British Journal of Pharmacology (1996) 119, 1217–1222

Angiotensin II receptor expression and inhibition in the chronically hypoxic rat lung

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1 Angiotensin II (AII) binding density and the effect of chronic AII receptor blockade were examined in the rat model of hypoxia-induced pulmonary hypertension.

2 [^{125}I]-[Sar^1,Ile^8]AII binding capacity was increased in lung membranes from rats exposed to hypoxia (10% fractional inspired O_2) for 7 days compared to normal rats (B_{max} 108±12 vs 77±3 fmol mg^{-1} protein; $P<0.05$), with no significant change in dissociation constant. Competition with specific AII receptor subtype antagonists demonstrated that AT_1 is the predominant subtype in both normal and hypoxic lung.

3 Rats treated intravenously with the AT_1 antagonist, GR138950C, 1 mg kg^{-1} day^{-1} rather than saline alone during 7 days of exposure to hypoxia developed less pulmonary hypertension (pulmonary arterial pressure: 21.3±1.7 vs 28.3±1.1 mmHg; $P<0.05$), right ventricular hypertrophy (right/left ventricle weight ratio: 0.35±0.01 vs 0.45±0.01; $P<0.05$) and pulmonary artery remodelling (abundance of thick-walled pulmonary vessels: 9.6±1.4% vs 20.1±0.9%; $P<0.05$).

4 The reduction in cardiac hypertrophy and pulmonary remodelling with the AT_1 antagonist was greater than that achieved by a dose of sodium nitroprusside (SNP) that produced a comparable attenuation of the rise in pulmonary arterial pressure during hypoxia.

5 The data suggest that AII, via the AT_1 receptor, has a role in the early pathogenesis of hypoxia-induced pulmonary hypertension in the rat.

Keywords: Pulmonary hypertension; AT_1 receptor; sodium nitroprusside

Introduction

The pulmonary vasculature responds to chronic hypoxia by vasoconstriction and structural remodelling (Riley, 1991). The pulmonary arteries of rats kept in a normobaric hypoxic chamber (10% fractional inspired O_2) show progressive hypertrophy of the muscular coat in the arterial media and the abnormal extension of smooth muscle into normally thin-walled pulmonary arterioles, with consequent narrowing of the vessel lumen. These changes are apparent within 3 days, progress to a maximum over 2 weeks and contribute to the development and persistence of pulmonary hypertension and right ventricular hypertrophy.

Evidence that angiotensin II (AII) is an important mediator of systemic vascular remodelling has stimulated interest in the contribution of this peptide to the pulmonary vascular response to hypoxia. However, studies based on changes in AII levels and angiotensin-converting enzyme (ACE) activity have failed to define its role. Circulating AII levels have been reported to increase during the first week of exposure to hypoxia but thereafter decline to baseline (Zakheim *et al.*, 1976). Several early studies indicated that whole lung ACE activity is reduced by chronic exposure to hypoxia (Keane *et al.*, 1982; Kay *et al.*, 1985; Jederlinic *et al.*, 1988), but recently a local increase in ACE levels has been observed in the walls of newly muscularized pulmonary arteries in hypoxic rat lung (Morrell *et al.*, 1995a). Treatment with ACE inhibitors has been reported to reduce pulmonary arterial pressure and remodelling the right ventricular hypertrophy in the chronically hypoxic rat (Zakheim *et al.*, 1975; Clozel *et al.*, 1991). Paradoxically, chronic infusion of AII has been reported to attenuate the development of pulmonary hypertension and its attendent vascular remodelling during chronic exposure to hypoxia (Rabinovitch *et al.*, 1988). This is surprising given that the pressor response to AII is increased in chronically hypoxic rat lung (Caldwell & Blatteis, 1983; Jin *et al.*, 1987).

