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The Inhibition of Angiotensin Converting Enzyme Attenuates the Effects of Chronic Hypoxia on Pulmonary Blood Vessels in the Rat

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Summary

The effect of chronic administration of angiotensin converting enzyme inhibitor on the development of hypoxic pulmonary hypertension was studied in rats. Male Wistar rats were exposed for 3 weeks to isobaric hypoxia (10 % O_2) and treated with 10 mg/kg b.w. of Ramipril daily. The haemodynamic properties of the pulmonary vasculature were then measured in isolated blood-perfused lung preparation. Ramipril administration during the sojourn in hypoxia resulted in lower baseline perfusion pressure and lower slope of perfusion pressure-flow relationship compared to non-treated hypoxic rats. Partitioning of the distribution of pulmonary vascular resistance across the vascular bed by the occlusion technique showed that it was mainly due to a decrease of arterial and venous vascular resistances to blood flow. It is suggested that Ramipril attenuates the process of morphological reconstruction of pulmonary vasculature by chronic hypoxia rather than the level of vascular smooth muscle tone.

Key words

Pulmonary hypertension – Collagen – Chronic hypoxia – Angiotensin II – Angiotensin converting enzyme

Introduction

Exposure to chronic hypoxia results in hypoxic pulmonary hypertension mainly due to structural reconstruction of the pulmonary vascular bed (Reid 1986). Growth of vascular smooth muscles in peripheral vessels and proliferation of collagen and elastin fibres in the matrix of their walls is the main cause of increased pulmonary vascular resistance to lung blood flow.

The effect of angiotensin II on acute hypoxic pulmonary vasoconstriction has been studied extensively (Voelkel *et al.* 1980, Kiely *et al.* 1995). Less is known, however, about its effects on chronic hypoxia-induced remodelling of pulmonary blood vessels. It was discussed recently that the proliferative ability of vascular mesenchymal cells is related to the trophic action of angiotensin II (Peacock and Matthews 1992). Angiotensin II may act as a modulator of cellular growth and thus may play a role in the development and maintenance of myocardial and vascular hypertrophy (Abraham *et al.* 1995). Angiotensin II causes vascular smooth muscle hypertrophy (Geisterfer *et al.* 1988, Owens 1989) and seems to be closely related to cell growth regulation (Bobik *et al.* 1990, Baker and Aceto 1990). Inhibition of cellular proliferation may be the long-term mechanism of action of angiotensin converting enzyme (ACE) inhibitors. Chronic treatment with ACE inhibitors reduced the pulmonary vascular changes in monocrotaline and hypoxic pulmonary hypertensions (Kentera *et al.* 1981, Molteni *et al.* 1986, Zakheim *et al.* 1975).

In the present study, we have assessed the effect of chronic administration of ACE inhibitor Ramipril on the haemodynamic properties of

pulmonary vascular bed during the development of hypoxic pulmonary hypertension in rats exposed for 3 weeks to hypoxic environment.

Methods

Twenty-four male Wistar rats were used. They were divided randomly into four groups. Two groups were exposed to chronic hypoxia in the isobaric hypoxic chamber (10 % O_2) (Hampl and Herget 1991). The remaining two groups of rats lived in atmospheric air. One group of rats exposed to hypoxia and one group kept in air were treated throughout the experiment by daily gastric instillation of 10 mg/kg of Ramipril (Hoechst A.G.) under light ether anaesthesia. Corresponding control groups were given the same volume of distilled water. The hypoxic chamber was opened for 15–20 min each day for the Ramipril applications, food and water supply and cleaning of cages.

