Angiotensin II Receptor Blockade and Effects on Pulmonary Hemodynamics and Hypoxic Pulmonary Vasoconstriction in Humans*

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Study objective: We examined the hypothesis that angiotensin II (ANG II) is a modulator of pulmonary vascular tone by examining the effects of ANG II blockade on pulmonary hemodynamics during normoxemia and hypoxemia in normal volunteers with an activated renin angiotensin system (RAS).

Participants and interventions: Eight normal volunteers, pretreated with furosemide, were studied on two separate occasions and received either an infusion of saralasin, 5 μg/kg/min, or placebo. After 20 min, they were rendered hypoxic, by breathing N2O2 mixture for 20 min to achieve arterial oxygen saturation (SaO2) of 85 to 90% adjusted for a further 20 min to achieve SaO2 of 75 to 80%. Doppler echocardiography was used to measure mean pulmonary artery pressure (MPAP), cardiac output, and hence total pulmonary vascular resistance (TPR).

Results: Saralasin compared with placebo resulted in a significant (p<0.05) reduction in MPAP during normoxemia, 6.70±1.0 vs 11.7±1.3 mm Hg; at SaO2 of 85 to 90%, 14.7±1.4 vs 20.5±1.0 mm Hg; and at SaO2 of 75 to 80%, 18.1±1.9 vs 27.8±1.9 mm Hg, respectively. Likewise saralasin compared with placebo resulted in a significant reduction in TPR during normoxemia, 104±14 vs 180±20 dyne·s·cm⁻²; at SaO2 of 85 to 90%, 222±24 vs 295±21 dyne·s·cm⁻²; and at SaO2 of 75 to 80%, 238±21 vs 362±11 dyne·s·cm⁻², respectively. The ΔMPAP response to hypoxemia was likewise significantly (p<0.01) attenuated by saralasin infusion compared with placebo: mean difference 5.0 mm Hg, 95% confidence interval (CI) 1.9 to 8.08, and there was a trend toward attenuation of the ΔTPR response to hypoxemia (0.05<p<0.10): mean difference 47 dyne·s·cm⁻², 95% CI, -10 to 105.

Conclusion: In addition to causing pulmonary vasodilatation in the presence of an activated RAS, our results suggest that ANG II receptor blockade attenuates acute hypoxic pulmonary vascular constriction and that ANG II may play a role in modulating this response in normal man.

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Key words: angiotensin II blockade; hypoxemia; pulmonary circulation

Abbreviations: ACE=angiotensin-converting enzyme; ANG II=angiotensin II; CI=confidence interval; CO=cardiac output; CSA=cross-sectional area; HPV=hypoxic pulmonary vasoconstriction; HR=heart rate; MAP=mean arterial BP; MPAP=mean pulmonary artery pressure; PAT=pulmonary acceleration time; PCWP=pulmonary capillary wedge pressure; PRA=plasma renin activity; RAS=renin angiotensin system; RIA=radioimmunoassay; SaO2=arterial oxygen saturation; SV=stroke volume; SVI=aortic systolic velocity integral; SVR=systemic vascular resistance; TPR=total pulmonary vascular resistance

In man, hypoxemia usually arises as a result of pulmonary disease or adverse environmental conditions such as altitude. The effects of hypoxemia on the pulmonary vasculature have been studied extensively since Von Euler and Liljestrand¹ first described the phenomenon of hypoxic pulmonary vasoconstriction (HPV). Although HPV has beneficial effects, the stimulus of chronic hypoxia results in an elevation of pulmonary artery pressure leading to the development of cor pulmonale.²³

These patients have activation of the renin angiotensin system (RAS),⁴⁶ with elevated levels of angiotensin II (ANG II). ANG II and hypoxia have both been shown to be potent pulmonary vasoconstrictors in man.⁷ In vitro studies have shown that ANG II facilitates HPV in the rat and potentiates this response in dogs.⁸⁹ Furthermore, the use of angiotensin-converting enzyme (ACE)-inhibitors in chronically hypoxic rats has been shown to attenuate the development of pulmonary hypertension.¹⁰
Studies in humans are rare, although recent data have shown that pretreatment with the long-acting ACE inhibitor lisinopril blunts acute HPV in normal volunteers compared to placebo. The effect of ACE inhibition could in theory be due to lowering levels of ANG II (a vasoconstrictor) or augmenting levels of bradykinin (a vasodilator) by suppressing the activity of kininase II, which normally contributes to the degradation of kinins.