The AII receptor represents an important level of regulation of AII activity but there are no data on the effect of hypoxia on AII receptor expression. Two major AII receptor subtypes have been cloned, known as AT_1 and AT_2. The AT_1 receptor is the predominant subtype in the adrenal cortex, vasculature and kidney of several species (Harris & Inagami, 1995) and is thought to mediate the hypertensive and trophic effects of AII (Chiu *et al.*, 1991). In the rat, the AT_2 subtype is found mainly in the adrenal medulla and ovary; interestingly, it is more widely expressed in the developing foetus (Viswanathan *et al.*, 1991) and may have a role in vascular neointima formation (Janiak *et al.*, 1992). Morrell *et al.* (1995b) found that rats treated with an AT_1 receptor antagonist during exposure to hypobaric hypoxia for 14 days develop less pulmonary hypertension and remodelling compared to animals treated with an AT_2 antagonist or saline. However, the AT_1 antagonist, losartan, employed in this study is also a competitive antagonist at thromboxane A_2 (TxA_2) receptors (Liu *et al.*, 1992) and inhibits TxA_2-induced pulmonary hypertension in rats (Bertolino *et al.*, 1994).

The aims of the present study were: (i) to quantify changes in pulmonary AII receptor density in the normobaric chronically hypoxic rat; (ii) to examine the effect on pulmonary vascular remodelling of an alternative AT_1 antagonist (GR138950C; a novel bromobenzofuran trifluoromethansulphonomide) used in a dose that does not affect TxA_2-mediated vascular activity; and (iii) to compare the response with the effect of chronic infusion of sodium nitroprusside, a vasodilator that acts through a different mechanism, via stimulation of soluble guanylyl cyclase.

Methods

Rat model

Male Wistar rats (250–280 g) were kept in a normobaric hypoxic chamber for periods of up to 7 days. The fractional

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inspired O_2 concentration was kept at 10% and excess humidity and CO_2 removed by means of scrub filters, as previously described (Zhao *et al.*, 1991). Food and water were allowed *ad libitum*. Control animals were kept in the chamber but allowed to breathe normal air.

Ligand binding studies

Membrane preparation Rats exposed to hypoxia for 7 days and their controls ($n=4$ each group) were killed with an overdose of Hypnorm anaesthetic (fentanyl/flunasone) and the lungs were removed and snap-frozen in liquid nitrogen. Cell membranes were prepared by differential centrifugation as previously described (Brown *et al.*, 1995). The final pellets were resuspended in 50 mM HEPES pH 7.6 at a concentration of 2–10 mg protein ml^{-1}, divided into aliquots and stored at −80°C.

Receptor binding assay Membranes from each rat were incubated for 90 min at 25°C in 0.5 ml of binding buffer (50 mM HEPES pH 7.4, 10 mM $MgCl_2$, 1 mg ml^{-1} bacitracin, 50 mg l^{-1} pepstatin and 0.2% bovine serum albumin) containing 600 Bq (14 pM) [^{125}I]-[Sar^1,Ile^8]AII (Amersham Int, UK), in siliconised microcentrifuge tubes. Bound and free label were separated by centrifugation at 15,000 *g* for 2 min at 4°C. Pellets were washed with 0.5 ml binding buffer at 4°C and counted. Non-specific binding was determined in the presence of 1 mM unlabelled peptide. Specific binding was defined as total binding minus non-specific binding. In competition studies, the concentration of unlabelled peptide or ligand was varied from 0 to 10^{-5} M. Binding data were analysed by non-linear regression using the 'Receptor-Fit' programme (London Software, Cleveland, OH, U.S.A.) to calculate the dissociation constant (K_D) and the concentration of binding sites (B_{max}). The integrity of the radioligand after incubation with the membranes was determined by fast protein liquid chromatography using reversed-phase C_2/C_{18} columns (pep RPC HR5/5, Pharmacia Biotech, St Albans, Herts UK); >95% co-eluted with standard (results not shown).