Isolated lung preparation

The haemodynamic properties of the pulmonary vasculature were measured on a preparation of isolated perfused rat lungs (Herget and McMurtry 1987). Rats were anaesthetized by thiopental (100 mg/kg b.w, i.p.) and ventilated *via* a tracheal cannula with air containing 5 % CO_2 at 65 breaths per min. The pulmonary artery and the left ventricle were cannulated after sternotomy and heparinization. The lungs and heart were removed from the chest en block and suspended in a humid and thermostated chamber (38 °C). They were perfused in a recirculating manner with blood at a rate of 0.06 ml/min/g b.w. Blood was obtained from a separate group of rats under ether anaesthesia by cardiac puncture. After 20 min of stabilization the lungs were challenged with 10 min of ventilatory hypoxia (3 % O_2 + 5 % CO_2). The perfusion pump was then stopped for about 30 s after which the perfusion flow was increased in 4–5 steps (30–90 s each) up to 32–37 ml/min. After the perfusion pressure-flow (P/Q) relationship measurement had been completed, the baseline perfusion flow was adjusted again for 5 min. Then the distribution of pulmonary vascular resistance was partitioned by rapid occlusion of the inflow and outflow cannulas of the preparation (Hakim *et al.* 1982, Herget and Kuklík 1995). Ventilation was arrested at the end of expiration (alveolar pressure 2.5 cm H_2O) and the inflow cannula was rapidly occluded with an electromagnetic device. After 3 min of stabilization, a similar occlusion was applied to the outflow cannula and then arterial and venous cannulas were occluded simultaneously. The pressure drops across the arterial segment, the venous segment and the middle vascular segment were calculated from the pressures obtained after the particular occlusions (Hakim *et al.* 1982, Herget and Hampl 1995).

Statistics

Differences in body weight and baseline perfusion pressure in isolated lungs between the groups were analyzed by one-way ANOVA and the Scheffé test (Steel and Torrie 1960). Perfusion pressure-flow data were analyzed by linear regression. The relation was always found to be linear ($r = 0.942$). Therefore, the relationship was described by its slope and extrapolated intercept with the pressure axis. The values of $P<0.05$ were considered significant. The results are presented as means ± S.E.M.

Table 1
Characteristics of the pressure-flow relationships in isolated perfused lungs

GROUP	P_{ap} (mm Hg)	INTERCEPT (mm Hg)	SLOPE (mm Hg/ml)
HR	17±1	8.76±0.81	0.287±0.011
H	22±3*	8.85±0.82	0.641±0.053*
NR	16±1	8.12±1.00	0.267±0.039
N	16±1	5.66±0.89	0.304±0.026

*Significant differences ($P<0.05$): * vs all other groups, HR = Chronic hypoxia + Ramipril, H = Chronic hypoxia, NR = Normoxia + Ramipril, N = Normoxia, P_{ap} = Baseline perfusion pressure, INTERCEPT = extrapolated intercept of pressure-flow plot with the perfusion pressure axis, SLOPE = slope of the pressure-flow plot.*

Results

There was no mortality in either group of animals. The body weight in all groups did not differ at the beginning of the experiment. At the end of experiment the body weight of rats exposed to hypoxia (Ramipril – 329±17 g, saline – 316±10 g) was lower ($p<0.05$) than in the corresponding controls (359±11 g and 378±8 g). The body weights of normoxic and hypoxic rats were not affected by Ramipril treatment.

Perfusion flow-perfusion pressure relationship

The baseline perfusion pressure in the non-treated rats exposed to chronic hypoxia was significantly higher ($p<0.05$) than that in all other groups (Table 1). The perfusion flow-perfusion pressure plots in the groups of rats studied are given in Fig. 1, the results of the linear regression analysis are presented in Table 1. The slope of the relation was significantly higher ($p<0.05$) in rats exposed to hypoxia and not treated by Ramipril than that in all other groups where it did not differ significantly. The intercepts with the perfusion pressure axis did not differ significantly between the groups.