The purpose of this study was to determine the role of ANG II in the pulmonary circulation during both normoxemia and hypoxemia by competitive inhibition of ANG II with its analogue, saralasin (1-sar-5-val-8-al-ANG II), which has been shown to be a competitive antagonist of ANG II in the presence of an activated RAS, but has no effect on bradykinin degradation.

**Materials and Methods**

**Subjects**

Eight healthy male volunteers, age (mean±SEM) 29±3 years, were studied on 2 separate occasions. There was no abnormality present on clinical history, examination, 12-lead ECG, echocardiography, biochemical screening, or hematologic screening. No medications were permitted during and for 1 month prior to the study. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

**Study Protocol**

Subjects attended the laboratory at the same time of the day on 2 separate occasions, at least 1 week apart. Subjects were pretreated with 4 daily doses of furosemide, 40 mg, to activate the RAS such that saralasin would function as a pure antagonist of ANG II devoid of partial agonist activity. An indwelling IV cannula was inserted into each antecubital fossa, one for infusion of saralasin or placebo and the other for blood sampling. Subjects then rested in a supine position for 30 min to obtain stable resting hemodynamics (T0). They then received either an infusion of 5% dextrose or saralasin 5, pg/kg/min (Sigma Chemical Company; St. Louis). They were restudied after 20 minutes (T1), and then rendered hypoxic by breathing a variable mixture of oxygen and nitrogen sufficient to render arterial oxygen saturation (SaO2) between 85 and 90% (T2) for 20 min adjusted for a further 20 min to achieve an SaO2 of 75 to 80% (T3). The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves. Gases were mixed in a 25-L Douglas bag, from which the subjects breathed through a mouthpiece connected by a series of one-way valves, while wearing an occlusive nose clip. Measurements of pulmonary and systemic hemodynamic variables and venous blood samples for plasma renin activity (PRA) were taken at T0, T1, T2, and T3. Serum electrolytes were measured at T0.

**Measurements**

**Oxygenation:** Arterial blood oxygen saturation was continuously monitored by transcutaneous oximetry (CSI 503; Criticare Systems Inc; Waukesha, Wls).

**Hemodynamics:** Heart rate (HR) and mean arterial BP (MAP) was measured by semiautomatic sphygmomanometer as the mean of three consecutive readings (Vital Signs Monitor; Critikon; Tampa, Fla). Pulmonary acceleration time (PAT) in milliseconds was measured as previously described from pulmonary arterial flow by pulsed-wave Doppler echocardiography (Vingmed SD50; Vingmed Sound; Horten, Norway) from the left third/fourth intercostal space. The mean of three consistent waveforms at each time point was used for the purpose of analysis. Mean pulmonary artery pressure (MPAP) (mm Hg) was calculated as MAP=73–(0.42×PAT). Aortic cross-sectional area (CSA) was measured by M-mode echocardiography (Vingmed SD50). The aortic systolic velocity integral (SVI) was measured by on-line computer-assisted determination using pulsed-wave Doppler echocardiography of ascending aortic blood flow from the suprasternal notch. On-line calculations of stroke volume (SV=SVI×CSA) and cardiac output (CO) as the product of SV and HR were also made. Total pulmonary vascular resistance (TPR) was calculated as follows: TPR=MPAP/CO×80 dyne-s-cm⁻². We have shown previously the short-term coefficients of variability for measurement of PAT and SVI in our hands to be 1.7% and 1.2%, respectively.

**Electrolytes:** Samples for serum electrolytes were collected in chilled lithium-heparin tubes and were centrifuged at 4°C immediately and separated plasma was stored at -20°C until measured in one batch at the end of the study using an internal caesium
standard flame photometer (Instrumentation Laboratory; Milan, Italy).

RAS Activity: Samples for measurement of plasma renin activity (PRA) were collected into chilled EDTA tubes. They were centrifuged at 4°C immediately and separated plasma was stored at -20°C until assayed in one batch at the end of the study. PRA was assayed using commercially available radioimmunoassay (RIA) kits (Sorin Biomedica; Saluggia, Italy) that assayed PRA by measurement of amount of angiotensin I generated per hour.