Challenge with TxA_2 agonist, U-46619

Normal rats were anaesthetized with Hypnorm (Fentanyl/Flunasone) and a femoral vein and pulmonary artery cannulated in each animal. The pulmonary artery catheter was inserted via the jugular vein and the right ventricle and connected to a pressure transducer and a MacLab integrated data acquisition system. The position of the catheter was confirmed by the characteristic waveform of the tracing from the pressure transducer. The femoral line was used for the administration of drugs.

Each rat received 2 doses of U-46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$) 5 μg kg^{-1}. The second dose was given 5 min after one of the following: (i) saline; (ii) the AT_1 antagonist, losartan, 3 or 30 mg kg^{-1}; or (iii) an alternative AT_1 antagonist, GR138950C, 0.3 or 3 mg kg^{-1}. All compounds were given in 100 ml saline over 1 min. The dose of U-46619 administered was based on preliminary dose-response experiments (data not shown) which confirmed the observations of Bertolino *et al.* (1994). The doses of losartan examined bracketed the dose used by Morrell *et al.* (1995b) (20 mg kg^{-1} day^{-1}) and those of GR138950C exceeded the dose of this agent chosen for chronic dosing studies as described below.

Chronic dosing studies

Separate groups of rats were randomized to receive (i) the AT_1 receptor antagonist, GR138950C 1 mg kg^{-1} day^{-1}; (ii) sodium nitroprusside 4.3 mg kg^{-1} day^{-1}; (iii) sodium nitroprusside 14.4 mg kg^{-1} day^{-1}; or (iv) saline. These were administered via the right jugular vein by 14 day Alzet osmotic mini-pumps (delivering 10 μl h^{-1}) implanted subcutaneously. The mini-pumps were implanted 24 h before the rats were placed in the chamber. The rats were kept in the chamber for 7 days under hypoxic (FiO_2 10%), normobaric conditions. Control rats received treatments (i), (ii) or (iv) and were allowed to breathe normal air. The dose of GR138950C was chosen on the basis of dose-response curves in normal and hypertensive rats; 1 mg kg^{-1} day^{-1} reduced systemic blood pressure in the renal artery-ligated rat (where the renin-angiotensin system is stimulated) but not in normal rats (Hilditch *et al.*, 1995). The doses of SNP were based on evidence that infusions of 4 μg min^{-1} exert demonstrable haemodynamic effects in rats and that 15 mg kg^{-1} day^{-1} is probably close to the maximum tolerated dose of this compound in this species (Thomas *et al.*, 1988; Volker & Kreye, 1980).

After 7 days in the chamber, the animals were anaesthetized with pentobarbitone to permit cannulation of the pulmonary artery and systemic blood vessels. The pulmonary artery was cannulated with a preformed cannula via the right jugular vein, atrium and ventricle as previously described (Zhao *et al.*, 1991) and a catheter inserted in a femoral vein for administration of AII. The animals that received the AT_1 antagonist and the saline controls were challenged with AII (given as a bolus injection) in increasing doses (0.001–10 μg kg^{-1}); the peak pressor response after each dose was recorded, using a MacLab integrated data acquisition system. The animals remained anaesthetized throughout this procedure.

Haematocrits were measured and then hearts were removed for weighing; the right and left ventricles were dissected free (the septum with the left venticle) and the chambers were weighed separately. The lungs were perfusion-fixed for histological assessment of vascular remodelling as previously described (Winter *et al.*, 1991). Briefly, 3 μm sections were stained with elastic Van Gieson and examined systematically using the ×40 magnification objective in a 'blind' manner. All vessels 25–55 μm diameter with a definite elastic coat and adjacent to alveoli and alveolar ducts were counted. Those with a double elastic lamina making up 35 to 100% of the circumference (indicating a muscular media) were designated thick-walled peripheral lung vessels (TWPV) and the proportion expressed as a percentage of the total vessels counted.

Statistical analysis

Results are expressed as mean ± s.e.mean. The data were examined by two-way ANOVA followed by Scheffe's test to examine for statistical significance. The significance level was set at 0.05.

