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Distribution in Tissues and Effects by Acute Hypoxia on Angiotensin I Converting Enzyme Activity in Guinea Pig and Chicken

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Angiotensin I converting enzyme activity was measured in homogenates of guinea pig and chicken organs (lung, kidney, heart, ileum, diaphragm and liver), using a spectrophotometric assay for hydrolysis of hippuril-L-histidyl-L-leucine. High specific activities were found in lung, kidney and diaphragm, but the highest corresponded to guinea pig lung and chicken kidney. Acute hypoxia decreased angiotensin I converting enzyme activity in guinea pig lung and chicken diaphragm, but the changes in kidney were considered non-significant in both the guinea pig and chicken.

Key words: Hypoxia, Angiotensin I Converting enzyme, Tissue distribution Guinea pig, Chicken.

Angiotensin I coverting enzyme (EC 3.4.15.1) is a peptidyl dipeptide hydrolase found in several mammalian tissues. This enzyme hydrolytically releases the dipeptide, His-Leu, from the carboxyl terminus of the decapeptide angiotensin I. The resulting octapeptide, angiotensin II, is a potent vasopressor. The enzyme also inactivates bradykinin, a vasodepressor.

The distribution of the angiotensin I converting enzyme activity in various tissues of the rat was studied by HUGGINS and THAMPI (4) and CUSHMAN and CHEUNG (1). The latter authors reported the highest enzyme activity in epididymis and fairly high concentrations in testis and lung. Converting enzyme activity was measured in other tissues like hypothalamus, aorta, choroid plexus, neurohypophysis, adenohypophysis, kidney, heart, plasma, cerebrospinal fluid, medulla oblongata (3, 10).

Converting enzyme activity of the systemic vascular bed in anesthetized dogs was inhibited by acute hypoxia (12). Hypoxia also inhibits angiotensin I

converting enzyme activity in cultured endothelial cells (13), but mice exposed to chronic alveolar hypoxia showed elevations of serum and lung angiotensin I converting enzyme activity during the second week of exposure (7).

As nothing is known about the distribution and the effect of hypoxia on that enzyme activity in birds, this study reports the activity in tissues of chicken and guinea pig and the changes of activity in lung, kidney and diaphragm after acute hypoxia.

Materials and Methods

Ten guinea pigs weighing 250-300 g and ten chickens with a weight of 1.5-2.0 kg were used in hypoxia experiments. The same number of animals were taken as controls. The birds and the guinea pigs were housed and kept in cages under natural conditions with standard dry food and water *ad libitum*. The experimental animals were placed in a low pressure chamber constructed by us and exposed to a simulated altitude of 4,000 m (462 Torr) for 48 hours.

Organs were removed immediately after being killed and rinsed gently with chilled saline solution. They were chopped into small pieces and homogenized in 5 vol. of 100 mM potassium phosphate buffer, 300 mM NaCl, pH 8.3, using a Potter-Elvehjem type homogenizer. The extract was centrifuged at 3,000 *g* for 40 min Protein concentration of supernatant fluid converting enzyme in homogenates was measured using the spectrophotometric procedure of CUSHMAN and CHEUNG (2) modified for us as follows: hippuryl-L-histidyl-L-leucine was 0.5 mM instead of 5 mM; the assay mixture was incubated at 37° C for 60 min instead of 30 min, and the reaction was stopped by adding 0.25 ml of 1N HCl. The hippuric acid was extracted with 1.5 ml of ethyl acetate. The concentration of hippuric acid was determined by measuring the absorbance at 228 nm against a zero time blank prepared by adding 0.25 ml of 1N HCl to the assay mixture before the reaction.

Hippuryl-L-histidyl-L-leucine was purchased from Sigma and other chemicals used in the experiments were of reagent grade. Activities of angiotensin I converting enzyme are calculated as specific activities, (μM hippuric acid released from hippuryl-L-histidyl-L-leucine per minute and mg of protein at 37° C). 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 significant.

Results

Comparative activities of angiotensin I converting enzyme in guinea pig and chicken tissues are shown in figure 1. The percentages are calculated with ref-

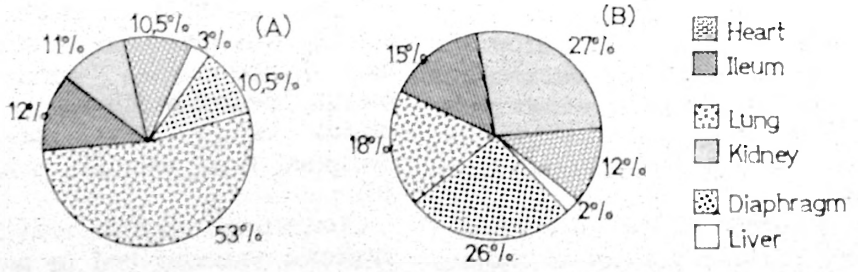

Fig. 1. *Distribution of the angiotensin I converting enzyme activity in lung, kidney, heart, ileum, diaphragm and liver of the guinea pig (A) and chicken (B).*

Table I. *Activities of angiotensin converting enzyme in lung, kidney and diaphragm of guinea pig and chicken of controls and hypoxic animals.*
Values are expressed as specific activities* (Mean ± S.E.) · 10^3. Number of experiments are shown in parentheses.