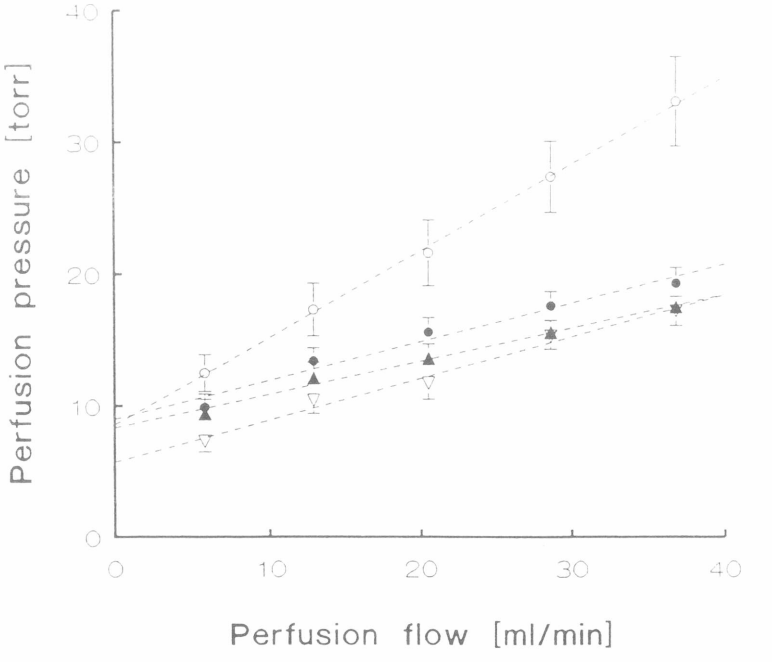

Fig. 1
Perfusion pressure-flow plots in control and hypoxic rats treated with Ramipril. For the group denominations see Table 1.

Table 2
Pressure drops across the pulmonary vascular bed measured by occlusion technique in isolated perfused lungs

GROUP	P_t (mm Hg)	P_a (mm Hg)	P_v (mm Hg)	P_m (mm Hg)
HR	19.9±1.3	7.4±0.9	8.4±8.9	4.6±1.3
H	25.8±2.5$^{\times}$	9.1±1.1^{+}	11.3±1.2*	6.6±1.7
NR	17.4±1.9	5.4±0.8	7.1±0.7	6.9±0.7
N	17.8±0.6	6.6±0.5	7.4±0.6	4.2±0.8

*Significant differences ($P<0.05$): * vs all other groups, # vs groups kept in normoxia (NR and N), $^{+}$ vs NR. The group denominations are the same as in Table 1.*

Occlusion measurements

Occlusion experiments resulted in significantly larger perfusion drops across the pulmonary vascular bed (P_t) in non-treated rats exposed to hypoxia than in both normoxic groups (Table 2). The value in the hypoxic group treated with Ramipril did not differ significantly from the other groups studied. The

pressure drops across the arterial segment (P_a) significantly differed between the non-treated hypoxic group and Ramipril-treated normoxic group only, P_a values being larger in the hypoxic animals. Marked differences were found in the drops across the venous part of pulmonary vasculature (P_v), where the pressure drops in the non-treated hypoxic rats were significantly greater than those in all other groups. There were no significant differences in the pressure drops across the middle portion of pulmonary vasculature (P_m) between the groups. The estimated "pulmonary capillary pressure" measured by the simultaneous occlusion of inflow and outflow cannulas was significantly higher in the non-treated hypoxic group ($P_c = 11.2 \pm 1.7$ mm Hg) than that in the other groups (hypoxia + Ramipril 8.1 ± 0.9, normoxia 6.7 ± 0.5 and normoxia + Ramipril 7.4 ± 1.2 mm Hg).

Discussion

The baseline perfusion pressure in isolated lungs from the group of rats exposed to hypoxia and treated with Ramipril was significantly lower than that in non-treated hypoxic rats and did not differ from that in the controls. According to our previous experience, the baseline perfusion pressure in isolated perfused lungs correlates well with the pulmonary artery blood pressure *in vivo* (Herget and Hampl 1995). We therefore assume that Ramipril treatment during the sojourn of experimental rats in a hypoxic environment inhibited the development of hypoxic pulmonary hypertension. However, for the technical reasons it was not possible to perform additional measurements in the present study which would be necessary for proving the presence of pulmonary hypertension.