Results

Ligand binding studies

Initial binding conditions were established using lung membranes from a normal rat. The binding of [^{125}I]-[Sar^1 Ile^8]AII to lung membranes was time-dependent, reaching a plateau at 60 min, and increased linearly with increasing amounts of protein over the range 25 to 100 μg (Figure 1). All subsequent experiments were performed with 50 μg protein at 25°C. Under these conditions approximately 90% of [^{125}I]-[Sar^1,Ile^8]AII binding was specific.

Non-linear regression analysis of the competition binding experiments suggested a single class of receptors in membranes from normal rat lungs with a K_D of 0.78 ± 0.07 nM and a B_{max} of 77 ± 3 fmol mg^{-1} protein ($n=4$). Studies with AT_1 specific ligands, losartan and GR138950C, demonstrated competition binding with an IC_{50} of 9×10^{-11} M and 5×10^{-9} M respectively (Figure 2a). Studies with an AT_2 specific ligand, PD123319 (((S)-1-[[4-dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid, ditrifluroacetate,dihydrate), showed competition binding at concentrations above 10^{-7} M with an IC_{50} of 2.7×10^{-7} M. These data suggest that the AT_1 subtype is the predominant AII receptor form in normal rat lung. Similar studies on membranes from 7 day hypoxic rat lung showed that the main receptor subtype was also AT_1; the IC_{50} values for losartan, GR138950C and

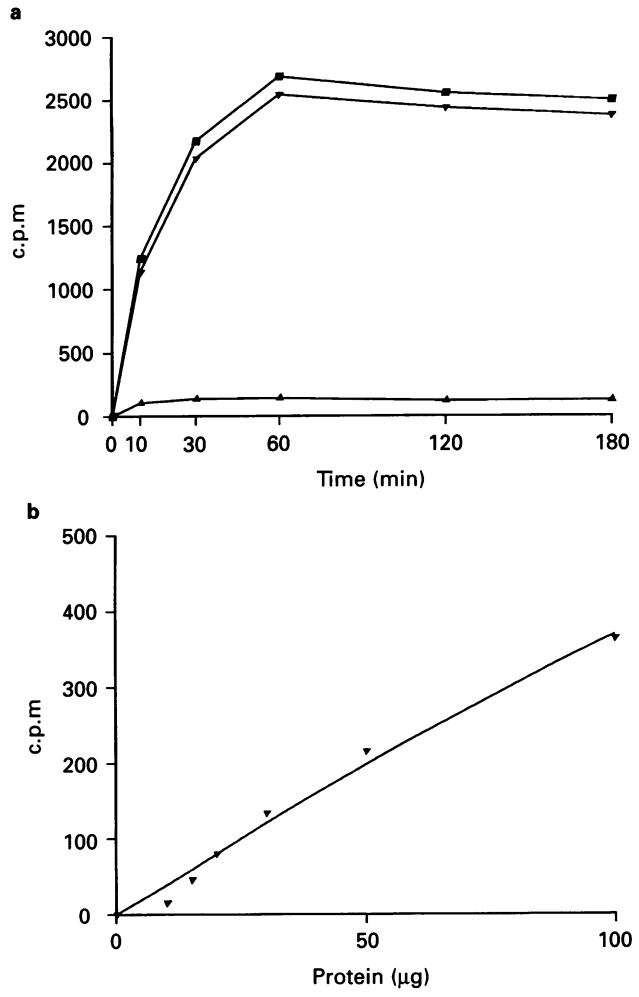

Figure 1 Effect of (a) time and (b) increasing protein concentration on [^{125}I]-[Sar^1,Ile^8]AII binding to lung membranes prepared from a normal rat: (■) total, (▼) specific and (▲) non-specific binding.

