

Lung angiotensin converting enzyme activity in chronically hypoxic rats

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ABSTRACT A study was carried out to test the hypothesis that the reduced lung angiotensin converting enzyme (ACE) activity which occurs in chronic hypoxia is related to the development of pulmonary hypertension rather than to hypoxia per se. Right ventricular mean systolic pressure (\bar{P}_{rvs} , mm Hg) and ACE activity (nmol/mg protein/min) in lung tissue homogenates were measured in seven groups of four rats placed in a hypobaric chamber (380 mm Hg; 51 kPa) for two to 24 days. Identical measurements were made on 11 groups of four rats, which were placed in the chamber for 24 days and then allowed to recover in room air for one to 153 days. After two days of hypoxia the mean \bar{P}_{rvs} (25.5 (SD 3.7)) and the mean lung ACE activity (56 (4.6)) did not differ significantly from control values. Exposure to hypoxia for four to 24 days caused a progressive increase in mean \bar{P}_{rvs} to 44.4 (5.9) and a progressive reduction in mean lung ACE activity to 34 (4.0). During recovery lung ACE activity increased and \bar{P}_{rvs} decreased, so that normal values were achieved by 15 and 56 days respectively. Decreased lung ACE activity may be related to haemodynamic factors associated with pulmonary hypertension rather than to hypoxia.

Acute alveolar hypoxia has been shown in dogs to reduce the ability of the pulmonary vasculature to convert angiotensin I to angiotensin II¹ and to degrade bradykinin.² Chronic alveolar hypoxia in rats has been shown to reduce the lung angiotensin converting enzyme (ACE) activity.^{3,4} Recently there has been controversy⁵ about whether the hypoxia induced inhibition of ACE activity is due to a direct effect of hypoxia on the endothelial cell membrane⁶ or whether it is secondary to pulmonary haemodynamic changes.⁷⁻⁹ We have investigated the hypothesis that the reduced lung ACE activity which occurs in chronic hypoxia is related to the development of pulmonary hypertension rather than to hypoxia per se. We measured the right ventricular mean systolic pressure (\bar{P}_{rvs}), right ventricular hypertrophy, and lung ACE activity in groups of rats exposed to chronic hypoxia for two to 24 days to determine whether the lung ACE activity diminished immediately with the onset of hypoxia or whether it diminished gradually as pulmonary

hypertension developed. We also measured \bar{P}_{rvs} , right ventricular hypertrophy, and lung ACE activity in groups of rats that were exposed to chronic hypoxia for 24 days and then allowed to recover in room air for one to 153 days. We wished to determine whether the lung ACE activity returned to normal immediately the rats were returned to room air or whether it gradually increased as the pulmonary hypertension slowly regressed.

Methods

We evaluated pulmonary hypertension, right ventricular hypertrophy, serum ACE activity, and lung ACE activity in seven groups of four young female Wistar rats, which were placed in a hypobaric chamber (380 mm Hg; 51 kPa) for two, four, eight, 11, 14, 18, and 24 days respectively. Identical measurements were made on 11 groups of four similar rats placed in the hypobaric chamber⁴ for 24 days and then allowed to recover in room air for one, three, seven, 15, 24, 34, 56, 76, 104, 132, and 153 days respectively. Thirty eight young female Wistar control rats matched for age were also studied during the course of the experiment. The mean initial body weights of the groups of control and test rats

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were 101 (SD 7.7) g and 104 (9.1) g respectively. Right ventricular mean systole pressure (\bar{P}_{rvs}) was measured by cannulating the right external jugular vein under light ether anaesthesia, a modified 21 gauge disposable needle being used.¹⁰ The tip of the cannula was guided through the right atrium and tricuspid valve into the right ventricle. The pressure was measured with a Hewlett-Packard transducer (1280C) attached to a pressure coupler (8805C) and thermotip recorder (7702B). The pressure was measured in the chronically hypoxic rats from 30 to 60 minutes after removal from the hypobaric chamber. After measurement of \bar{P}_{rvs} the abdomen was opened and a sample of blood was obtained from the inferior vena cava. The serum was separated, frozen, and stored for one week to four months to await ACE assay. The rats were then killed. The left lung was removed, washed in physiological saline, frozen, and stored for one week to two months to await ACE assay. ACE is stable for at least one year when stored at -20°C . The lungs and sera were prepared for assay by methods previously described in detail.^{4,11} Briefly, the left lung was thawed, weighed with a Sartorius semi-micro analytical balance, diced by hand, and then homogenised in a Brinkman Polytron PT 10-35. The homogenate was filtered and the filtrate was centrifuged at 1250 g for 5 minutes. The protein concentration was measured in the supernatant fluid that was then used for enzyme assay. ACE activity was measured by means of a radioenzymatic modification of the assay described by Cushman and

