PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 263–265 (1974)

# The Effect of Chronic Alveolar Hypoxia on Lung and Serum Angiotensin I Converting Enzyme Activity (38323)

AGOSTINO MOLTENI, RICHARD M. ZAKHEIM, KARY B. MULLIS, AND LEONE MATTIOLI

*Departments of Pathology and Pediatrics, University of Kansas Medical Center, Kansas City, Kansas 66103*

The renin angiotensin aldosterone system is involved in the response to alveolar hypoxia. Elevated indices of granulation of the cells of the juxtaglomerular apparatus (JGA) were found in populations living at high altitudes and in animals kept in hypobaric chambers (1, 2). Gould and Goodman have shown that rats exposed to chronic alveolar hypoxia have high plasma renin concentrations, high renin substrate and increased plasma renin activity during the first and second weeks of exposure (3). Gould *et al.* have also shown that renin is necessary for the erythropoietic response to hypoxia in nephrectomized rats (4). Another enzymatic component of the renin angiotensin–aldosterone system, the angiotensin I converting enzyme, has not been previously studied under the same experimental conditions. The enzyme is synthesized predominantly in the capillary endothelial cells of the lung (5) where 90% of the physiologic conversion of angiotensin I to angiotensin II takes place (6). This study reports changes of angiotensin I converting enzyme activity in lung and serum of chronically hypoxic mice and parallel changes in renal renin.

*Materials and Methods.* Seventy-two female Swiss Webster mice, 35 days old, were used. Twelve mice were considered controls. Twelve other mice were put in the hypobaric chamber at 0.5 atm on day 0 of the experiment. Twelve additional mice were placed in the chamber on days 2, 4, 7, and 9 of the experiment. At 11 days, all experimental and control mice were killed. We had, therefore, five groups of hypoxic mice kept in the hypobaric chamber, respectively, for 2, 4, 7, 9, and 11 days, and a group of controls, all of whom were of the same age. All mice were allowed regular rat Purina Chow and drank tap water *ad libitum*. Both the hypoxic and control mice were maintained at the same temperature with identical light cycles of 12 hr. Every 72 hr the chambers were opened for 10 min for animal maintenance. Control and experimental animals were housed 12 per cage in identical cages. The mice were killed by decapitation under ether anesthesia within 30 min of removal from the chamber. Blood, lungs and kidneys were immediately collected for enzymatic determinations. The blood was collected from the animals in chilled tubes on ice and after 20 min, centrifuged. The serum was stored at 4° until determinations were performed. Each determination was made on the pooled blood of 3 animals. Data from our laboratory has shown that under these conditions serum converting enzyme activity is stable for one week. Kidneys were immersed in Helly's fixative for 24 hr. Lungs were collected in chilled tubes, immediately frozen, and stored until determinations of converting enzyme activity were done. An acetone powder was prepared from the lungs by homogenization at 0° for one min in 20 ml of acetone. The powder was suction filtered to dryness and kept frozen until extraction of the enzyme. A weighed portion of the acetone powder (approximately 50 mg) was extracted with 20 ml of 50 m*M* phosphate buffer, pH 8.3, by homogenization at 0°. The homogenate was centrifuged and the clear supernatant analysed for converting enzyme activity by the method of Cushman and Cheung (7). The serum was assayed directly using the same method. The fixed kidneys were imbedded in paraffin, cut at 5 $\mu$m sections and stained with Bowie's stain according to the Wilson's technique (8). Indices of granulation of the cells of the JGA were counted on slides according to procedures of Hartroft and Hartroft (9). The significance of data was determined according to the Student's *t* test and the difference of $P < 0.05$ were considered significant and $P < 0.01$ highly signifi-

cant. Correlation coefficients (Pearson's) were calculated on Olivetti Underwood Programma 101 (10).

*Results.* Mice kept in the hypobaric chamber showed the expected weight loss while controls continued to gain weight. At the end of 11 days the weight of the hypoxic mice was approximately 60% of that of the controls (24 ± 1 g vs 38 ± 2 g, $P < 0.001$). The hypoxic animals also showed a progressive rise in hematocrit values and at 11 days their hematocrit was 73 ± 3 vs 42 ± 1 for that of the controls ($P < 0.001$).

In Table I are reported the results of the measurements of serum and lung angiotensin I converting enzyme activity as well as the concentrations of renal renin expressed as indices of granulation of the cells of the JGA. It can be seen from the table that both serum and lung angiotensin I converting enzyme activity diverged significantly from the control values by the ninth day of hypoxia. The increment of activity of the lung and serum converting enzyme was of the same order of magnitude and occurred at approximately the same time ($r = 0.90$, SEE 0.025 units/ml). The increase in renal renin granules was closely correlated with both lung ($r = 0.97$, SEE 0.98 units/g acetone powder) and serum ($r = 0.91$, SEE 0.025 units/ml) converting enzyme activity.