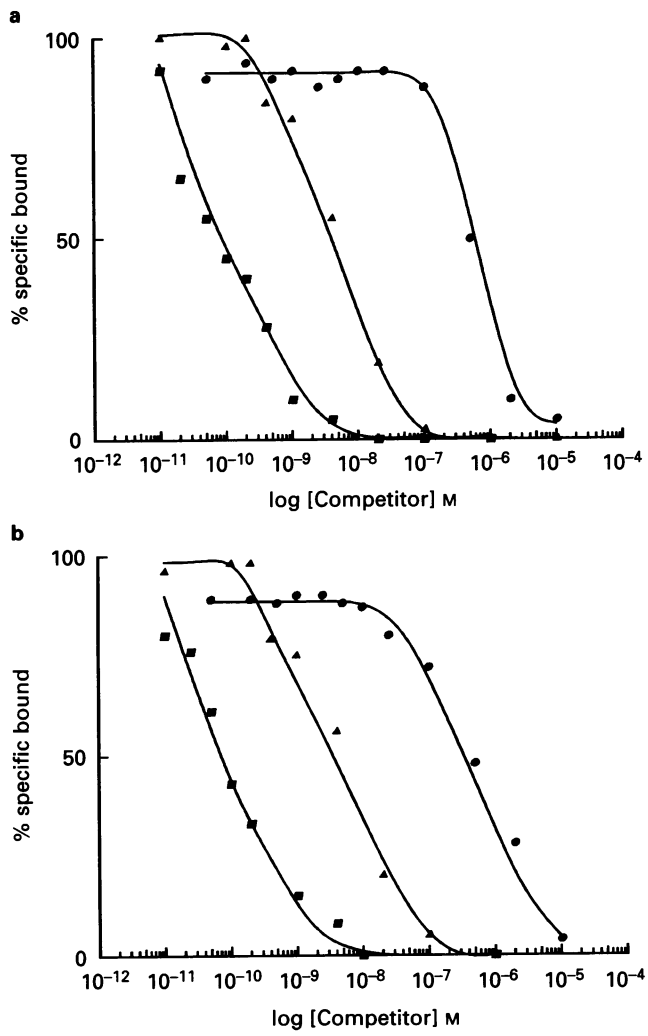

Figure 2 (a) Effect of AT_1 (losartan ■, GR138950C, ▲) and AT_2 (PD123319, ●) specific antagonists on [^{125}I]-[Sar^1, Ile^8]AII binding to lung membranes prepared from a normal rat. (b) Effect of AT_1 (losartan, ■), GR138950C, ▲) and AT_2 (PD123319, ●) specific antagonists on [^{125}I]-[Sar^1,Ile^8]AII binding to lung membranes prepared from a 7 day hypoxic rat.

PD123319 were 9.9×10^{-11} M, 4.2×10^{-9} M and 2.8×10^{-7} M respectively (Figure 2b). However, B_{max} was increased by approximately 25% in 7 day hypoxic rat lung (to 108 ± 12 fmol mg^{-1} protein) when compared to normal controls ($n=4$ rats each group; $P<0.05$), with no significant change in dissociation constant (K_D 0.64 ± 0.15 nM).

Challenge with TxA_2 agonist, U-46619

U-46619 5 μg kg^{-1} produced a transient rise in mean pulmonary arterial pressure in all rats (Table 1). This pressor response was unaffected by saline or the 2 doses of GR138950C studied (0.3 and 3 mg kg^{-1}). The lowest dose of losartan (3 mg kg^{-1}) used had no effect on the rise in pulmonary arterial pressure but, consistent with the observations of Bertolino *et al.* (1994), significant inhibition was observed at the higher dose (30 mg kg^{-1}).

Chronic dosing with AT_1 receptor antagonist

There was a significant increase in haematocrit in all animals exposed to hypoxia for 7 days compared to those allowed to breathe normal air (Table 2); there was no difference in response between the different hypoxic groups.