Cheung.¹² This method measures the rate of production of carbon 14 labelled hippuric acid from hippuryl-L-histidyl-L-leucine. Lung ACE activity was expressed in nmol/mg protein/min. The heart was dissected after fixation in 10% formol saline and weighed with a Sartorius semimicro analytical balance. Right ventricular hypertrophy was evaluated by expressing the weight of the free wall of the right ventricle (RV) as a percentage of the weight of the left ventricle and interventricular septum (LV+S). The results of the experiments, expressed as means with standard deviations in parentheses, were evaluated with the *t* test for unpaired data and linear regression analysis. Differences in values were considered to be significant when $p < 0.05$.

Results

The \bar{P}_{rvs} in the 38 control rats was 27.0 (4.2) mm Hg. After two days of hypoxia the mean \bar{P}_{rvs} (25.5 (3.7) mm Hg) was normal (fig 1). Exposure to hypoxia for four to 24 days caused a progressive increase in the mean \bar{P}_{rvs} to 44.4 (5.9) mm Hg. During normoxic recovery the mean \bar{P}_{rvs} gradually decreased, until normal values were achieved 56 days after the animals' removal from the hypobaric chamber (fig 1).

In the 38 control rats the ratio of right to left ventricular weight (RV/(LV+S)) was 26.5% (2.7%). This ratio was significantly increased to 32.5% (5.5%) in the group of rats killed after being in the hypobaric chamber for two days (fig 2). Right ventricular hypertrophy progressively increased so that after 24 days of hypoxia the mean RV/(LV+S)

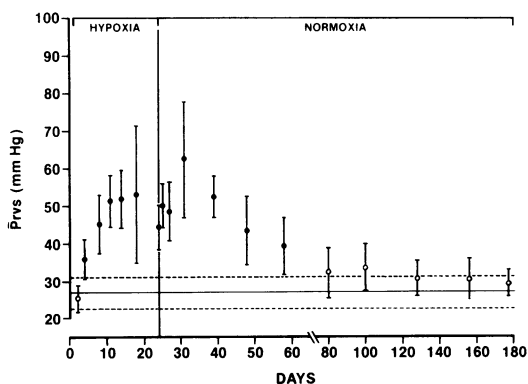


Fig 1 Effect of chronic hypoxia and subsequent normoxic recovery on mean right ventricular systolic pressure (\bar{P}_{rvs}). The mean value of the results derived from the 38 control rats is shown as a continuous horizontal line; each interrupted horizontal line indicates 1 SD. The results from the groups of four test rats are given as mean and SD values. A closed circle indicates that the value obtained from that group of test rats differs significantly from the control value ($p < 0.05$).

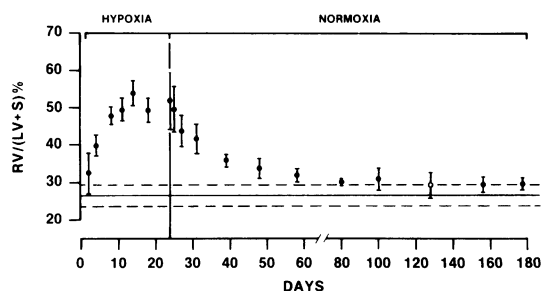


Fig 2 Effect of chronic hypoxia and subsequent normoxic recovery on the ratio of the weight of the free wall of the right ventricle (RV) to the weight of the left ventricle and interventricular septum (LV+S). The mean value of the results derived from the 38 control rats is shown as a continuous horizontal line; each interrupted horizontal line indicates 1 SD. The results from the groups of four test rats are given as mean and SD values. A closed circle indicates that the value obtained from that group of test rats differs significantly from the control value ($p < 0.05$).

