for the first time, demonstrated a dose-dependent pulmonary pressor response to cumulative doses of human urotensin-II in vivo. Human urotensin-II induced a moderate rise in pulmonary pressures in control rabbits (and an increase in cardiac index) and human urotensin-II produced a significantly greater rise in pulmonary pressure in ligated rabbits (with no change in cardiac index). It could be argued that the small human urotensin-II-induced increase in pulmonary pressure seen in control rabbits might have been due to the increase in cardiac output rather than a change in pulmonary resistance. However, the fact that human urotensin-II induced an increase in pulmonary pressure in ligated rabbits without changes in cardiac output or heart rate suggests that the effect is likely to be a direct effect on pulmonary vascular resistance. This is compatible with a study showing that human urotensin-II can cause increased systemic microvascular tone in patients with heart failure (Lim et al., 2004). However, no studies have examined the pulmonary effects in such patients and it is possible that this effect may be species dependent, as are so many of the effects of human urotensin-II.

The pressor response to human urotensin-II was moderate (20–27% rise over basal pressures in both control and ligated rabbits). For example, we have previously demonstrated that serotonin can induce an increase in pulmonary pressure of ~40% in control rabbits and ~50% in ligated rabbits (Deuchar et al., 1998). To investigate the possibility that there had been desensitisation to human urotensin-II in our study, we investigated the effects of bolus injections of high human urotensin-II concentrations. These were of the same magnitude as doses given cumulatively, hence no desensitisation of human urotensin-II occurred as a result of the cumulative additions.

Human urotensin-II had only a small pulmonary pressor response in control rats and had no effect on right ventricular pressure in hypoxic rats. It slightly decreased systemic pressure (~14%) in normoxic rats, in the absence of any effects on cardiac output or heart rate. Basal right ventricular pressure was markedly elevated by hypoxia (~100%) in the hypoxic rats and human urotensin-II had no additional effect on this elevated right ventricular pressure. Human urotensin-II had no effect on systemic pressures or heart rate in the hypoxic rat. Bolus injections of high human urotensin-II concentrations were of the same magnitude as doses given cumulatively. Hence, desensitisation cannot explain the absence of pressor responses to human urotensin-II concentrations. In order to check that the batch of human urotensin-II being used was viable, we also tested its effects on rat main pulmonary arteries and showed a significant contractile response. Whilst human urotensin-II does not have a direct pressor response on the pulmonary circulation in vivo, it remains a possibility that endogenous urotensin-II may contribute to the increased right ventricular pressure induced by hypoxia. Studies of a selective urotensin-II antagonist would be required to investigate this. However, it should be noted that at the time of this study, no selective urotensin-II antagonists are available for study.

We show that human urotensin-II does not induce a significant contractile response in rat isolated small pulmonary arteries either before or after exposure to hypoxia or after L-NAME pre-treatment. This is in contrast to the contractile response we observed in the rat isolated main pulmonary artery. It is curious that human urotensin-II induced a pressor response in vivo but was unable to constrict rat isolated pulmonary resistance arteries. A possible explanation is that circulating in

vivo factors, which are absent from the in vitro set-up, may synergise with urotensin-II in vivo to induce vasoconstriction.

We previously identified human urotensin-II as a potent vasodilator of human pulmonary and abdominal resistance arteries (Stirrat et al., 2001). In contrast, results here show no vasodilator responses to human urotensin-II in the rat isolated small pulmonary artery. This was not due to endothelial damage as vasodilator responses to acetylcholine were evident. This again highlights the species dependent effects of urotensin-II.

Human urotensin-II induced a fall in systemic pressure in normoxic rats consistent with previous studies in the anaesthetised rat where i.v. urotensin-II has been shown to decrease systemic blood pressure by ~19% (Abdelrahman and Pang, 2002) and the anaesthetised monkey where it induced a ~18% fall in mean blood pressure (Zhu et al., 2004). However, consistent with the species dependence of urotensin-II actions, this is in contrast to the effect of i.v. urotensin-II in cats where it caused a pressor effect (Behm et al., 2004). Indeed we show in this study that urotensin-II had no effect on systemic pressures in either control rabbits or ligated rabbits (although the possibility of a systemic effect in rabbits at higher doses cannot be ruled out).

As discussed in the introduction, L-NAME has been shown to influence pulmonary and systemic responses to urotensin-II and hence we wished to investigate its effects on human urotensin-II-induced responses in vivo. L-NAME itself increased pulmonary pressure in the control rabbits, as we have shown previously (Deuchar et al., 2002). This is consistent with a degree of basal NO synthase in rabbits and/or a degree of pulmonary vascular tone. L-NAME itself cause an even greater increase in pulmonary pressure in the ligated rabbits than in their controls, suggesting that an increase in the degree of basal NO synthase and/or degree of pulmonary vascular tone accompanies the development of pulmonary hypertension in this model.

In the control rat, L-NAME had no effect on basal right ventricular pressure. This could signify either that there is no basal release of NO in the pulmonary circulation or that there is no vasoconstrictor-induced pulmonary vascular tone which would induce NO synthase activity. In the normoxic rat, L-NAME did not uncover any further effect of human urotensin-II in vivo. It did, in fact, appear to inhibit the small rise in right ventricular pressure induced by human urotensin-II. L-NAME also had no effect on the very small contractile response observed in isolated small pulmonary arteries removed from the hypoxic rats indicating that NO activity does not influence human urotensin-II constriction in these vessels. Consistent with species variation, in both control rabbits and ligated rabbits there was actually a trend towards a larger increase in the pulmonary arterial pressure response to human urotensin-II in the presence of L-NAME suggesting that, in rabbits, when the NO system is compromised, the in vivo pulmonary vascular response to urotensin-II may be increased.

In considering the effect of L-NAME on responses to human urotensin-II, the effect of L-NAME on cardiac output needs to be considered. L-NAME on its own reduced cardiac index in the normoxic rat without a change in heart rate, suggesting a decrease in stroke volume. L-NAME also reduced cardiac index (with no change in heart rate) in both control rabbits and ligated rabbits. Endothelial NOS is expressed in cardiac myocytes indicating that NO has the capacity to directly affect cardiac functions (Seki et al., 1996). Indeed, L-NAME has been shown to reduce overall ventricular performance in dogs by reducing left ventricular contractility, an effect which was suggested to be due to its effects on increasing afterload (Murray et al., 1999). Thus the fall in cardiac index shown here could be explained by a direct detrimental effect on the haemodynamic performance of the left ventricle in the face of increased afterload, rather than being as the result of a reflex mechanism.

In conclusion, human urotensin-II exerts a pulmonary pressor response in rabbits in vivo and this is increased in the rabbit model of pulmonary hypertension secondary to left ventricular dysfunction. Human urotensin-II only induces a very small pulmonary pressor response in control rats in vivo and this is not increased after exposure to hypoxia.

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