A 1.8 fold rise in mean pulmonary arterial pressure was recorded in the hypoxic group that received saline compared to that measured in rats allowed to breathe air (Table 2). Chronic infusion of the AT_1 antagonist, GR138950C, attenuated significantly the rise in pulmonary arterial pressure in hypoxic rats. Consistent with effective AT_1 receptor antagonism, the pulmonary pressor response to AII in rats receiving GR138950C was blunted markedly (Figure 3). Both doses of SNP (4.3 and 14.4 mg kg^{-1} day^{-1}) also reduced significantly the rise in mean pulmonary arterial pressure during exposure to hypoxia, the higher dose to a level comparable to that recorded in the AT_1-treated group. There were no significant differences in mean systemic blood pressure between the different treatment groups (Table 2).

Right ventricular hypertrophy was observed in the saline-treated hypoxic animals compared to normal rats (Table 2). This was significantly reduced by the AT_1 receptor antagonist but not by treatment with SNP. AT_1 receptor blockade attenuated markedly hypoxia-induced pulmonary vascular remodelling as assessed by the percentage increase in thick-walled pulmonary vessels (Table 2). Pulmonary vascular remodelling was also reduced by SNP but the effect was quantitatively less marked than with the AT_1 antagonist.

Discussion

This study demonstrates that the AT_1 subtype is the major AII receptor present in normal and 7 day hypoxic rat lungs. Fur-

Table 1 Response to challenge with U-46619: Rise in mean pulmonary arterial pressure (PAP; mmHg) above baseline in response to U-46619 5 $\mu g\,kg^{-1}$ given as a bolus injection before and 5 min after intervention with saline or AT_1 receptor antagonist (Los, losartan; GR, GR138950C)

Baseline PAP	*Rise in PAP after U46619*	*Intervention*	*PAP after intervention*	*Rise in PAP after U46619*
20.5±2.0	9.5±4.9	Saline	23.0±2.0	12±2.8
18.3±0.7	7.7±0.6	Los 3 $mg.kg^{-1}$	17.0±0.6	7±1.7
21.3±2.0	11.3±1.5	Los 30 $mg.kg^{-1}$	17.0±2.9	1.7±0.6*
16.0±1.5	5.7±2.5	GR 0.3 $mg.kg^{-1}$	14.0±2.1	6.0±1.0
20.3±3.8	12.3±3.2	GR 3.0 $mg.kg^{-1}$	21.3±2.3	12.0±0.5

*$P<0.05$ compared to U-46619-induced rise in PAP before intervention. $n=4$ each group.

Table 2 Response to chronic hypoxia: Effect of chronic administration of saline, AT_1 receptor antagonist (GR13 8950C; 1 mg $kg^{-1}\,day^{-1}$) or sodium nitroprusside (SNP; low dose = 4.3 mg $kg^{-1}\,day^{-1}$; high dose = 14.4 mg $kg^{-1}\,day^{-1}$) on response to exposure to hypoxia for 7 days

	Normal air			*Hypoxia*			
	Saline	*GR138950C*	*SNP (high dose)*	*Saline*	*GR138950C*	*SNP (low dose)*	*SNP (high dose)*
Hct (%)	47±1	47±1	46±1	66±1†	65±1†	64±1†	65±1†
PAP (mmHg)	16.0±0.5	16.0±0.5	16.3±0.4	28.3±1.1†	21.3±1.7†*	23.5±0.4†*	22.0±0.4†*
SBP (mmHg)	125±8	127±12	123±6	131±8	118±11	109±7	115±7
RV/LV	0.31±0.01	0.31±0.01	0.32±0.01	0.45±0.01†	0.35±0.01†*	0.39±0.01†	0.41±0.02†
TWPV (%)	4.7±0.2	2.2±0.3	2.0±0.1	20.1±0.9†	9.6±1.4†*[a]	14.4±0.9†*	14.2±0.6†*

Hct, haematocrit; PAP, pulmonary arterial pressure; SBP, systemic blood pressure; RV, right ventricle; LV. left ventricle; TWPV, thick-walled pulmonary vessels. †$P<0.05$ compared to equivalent treatment group allowed normal air; *$P<0.05$ compared to saline-treated hypoxic rats; [a]$P<0.05$ compared to SNP-treated rats. $n=6$ each group.