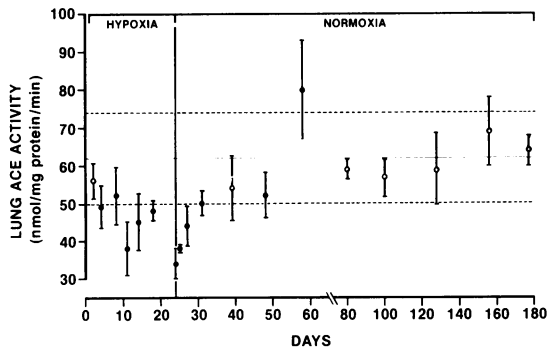


Fig 3 Effect of chronic hypoxia and subsequent normoxic recovery on lung angiotensin converting enzyme (ACE) activity. The mean value of the results derived from the 38 control rats is shown as a continuous horizontal line; each interrupted horizontal line indicates 1 SD. The results from the groups of four test rats are given as mean and SD values. A closed circle indicates that the value obtained from that group of test rats differs significantly from the control value ($p < 0.05$).

was 51.8% (7.7%). During normoxic recovery the mean RV/(LV+S) progressively decreased until a normal mean value (29.3% (3.5%)) was achieved 104 days after removal from the hypobaric chamber (fig 2).

The mean specific activity of ACE in the lung tissue homogenates derived from the 38 control rats was 62 (12.1) nmol/mg protein/min. After two days of hypoxia the mean lung ACE activity (56 (4.6) nmol/mg protein/min) did not differ significantly from the control value. Exposure to hypoxia for four to 24 days caused an uneven reduction in mean lung

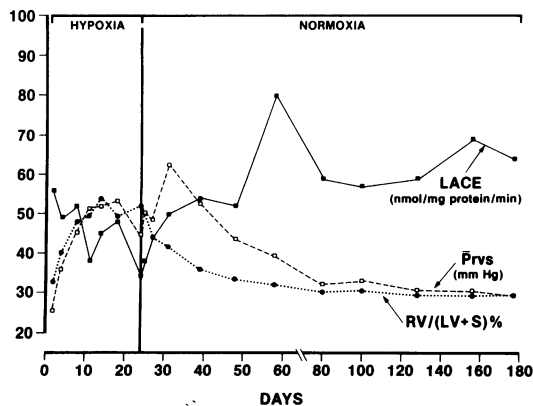


Fig 4 Effect of chronic hypoxia and subsequent normoxic recovery on lung angiotensin converting enzyme activity (LACE), mean right ventricular systolic pressure (Prvs), and ratio of right to left ventricular weight (RV/(LV+S)%). Each point represents the mean value from a group of four test rats.

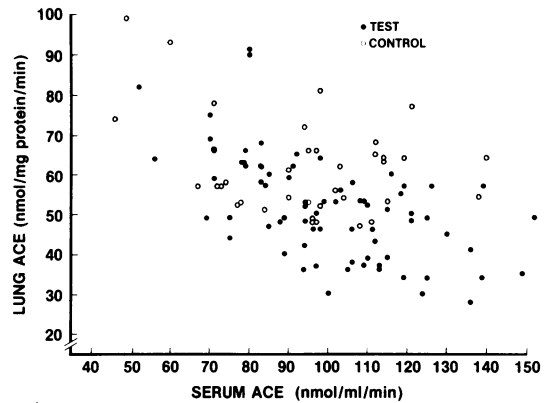


Fig 5 Lung and serum ACE activity in control rats, chronically hypoxic test rats, and test rats exposed to chronic hypoxia followed by normoxic recovery. Values from individual rats are shown.

ACE activity to 34 (4) nmol/mg protein/min (fig 3). During normoxic recovery the mean lung ACE activity gradually increased until a normal value (54 (8.6) nmol/mg protein/min) was attained after 15 days. On the 34th day of normoxic recovery the mean lung ACE was significantly raised to 80 (12.9) nmol/mg protein/min. This "rebound" was followed by normal mean values for the recovery period (fig 3). The relation between Prvs, RV/(LV+S)%, and lung ACE activity during the hypoxic and recovery phases of the experiment is shown in figure 4. Throughout the hypoxic and recovery phases of the experiment, in the test rats the lung ACE activity had a significant negative correlation with the Prvs ($r = -0.39$) and RV/(LV+S)% ($r = -0.71$).

In the 38 control rats the mean serum ACE activity was 94 (21.7) nmol/ml/min and there was a significant negative correlation between the serum ACE value and the duration of the experiment in days ($r = -0.54$). Figure 5 shows that serum ACE activity does not reflect lung ACE activity in female rats. In the test rats the serum ACE values did not appear to differ from the values in the control rats during either the hypoxic or the recovery phases of the experiment.