Data Analysis

Comparison of values between study days or between serial time points on the same study day was made by multifactorial analysis of variance. A probability value of $p<0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables, and figures as means and SEM, and where a difference between means is quoted, the 95% confidence interval (CI) for this difference is given.

RESULTS

Pulmonary Hemodynamics

There was no significant difference in PAT, MPAP, or TPR at baseline ($T_0$) between study days. Infusion of saralasin compared to the placebo resulted in a significant ($p<0.05$) reduction in MPAP during normoxemia ($T_1$), mean difference 4.6 mm Hg (95% CI, 1.25 to 8.0); at an SaO$_2$ of 85 to 90% ($T_2$), mean difference 6.1 mm Hg (95% CI, 1.4 to 10.8); and a significant ($p<0.0005$) difference at an SaO$_2$ of 75 to 80% ($T_3$), mean difference 9.6 mm Hg (95% CI, 6.0 to 13.2), respectively (Fig 1, top). Likewise saralasin infusion compared to placebo resulted in a significant ($p<0.05$) reduction in TPR at $T_1$, mean difference 76 dyne-s-cm$^{-5}$ (95% CI, 29 to 123); at $T_2$, mean difference 72 dyne-s-cm$^{-5}$ (95% CI, 8 to 136); and a significant ($p<0.0005$) reduction at $T_3$, mean difference 123 dyne-s-cm$^{-5}$ (95% CI, 82 to 165), respectively (Fig 1, bottom). Hypoxemia caused a significant ($p<0.05$) increase in MPAP and TPR at $T_2$ and $T_3$ compared to baseline on both study days. Saralasin compared to placebo significantly reduced PAT during normoxemia and moderate and severe hypoxemia (Table 1). In terms of change in MPAP ($\Delta$MPAP) from baseline ($T_0$) to severe hypoxia ($T_3$), the $\Delta$MPAP response was significantly ($p<0.005$) attenuated by saralasin infusion compared to placebo: mean difference 7.3 mm Hg; 95% CI, 3.9 to 10.7. Likewise the $\Delta$TPR response from $T_0$ to $T_3$ was significantly ($p<0.05$) attenuated by saralasin infusion compared with placebo: mean difference 89 dyne-s-cm$^{-5}$; 95% CI, 26 to 152. Because there was a significant fall in MPAP and TPR at $T_1$ compared with baseline ($T_0$) after saralasin infusion compared to placebo, we also assessed the $\Delta$MPAP and $\Delta$TPR response from $T_1$ to $T_3$. The $\Delta$MPAP response was likewise significantly ($p<0.01$) attenuated by saralasin infusion compared with placebo: mean difference 5.0 mm Hg; 95% CI, 1.9 to 8.0; and there was a trend toward attenuation of the $\Delta$TPR response from $T_1$ to $T_3$ (0.05<p<0.10): mean difference 47 dyne-s-cm$^{-5}$; 95% CI, −10 to 105.

Systemic Hemodynamics

Although, saralasin infusion compared to placebo did not significantly alter systemic hemodynamic parameters either at baseline or during hypoxia, saralasin infusion had significant effects on systemic hemodynamics compared with baseline measurements (Table 1). Compared to baseline ($T_0$), a significant ($p<0.05$) increase in CO in response to hypoxemia ($T_3$) was noted on both study days. A fall in systemic vascular resistance (SVR) in response to hypoxemia ($T_3$) was