thermore, there was a small increase in abundance of this receptor during the first week of exposure to hypoxia. This occurred in spite of evidence that circulating AII levels increase during this time (Zakheim *et al.*, 1976), a situation which might be expected to down-regulate AII receptors. The increase in AII binding sites does not appear to be part of a generalized cellular response to hypoxia; previous studies have reported no change in the density of vasointestinal polypeptide binding sites and the selective downregulation of the atrial natriuretic peptide clearance receptor in hypoxic rat lungs (Li *et al.*, 1995; Zhao *et al.*, 1996). No attempt was made to localize AII receptors to particular cell types in the lung. However, the inhibition of the pressor response to AII in the pulmonary circulation by the AT_1 receptor antagonist *in vivo* supports the presence of AT_1 receptors in the contractile elements of the pulmonary vasculature.

Chronic treatment with a selective AT_1 receptor antagonist, starting before exposure to hypoxia, produced a 58% reduction in the rise in pulmonary arterial pressure and a 71% and 68% reduction in right ventricular hypertrophy and thick-walled pulmonary arterioles respectively in the hypoxic rat. Until recently, studies examining the contribution of the renin-angiotensin system to the development of pulmonary hypertension and its consequences in this model have relied on studies of the effects of chronic ACE inhibition (Kentera *et al.*, 1981; McKenzie *et al.*, 1984; Clozel *et al.*, 1991). Captopril has been reported to reduce pulmonary arterial pressure and right ventricular hypertrophy in chronically hypoxic rats (Kentera *et al.*, 1981). Teprotide and cilazopril have been shown to reduce thickening of the media of the pulmonary vessel wall, the latter independent of changes in pulmonary arterial pressure (McKenzie *et al.*, 1984; Clozel *et al.*, 1991). These studies, however, are unable to differentiate the effects of reducing AII levels from increasing bradykinin concentrations.

Recently, Morrell *et al.* (1995b) have addressed this by demonstrating that the beneficial effects of captopril are not reversed by co-treatment with a bradykinin antagonist. In the same study, these authors showed that chronic administration

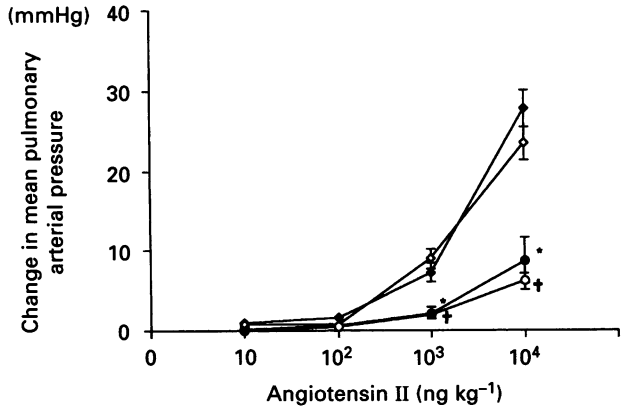

Figure 3 Effect of increasing doses of AII, given by bolus injection, on mean pulmonary arterial pressure in saline-treated normal (◇) and 7 day hypoxic (◆) rats and in AT_1 antagonist-treated (GR138950C; 1 mg $kg^{-1}\,day^{-1}$) normal (○) and 7 day hypoxic (●) rats. †$P<0.05$ compared to saline-treated normal controls. *$P<0.05$ compared to saline-treated hypoxic controls. $n=6$ each group.