*Discussion.* This experiment has confirmed previous observations of the effect of chronic alveolar hypoxia on renal renin (1–3). In addition, we have shown that another enzymatic component of the renin angiotensin aldosterone system (angiotensin I converting enzyme) is also influenced by hypoxia. Further, it was demonstrated that increases in serum converting enzyme activity closely parallel changes in lung converting enzyme activity.

The significance of the stimulation of the renin angiotensin aldosterone system in the response to hypoxia remains unknown. It may be an adjustment to the stress of hypoxia or be necessary to the erythropoietic response. The observed increase in the activity of the renin angiotensin aldosterone system may be related to the well-known pulmonary vascular changes of chronic alveolar hypoxia (11). Consistent with this is the recent proposal that angiotensin II plays a role in the acute pulmonary vasoconstrictive response to hypoxia (12). Yang *et al.* have suggested that angiotensin I converting enzyme activity may be the rate limiting step in the re-

TABLE I. Serum and Lung Angiotensin-I Converting Enzyme Activity (A-I-CEA) and Indices of Granulation of the Cells of Juxtaglomerular Apparatus (JGA) of Controls and Hypoxic Mice.

| | Controls | 2 days hypoxia | 4 days hypoxia | 7 days hypoxia | 9 days hypoxia | 11 days hypoxia |
|---|---|---|---|---|---|---|
| Serum A-I-CEA units/ml | 0.315 ± .008* | 0.298 ± .035 | 0.308 ± .004 | 0.336 ± .019*** | 0.403 ± .010*** | 0.407 ± .032** |
| Lung A-I-CEA units/g Acetone powder | 27.63 ± 1.20 | 25.10 ± .420 | 29.10 ± 4.80 | 32.00 ± 3.10 | 33.50 ± 1.10** | 34.80 ± 3.10** |
| Indices of granulation JGA cells | 38 ± 6 | 38 ± 6 | 55 ± 5 | 59 ± 6 | 71 ± 4*** | 70 ± 6*** |

* Standard error of the mean.
** $P < 0.005$.
*** $P < 0.001$.

lease of angiotensin II (13). Recently much experimental evidence has become available which indicates that converting enzyme inhibitors (SQ 20881) are effective in preventing hypertension sustained by hyperreninemia (14–18). The present findings show that converting enzyme activity varies along with renin in the condition of chronic hypoxia and this may be of physiologic significance.

*Summary*. Mice exposed to chronic alveolar hypoxia showed elevations of serum and lung angiotensin I converting enzyme activity during the second week of exposure. The increment of activity of the lung and serum converting enzyme was of the same order of magnitude and occurred at approximately the same time. An increase in renal renin granules was closely correlated with both lung and serum converting enzyme activity.

The authors thank Dr. R. Riley for allowing the use of the hypobaric chambers and Dr. John Glick for his advice and suggestions on the statistical analysis.

This work was supported by Kaw Valley Heart Association Grants Nos. 9783-01 and 9784-01.

1. Demopoulos, H. B., Highman, B., Altland, P. D., Gerving, M. A., and Kaley, G., Amer. J. Pathol. **46,** 497 (1965).

2. Oliver, W. J., and Brody, G. L., Circ. Res. **16,** 83 (1965).

3. Gould, A. B., and Goodman, S. A., Lab. Invest. **22,** 443 (1970).

4. Gould, A. B., Goodman, S. A., and Green, D., Lab. Invest. **28,** 719 (1973).

5. Smith, V., and Ryan, J., Fed. Proc. **32,** 1957 (1973).

6. Ng, K. K. F., and Vane, J. R., Nature (London) **218,** 144 (1968).

7. Cushman, D. W., and Cheung, H. S., Biochem. Pharmacol. **20,** 1937 (1971).

8. Wilson, W., Anat. Rec. **112,** 497 (1952).

9. Hartroft, P. M., and Hartroft, W. S., J. Exp. Med. **97,** 415 (1953).

10. Olivetti, Underwood Programma 101. Statistical Analysis Instructions, p. 127 (1968).

11. Jaenke, R. S., and Alexander, A. F., Amer. J. Pathol. **73,** 377 (1973).

12. Berkov, S., Circulation. Supp. IV. Vol. VII, Vol. VIII, IV. 133 (1973).

13. Yang, H. Y. T., Erdös, E. G., and Levin, Y., J. Pharmacol. Exp. Ther. **177,** 291 (1971).

14. Klein, G. R., Kirpou, J., Peterson, H. E., Murphy, B. F., Hassert, G. L., and Poutsiada, J. W., Proc. Soc. Exp. Biol. Med. **140,** 149 (1972).

15. Engel, S. L., Schaeffer, T. R., Gold, B. I., and Rubin, B., Proc. Soc. Exp. Biol. Med. **140,** 240 (1972).

16. Di Salvo, J., Brittori, S., Galvas, P., and Sanders, T. W., Circ. Res. **22,** 85 (1973).

17. Collier, J. G., Robinson, B. F., and Vane, J. R., Lancet, Jan. **13,** 72 (1973).

18. Cheung, H. S., and Cushman, D. W., Fed. Proc. **32,** 765 Abs. (1973).

Received March 22, 1974. P.S.E.B.M., 1974, Vol. 147.