The measurement of perfusion pressures over a reasonably wide range of perfusion flows (P/Q relationship) in isolated lungs provides sufficient information about the changes of haemodynamic properties of the pulmonary vascular bed (Barer 1976). As it is linear, it can be described by an extrapolated intercept with the pressure axis at zero flow and the slope of the P/Q line. The pressure axis intercept represents the mean critical closing pressure of pulmonary vasculature, the slope is related to the upstream incremental vascular resistance. In our current experiment, we found that the intercept with the pressure axis did not differ between the groups. This is not in agreement with our previous experiments (Herget and Kuklík 1995, Herget and Hampl 1995), and also with the results of others (Emery *et al.* 1981), where exposure of experimental rats to similar chronic hypoxia resulted in a significant rise of the pressure axis intercept. We do not have an explanation for this unexpected observation apart from the effect of biological variation in a small group of animals. The only difference between our previous and present experiments was the fact that the hypoxic chamber was opened more often in the present study. We cannot, however, offer any explanation why this intermittent hypoxic exposure may affect the closing pressure of pulmonary vasculature. Similarly as in our previous studies (Herget and Hampl 1995, Herget and Kuklík 1995), the slope of the P/Q line in rats exposed to chronic hypoxia was significantly steeper. We interpret this finding as a result of the increase in haemodynamic resistance and/or decrease of compliance of pulmonary vessels upstream from the collapsible vascular portion (middle vascular portion). The haemodynamic resistance in hypoxic pulmonary hypertension increases due to encroachment of hypertrophied smooth muscles into the vascular lumen (Finlay *et al.* 1986). The vascular compliance decreases (Emery *et al.* 1981), mainly due to a deposition of collagen and hypertrophy of smooth muscle cells in the walls of peripheral pulmonary vessels (Hislop and Reid 1976). The slope of the P/Q line in Ramipril-treated hypoxic rats was not increased. Inhibition of ACE therefore prevented the increase of upstream vascular resistance and/or the decrease of the compliance of pulmonary vessels induced by exposure to hypoxia. Both may be affected if the proliferation of mesenchymal cells in the vascular wall is inhibited. The possibility that Ramipril attenuates the structural reconstruction of the pulmonary vascular bed rather than affects the smooth muscle tone is supported by the fact that Ramipril decreased the slope but not the pressure axis intercept of the P/Q plot.

Occlusion experiments showed significantly higher pressure drops across the pulmonary vascular bed in rats exposed to chronic hypoxia. The increase resulted from the larger pressure drops in arterial and venous segments of pulmonary vasculature. This is in accordance with our previous findings (Herget and Kuklík 1995). In this previous study we also found a small increase of resistance in the middle vascular segment of chronically hypoxic rats. This was not the case in the present study. This difference may be related to the already discussed and unexplained absence of the increase in the pressure axis intercept in the group exposed to hypoxia. The effects of Ramipril administration on the distribution of pulmonary vascular resistance across the pulmonary vasculature in chronically hypoxic rats support the interpretation of changes in the P/Q relationships. These results are in accord with other studies (Zakheim *et al.* 1975, Abraham *et al.* 1995) where administration of angiotensin converting enzyme inhibitors to hypoxic rats prevented the morphological reconstruction of pulmonary vasculature. In contrast to our results, authors of the latter study found an increase in total lung vascular resistance after Cilazapril treatment in rats exposed to hypoxia. The cardiac output, however, decreased in their experimental rats. Their paper unfortunately did not give sufficient data for further analysis.

The present study does not provide information concerning the mechanism by which ACE inhibitors block the increase of the pulmonary vascular resistance in hypoxic animals. It is not clear whether the effect is attributable to the haemodynamic influence induced by the drug administration (lower production of vasoconstrictor angiotensin II) or whether it is related to the indirect or direct action on mesenchymal cell proliferation in the vascular wall. It has been shown that these two processes are linked. The increase of vascular wall tension stimulates the proliferation of pulmonary vascular cells (Riley and Gullo 1988). Angiotensin II itself has several actions (see above) which can be attributed to regulation of the mesenchymal cell cycle. Therefore, the direct relation of angiotensin II and morphological reconstruction of pulmonary blood vessels cannot be excluded. There is evidence that the renin-angiotensin system is activated during exposure to chronic hypoxia. Plasma renin activity is higher in high altitude climbers (Milledge *et al.* 1983) so that the decrease of ACE activity found in hypoxic rats (Jederlinic *et al.* 1988, Oparil *et al.* 1988) may be a compensatory reaction to a still unknown mechanism.

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