Discussion

Chronic hypoxic pulmonary hypertension results from a combination of vasoconstriction, organic narrowing of muscularised pulmonary arterioles, and increased blood viscosity due to polycythaemia. If a high altitude dweller breathes pure oxygen there is only a slight reduction in pulmonary artery pressure, presumably due to relief of vasoconstriction.¹³ Thus

we consider that a slight reduction in \bar{P}_{rvs} may have occurred during the 30–60 minutes between removal of the hypoxic rats from the hypobaric chamber and cardiac catheterisation in room air. We do not think that this affects our conclusions since the lung ACE levels were decreased in pulmonary hypertensive rats catheterised under normoxic conditions. One would have expected the lung ACE to increase rapidly to normal if its activity had been inhibited by hypoxia. The mean \bar{P}_{rvs} was normal in the group of rats examined after two days of hypoxia but the mean ratio of right to left ventricular weight (32.5% (5.5%)) was significantly greater than the control value of 26.5% (2.7%). This apparent discrepancy could have resulted from a raised \bar{P}_{rvs} reverting to normal during the 30–60 minutes between removal from the hypobaric chamber and cardiac catheterisation in room air. We believe, however, that the right ventricular hypertrophy in this group of rats is spurious and represents a statistical artefact related to the unusually low mean ratio of right to left ventricular weight in the control rats of this experiment. In all our previous studies the mean ratio has been about 30%.^{4 11 14} In favour of this explanation, the pulmonary arterial vessels were normal in the rats killed after two days of hypoxia, whereas there was muscularisation of the pulmonary arterioles in the rats killed after four days, when the mean \bar{P}_{rvs} was 36 mm Hg and the RV/(LV+S) ratio was 39.9%.

This experiment has shown that after two days of exposure to chronic hypobaric hypoxia rats have normal lung ACE activity and no pulmonary hypertension. Exposure to hypoxia for four to 24 days caused a progressive development of pulmonary hypertension associated with an uneven reduction in lung ACE activity. During normoxic recovery the lung ACE activity gradually increased and the pulmonary hypertension gradually decreased over periods of 15 and 56 days. We made direct measurements of ACE activity in lung tissue homogenates. Our findings confirm the recent observations of Caldwell and Blatteis,³ who exposed rats to hypobaric hypoxia for two weeks and then injected graded doses of angiotensin I and angiotensin II into the right atrium. Dose-arterial pressor response curves were constructed. Total vascular ACE activity was estimated by determining the mean proportion of the magnitudes of the angiotensin I systemic pressor responses and comparing these with equimolar doses of angiotensin II. They found that total vascular angiotensin I conversion was 55% in the chronically hypoxic rats and 84% in control rats. Furthermore, the total vascular conversion of angiotensin I to angiotensin II was reduced in the rats exposed to chronic hypoxia even one to three

days after they had been removed from the hypobaric chamber. Our results and those of Caldwell and Blatteis³ suggest that the decreased lung ACE activity may be related to haemodynamic factors associated with pulmonary hypertension, rather than to hypoxia per se. This proposal is in keeping with our observation that throughout the hypoxic and recovery phases of the experiment in the test rats the lung ACE activity had a significant negative correlation with the severity of pulmonary hypertension and degree of right ventricular hypertrophy. ACE is located on the luminal surface of pulmonary endothelial cells. Meyrick and Reid¹⁵ have described structural changes in the pulmonary endothelial cells of rats exposed to chronic hypobaric hypoxia. From the third day of exposure the endothelial cells of newly muscularised arteries appeared thickened with flocculent cytoplasm containing scanty small and elongated mitochondria, ribosomes, rough endoplasmic reticulum, a small Golgi apparatus, and pinocytotic vesicles. The pulmonary capillary endothelial cells were up to four times thicker than normal and had abnormally electron lucent cytoplasm. Perhaps these structural changes are related to decreased synthesis of ACE.

In 1976 Oparil and her coworkers¹⁶ suggested that plasma ACE activity may be a reflection of pulmonary conversion and that it can be altered by pulmonary disease. Our results do not support this idea. As we have shown before, rats with pulmonary hypertension and decreased lung ACE activity have normal levels in the serum.^{4 11} In this experiment our female control rats showed a significant negative correlation between the serum ACE value and the duration of the experiment. This finding confirms the observations of other workers that the serum ACE level is related to age both in human subjects^{17 18} and in rats.¹⁷

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