of losartan, an AT_1 antagonist, reduced hypoxia-induced pulmonary hypertension and the attendant structural remodelling (Morrell *et al.*, 1995b). The interpretation of their data and our own is dependent on the specificity of action of losartan and GR138950C. Both compounds exhibit high affinity for AT_1 receptors. However, Bertolino *et al.* (1994) have shown that losartan also inhibits TxA_2-mediated pressor responses in the pulmonary circulation, albeit at doses somewhat higher than those required to inhibit AII responses in this vascular bed. Nonetheless this property may contribute to the beneficial effects of high doses of losartan in the hypoxic rat. We chose a dose of GR138950C that did not influence the TxA_2-mediated pressor response. Moreover GR138950C ex-

hibits little or no affinity for a range of other receptors including adenosine, histamine, muscarinic receptors or adrenoceptors (Hilditch *et al.*, 1995). Our data together with the ligand binding studies suggest a prominent role for AII in the early development of hypoxia-induced pulmonary hypertension and remodelling mediated through the AT_1 receptor,

Paradoxically, elevation of AII concentrations to 'pharmacological' levels by infusion of the peptide prevented the development of pulmonary hypertension in rats exposed to hypoxic conditions for 7 days (Rabinovitch *et al.*, 1988). This effect was blocked by indomethacin and was attributed to the release of vasodilator prostaglandins. It is significant that the levels of AII produced during these infusions exceeded by ~10 fold levels of the peptide measured in hypoxic conditions. This appears to be another example of the principle that the response to specific pharmacological inhibition of a factor is more informative about its contribution to pathophysiology than the effect of pharmacologically enhancing its action.

The effects of AT_1 receptor antagonism were compared with those of SNP. This agent acts as a nitric oxide donor and relaxes vascular smooth muscle through stimulation of soluble guanylyl cyclase. Although there may be concerns about tolerance and toxicity, chronic administration produced a dose-related reduction in pulmonary arterial pressure at 7 days in the hypoxic rat. Significantly, pulmonary arterial pressure in the hypoxic group treated with the highest dose of SNP was very similar to that in the hypoxic GR138950C-treated group. However, this dose of SNP had no significant effect on right ventricular hypertrophy in hypoxic rats and the reduction in pulmonary vascular remodelling was significantly less than that observed with the AT_1 antagonist. No data are available on the time-course of blood pressure reduction by the two treatments. It is possible that the AT_1 antagonist achieved a greater reduction in blood pressure than SNP in the first few days of treatment but a more plausable explanation is that the AT_1 antagonist had an inhibitory effect on cardiac hypertrophy and vascular remodelling over and above that mediated via pressure reduction. AII exhibits trophic effects on a number of cell types in culture, including cardiac myocytes, vascular smooth muscle cells and fibroblasts (Sadoshima & Izumo, 1993; Villarreal *et al.*, 1993). In the rat these actions are mediated via the AT_1 receptor subtype. Pretreatment with an AT_1 antagonist has been shown to reduce cardiac hypertrophy in the aorta-banded rat independent of an effect on afterload (Everett *et al.*, 1994). Thus direct inhibition of the trophic actions of AII may contribute to the protective effect of GR138950C on right ventricular hypertrophy and vascular remodelling in the hypoxic rat.

It is clear that factors in addition to AII influence the cardiovascular response to hypoxia. Recent studies have shown that treatment with nitric oxide or an endothelin ET_A antagonist also reduces pulmonary hypertension and remodelling in the chronically hypoxic rat (Kouyoumdijan *et al.*, 1994; Bonvallet *et al.*, 1994). There is evidence from studies using other tissues that these factors do not operate in isolation. For example, the hypertrophic response to AII in cardiac myocytes in culture can be inhibited by anti-sense oligonucleotides directed against preproendothelin-1 (Ito *et al.*, 1993) suggesting that endothelin-1 may mediate this effect of AII in these cells. Interactions such as this in the pulmonary vasculature remain to be demonstrated but would explain the attenuation of the development of pulmonary hypertension with apparently different interventions.

The authors are grateful to Glaxo-Wellcome for supplying GR138950C, Merck, Sharpe and Dohme for losartan and Parke Davis for PD123319. Dr David M. Smith provided valuable assistance with the binding studies. The study was funded by a project grant from the British Heart Foundation (PG93/050).

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(Received May 7, 1996
Revised July 25, 1996
Accepted August 27, 1996)