| Table 1—Effects of ANG II Receptor Blockade on Systemic Hemodynamics and PAT* |
|------------------|------------------|------------------|------------------|
|                  | Preinfusion       | $>95\%$ ($T_0$)  | $85-90\%$ ($T_2$) | $75-80\%$ ($T_3$) |
| HR, beats/min    |                  |                  |                  |                  |
| P                | 71.0 ± 4.8       | 68.6 ± 4.2       | 73.1 ± 4.3       | 73.8 ± 5.6       |
| S                | 67.2 ± 3.8       | 64.1 ± 3.5       | 68.5 ± 4.5       | 71.8 ± 5.1       |
| MAP, mm Hg       |                  |                  |                  |                  |
| P                | 84.8 ± 4.5       | 81.9 ± 3.5       | 82.4 ± 3.8       | 86.5 ± 3.1       |
| S                | 83.4 ± 3.0       | 76.9 ± 1.9$^i$  | 79.4 ± 3.3       | 77.4 ± 4.2$^i$  |
| CO, L/min        |                  |                  |                  |                  |
| P                | 5.45 ± 0.48      | 5.20 ± 0.25      | 5.69 ± 0.38      | 6.18 ± 0.24$^i$ |
| S                | 5.36 ± 0.34      | 5.06 ± 0.17      | 5.42 ± 0.36      | 6.10 ± 0.39$^i$ |
| SVR, dyne-s-cm$^{-5}$ |                |                  |                  |                  |
| P                | 1,306 ± 132      | 1,262 ± 72       | 1,192 ± 48       | 1,128 ± 48       |
| S                | 1,332 ± 50       | 1,251 ± 54       | 1,260 ± 69       | 1,082 ± 73$^i$  |
| PAT, ms          |                  |                  |                  |                  |
| P                | 146 ± 2          | 146 ± 3          | 125 ± 2$^i$      | 106 ± 2$^i$      |
| S                | 152 ± 2          | 158 ± 2$^i$      | 139 ± 3$^i$      | 130 ± 5$^i$      |

*Absolute values (mean±SEM) of HR, MAP, CO, SVR, and PAT at each level of oxygen after pretreatment with placebo (P) or saralasin (S).

$^i$Significantly ($p<0.05$) different from baseline ($T_0$).

$^i$Significantly different between treatment with saralasin and placebo at that time point.
also noted on both study days, although this reached statistical significance (p<0.05) only during saralasin infusion. HR and MAP were unaffected by hypoxemia on the placebo day, although saralasin infusion resulted in a significant (p<0.05) decrease in MAP at T3 and T4 compared to baseline (T0).

**Electrolytes**

There was no significant difference in serum sodium or potassium levels at baseline (T0), between the study days when patients received placebo or saralasin. Serum sodium level was 137.4±0.8 (placebo) vs 138.9±0.8 (saralasin) mmol/L, and serum potassium level was 3.97±0.10 (placebo) vs 3.89±0.08 (saralasin) mmol/L.

**RAS Activity**

Saralasin infusion resulted in a significant (p<0.05) increase in PRA at T2 and T3 compared to baseline (T0): 5.25±1.36 and 5.60±1.31 vs 3.99±0.55 ng/mL/h, respectively. Hypoxia alone was not associated with a significant change in PRA at T2 and T3 compared to baseline (T0): 3.48±0.65 and 3.29±0.58 vs 3.84±0.77 ng/mL/h, respectively. There was no significant difference in PRA at any of the time points between the 2 study days.

**Discussion**

Our results demonstrate that the ANG II antagonist saralasin causes pulmonary vasodilatation in the presence of an activated RAS. In this respect, we have shown that absolute MPAP and TPR were significantly lower during hypoxemia after saralasin compared to placebo and similarly that the ∆MPAP and ∆TPR responses from baseline to each level of hypoxemia were also significantly attenuated by saralasin. There is also evidence to suggest that ANG II blockade may attenuate acute hypoxic pulmonary vasoconstriction reflected by a significant reduction in the ∆MPAP and a trend toward a reduction of the ∆TPR response from T1 to severe hypoxemia (T3).

Saralasin is a highly soluble and stable ANG II analogue that was developed as an ANG II antagonist. However, saralasin also possesses partial agonist-type activity. It has been demonstrated that saralasin functions as an agonist in the presence of low renin states, whereas elevated levels of PRA and ANG II are associated with antagonism. Consequently, we pretreated our study patients with diuretics to reduce total body sodium concentration and extracellular volume to achieve elevated PRA. We found PRA to be the same after furosemide pretreatment on both study days suggesting comparable degrees of RAS activation. 

PRA was elevated at baseline in all patients on both days, compared with our own normal reference range (0.2 to 2.8 ng/mL/h), allowing saralasin to function as an ANG II antagonist rather than agonist. This antagonistic activity is supported by the findings of a significant reduction in MAP at T1 and T3 compared to baseline (T0) when patients received saralasin. We also observed a significant increase in PRA at T2 and T3 compared to baseline (T0), supportive of the antagonistic activity of saralasin in which blockade of ANG II receptors on renin secreting cells would be expected to result in an increase in renin secretion due to inhibition of ANG II mediated negative feedback, occurring at the level of the juxtaglomerular apparatus.