	Lung		Kidney		Diaphragm	
	Guinea pig	Chicken	Guinea pig	Chicken	Guinea pig	Chicken
Control	301 ± 72 (9)	253 ± 47 (10)	100 ± 37 (8)	295 ± 36*** (10)	66 ± 42 (3)	828 ± 339*** (7)
Hypoxia**	241 ± 43 (10)	248 ± 51 (11)	117 ± 43 (10)	268 ± 67*** (11)	45 ± 23 (7)	404 ± 218*** (11)

* μM hippuric acid/min · mg protein. ** Po_2 = 462 Torr. *** $P < 0.001$.

erence to the total activity which is the total amount of the activities of the six organs. The activity in the lung of the guinea pig was 53 %, whereas that in the lung of the chicken was only 18 %. The highest activity in chicken was found in the kidney (27 %). The activity was lowest in the liver of both the guinea pig and the chicken.

Table I shows the converting enzyme activity in the lung, kidney and diaphragm in control and hypoxia conditions. The difference in activity for the same organ among the different species was highly significant in the kidney and diaphragm, but in the lung the difference was not significant. With regard to the changes due to hypoxia, it can be seen from the table I that a fall of activity was significative in the guinea pig lung ($P < 0.05$) and the chicken diaphragm ($P < 0.05$).

Discussion

The high concentrations of angiotensin I converting enzyme found in the lung of guinea pig are consistent with the findings of CUSHMAN and CHEUNG (1) but differ from those of HUGGINS and THAMPI (4), who found much lower activities in general and only small differences in the activities of the lung, liver, diaphragm and heart. The preliminary acidification and $(NH_4)_2SO_4$ fractionation employed by the latter authors could have resulted in a low yield of the enzyme.

Although the enzyme activity in the kidney of the guinea pig is low, this finding does not rule out, in this organism, an intra-renal role for angiotensin I converting enzyme; this low activity may be quite significant if the enzyme is located in site such as the afferent arteriole. Various authors (8) have demonstrated significant conversion of angiotensin I to angiotensin II in the renal vascular beds of dogs.

Angiotensin I converting enzyme activity in chicken kidney and diaphragm was higher than in the same organs of the guinea pig.

Both guinea pig lung and the chicken diaphragm decrease significatively ($P < 0.05$) converting enzyme activity by hypoxia. This experiment has confirmed that another enzymatic component of the renin-angiotensin-aldosterone system (angiotensin I converting enzyme) could also be influenced by hypoxia in both guinea pig and chicken.

MOLTENI *et al.* (7) in mice at short time hypoxia (2 days) found that angiotensin I converting enzyme decreased in serum and lung, but that mice exposed to chronic alveolar hypoxia

showed a rise of converting enzyme activity during the second week of exposure. ZAKHEIM *et al.* (15), demonstrated a rapid decline in arterial levels of angiotensin II after the onset of hypoxia in rabbits, which is caused by the inhibition of converting enzyme by hypoxia.

Studies of converting enzyme activity in cultures endothelial cells (13) show the same effect of hypoxia on enzyme activity as seen in these chicken and guinea pig experiments.

Resumen

Se mide en distintos órganos de cobaya y de pollo la actividad del enzima de conversión de angiotensina I por un método espectrofotométrico que hidroliza el tripéptido hipuril-L-histidil-L-leucina. Se encuentran actividades específicas altas en pulmón, riñón y diafragma, pero las mayores actividades corresponden al pulmón en cobayas y al riñón en pollos. La hipoxia aguda hace decrecer la actividad del enzima de conversión en pulmón de cobaya y en diafragma de pollo; pero los cambios en riñón no son significativos ni en cobaya ni en pollo.

References

1. CUSHMAN, D. W. and CHEUNG, H. S.: *Biochem. Biophys. Acta*, **250**, 261-265, 1971.
2. CUSHMAN, D. W. and CHEUNG, H. S.: *Biochem. Pharmacol.*, **20**, 1637-1648, 1971.
3. ERDOS, E. G. and OSHIMA, G.: *Acta Physiol. Lat. Amer.*, **24**, 507-514, 1974.
4. HUGGINS, C. G. and THAMPI, N. S.: *Life Sci.*, **7**, 633-639, 1968.
5. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J.: *J. Biol. Chem.*, **193**, 265-275, 1951.
6. MATTIOLI, L., ZAKHEIM, R. M., MULLIS, K. and MOLTENI, A.: *J. Pediatr.*, **87**, 97-101, 1975.
7. MOLTENI, A., ZKHEIM, R. M., MULLIS, K. B. and MATTIOLI, L.: *Proc. Soc. Exptl. Biol. Med.*, **147**, 263-265, 1974.
8. OPARIL, S., SANDER, C. A. and HABER, E.: *Circ. Res.*, **26**, 591-599, 1970.
9. OSHIMA, G., GECSE, A. and ERDOS, E. G.: *Biochem. Biophys. Acta*, **350**, 26-27, 1974.
10. RIX, E., GANTEN, D., SCHULL, B., UNGER, Th. and TAUGNER, R.: *Neurosci. Letters*, **22**, 125-130, 1980.
11. STALCUP, S. A., LIPSET, J. S., WONG, J. M., LEUENBERGER, P., TURINO, G. M. and MELLINS, R. B.: *Pediatr. Res.*, **12**, 569, 1977.
12. STALCUP, S. A., LIPSET, J. S., LEGANT, P. M., LEUENBERGER, P. J. and MELLINS, R. B.: *J. Appl. Physiol.*, **46**, 227-234, 1979.
13. STALCUP, S. A., LIPSET, J. S., WONG, J. M., LEUENBERGER, J. and MELLINS, R. B.: *J. Clin. Invest.*, **63**, 966-976, 1979.
14. TAKADA, Y., HIWADA, K. and KOKUBU, T.: *J. Biochem.*, **90**, 1309-1319, 1981.
15. ZAKHEIM, R. M., MOLTENI, A., MATTIOLI, L. and PARK, M.: *J. Appl. Physiol.*, **41**, 462-465, 1976.