Our results agree with animal studies suggesting a possible role for ANG II in modulating HPV. Berkov suggested that ANG II was required to facilitate HPV in the saline solution perfused rat lung, and studies in dogs suggest that ANG II infusion augments HPV. Recently studies in man have shown that ANG II infusion augments HPV although not in a synergistic manner. The finding that lisinopril pretreatment significantly attenuated acute HPV in normal volunteers suggested that ANG II plays an important role in modulating HPV in normal man. Although theoretically this could have been due to increased levels of bradykinin, this agent has not been shown to produce pulmonary vasodilatation in normal humans. Saralasin and lisinopril alleviate HPV to similar extents, providing further evidence for a role of ANG II in modulating HPV. Although ANG II undoubtedly has important pressor effects in the pulmonary circulation, studies have suggested that it is not the sole mediator of HPV. McMurtie has shown that ANG II is not required for HPV in vitro, and studies in man have shown that acute hypoxemia does not increase ANG II levels, although this may not necessarily reflect tissue ANG II activity in the lung. In this study, acute hypoxemia had no effect on PRA and ANG II blockade with saralasin only attenuated and did not abolish HPV. This supports the theory that ANG II has a role to play in modulating HPV rather than being the sole mediator of HPV.

How ANG II and hypoxia interact remains unclear, although much interest has surrounded in vitro studies showing that pulmonary but not mesenterial arterial myocytes close potassium channels in response to hypoxia. This results in membrane depolarization and inward calcium flux through voltage-dependent calcium channels. ANG II is known to increase intracellular calcium through the inositol trisphosphate pathway and it has recently been suggested that it may act directly on calcium channels through its receptor. It may be that one subtype of potassium channel acts as a hypoxia sensor in the pulmonary vasculature and ANG II may modulate acute HPV via its effects on calcium flux or on this potassium channel via mem-

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brane voltage changes, such that in the presence of ANG II blockade, the hypoxic signal, with respect to pulmonary vasoconstriction, is reduced.

We have used Doppler echocardiography to measure hemodynamic changes in the pulmonary circulation. These noninvasive techniques have been shown to be reproducible and a good correlation between Doppler PAT and MPAP as measured by right heart catheter is well established. We looked at two measures of pulmonary vasoconstriction: changes in MPAP and TPR. A possible limitation of this method is that the use of TPR does not account for any changes in the postcapillary vascular bed, as assessed by pulmonary capillary wedge pressure (PCWP). In this respect, we believe it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes. However, we believe this extra information is not essential. It is known from previous work that hypoxemia has no significant effects on PCWP, suggesting that changes in TPR are reflective of changes in true pulmonary vascular resistance in precapillary arterioles during hypoxia. To our knowledge, there is no information available regarding long-term dosing with diuretics in normal volunteers; the only study available looked at the acute hemodynamic consequences of IV ethacrynic acid, which showed a reduction in PCWP. However, it would be difficult to extrapolate these findings to long-term dosing, and although long-term diuretic therapy may affect PCWP in normal volunteers, it is important to note that patients were exposed to both hypoxia and pretreatment with furosemide on both study days, suggesting that these stimuli are unlikely to be responsible for the changes observed between study days. With respect to saralasin, its infusion has not been shown to affect PCWP. We believe, therefore, that the observed changes in TPR are a true reflection of changes in pulmonary vascular tone.

We have shown for the first time (to our knowledge) in man that ANG II blockade with a specific competitive ANG II antagonist causes pulmonary vasodilatation and alleviates acute HPV in patients with an activated RAS. This suggests the possibility that ANG II is a modulator of acute HPV in normal man. Although one must be careful when extrapolating results from normal volunteer studies, the ability to cause pulmonary vasodilatation and attenuate acute HPV, and therefore the stimulus for pulmonary hypertension in cor pulmonale, suggests that ANG II antagonists may have a role to play in chronic hypoxic lung disease either to prevent or treat the cardiopulmonary consequences of chronic hypoxemia. The availability of orally active ANG II antagonists such as losartan may therefore provide a novel therapeutic avenue for this patient group.

REFERENCES
7 Cargill RJ, Lipworth BJ. Acute effects of hypoxia and angiotensin II in the human pulmonary vascular bed. Pulm Pharmacol 1994; 7:305-10