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# Properties of Angiotensin I-Converting Enzyme Activity in the Rice Eel, *Monopterus albus*

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**Angiotensin I-converting enzyme (ACE) activity has been characterized in the rice eel, *Monopterus albus*. Peak activity of ACE in plasma from the rice eel was shown at around pH 10, which was more alkaline compared to that of mammals. Chloride requirements for the optimal ACE activity were different from species to species. ACE inhibitors, EDTA, teprotide (SQ 20,881), and captopril (SQ 14,225) showed dose-dependent inhibitions of ACE activity in plasma from the rice eel as well as mammals. ACE activity in the rice eel was increased by $CoCl_2$, and the enzyme activity was more unstable at high temperature as compared to mammals. The highest activity of ACE among the various tissues in the rice eel was found in the brain.**

**KEY WORDS: Rice eel, Angiotensin I-converting enzyme**

The renin-angiotensin system in mammals plays an important role in the homeostatic regulation of blood pressure, body fluid volume and electrolyte balance. Angiotensin I-converting enzyme (dipeptidyl carboxypeptidase, Kininase II), a component of renin-angiotensin system, is a membrane-bound glycoprotein, located mainly in the endothelial cells of pulmonary capillaries in mammals (Ryan et al., 1975). It mediates the cleavage of the dipeptide His-Leu from the inactive decapeptide angiotensin I, thus generating the powerful vasoconstricting angiotensin II (Lanzillo and Fanburg, 1977). This physiologically important enzyme plays a key role in the renin-angiotensin system. This enzyme was isolated and characterized from various tissues of mammals (Poth et al., 1975; Schweisfurth and Schiöberg-Schiegnitz, 1984; Velletri et al., 1985; Duggan et al., 1989). Although the components of the renin-angiotensin system have been found in all vertebrates phylogenetically above the fishes, the exact physiological roles of the system are not yet defined in non-mammalians (Taylor, 1977; Nishimura, 1980; Nishimura and Bailey, 1982; Cho et al., 1987a; Kim et al., 1987). Especially, little is known about the presence and nature of angiotensin I-converting enzyme in this animal groups except for the few studies (Stephens, 1981; Stephens and Creekmore, 1984; Cipolle and Zehr, 1984; Fernandez-Pardal et al., 1986; Cho et al., 1987b).

The present study was undertaken to characterize and to determine the distribution of angiotensin I-converting enzyme activity in plasma and various tissues of the rice eel, *Monopterus albus*.

## Materials and Methods

Blood samples were obtained from healthy rice eel, *Monopterus albus*, Sprague-Dawley rats and human subjects. Blood in heparinized tubes was centrifuged for 15 min at 3,000 rpm at 4°C, and plasma was stored at −20°C until assayed. Tissues were obtained from the rice eel by open surgery. Tissue homogenates were made by grinding the tissue in Polytron homogenizer, and gently

stirred for 1 hr in the presence of 0.5% Nonidet-P40. Homogenates were centrifuged for 5 min at 13,000 × g and the supernatants were stored at −20 C. ACE activity was determined by the method of Santos et al. (1985) with modifications. Ten microliters of plasma and tissue extracts were incubated with 490 μl of assay solution containing 5 mM Hip-His-Leu in 0.4 M sodium borate buffer for 15 min at 37 C. The reaction was stopped by the addition of 1.2 ml of 0.34 N NaOH. To form the fluorescent product, 100 μl of *O*-phthaldialdehyde reagent was added to each tube and mixed. Exactly 10 min later the reaction was terminated by the addition of 200 μl of 3 N HCl and the tube contents were mixed thoroughly. To eliminate the precipitation of a presumptive protein-*O*-phthaldialdehyde complex, the tube was centrifuged for 10 min at room temperature. The final product, His-Leu, was fluorometrically measured at 365 nm excitation and 495 nm emission by the spectrofluorometer.

## Results

### Effects of pH on ACE Activity

Plasma samples from the rice eel showed a peak activity at around pH 10, while peak activities of ACE in plasma samples from rats and humans were shown at pH 8.5 and 8.3, respectively (Fig. 1).

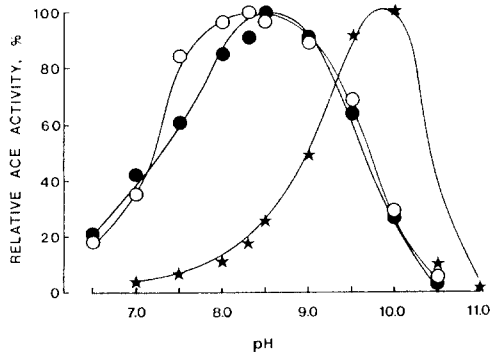


**Fig. 1.** Effects of pH on ACE activity in plasma samples from the rice eel (★—★), rats (●—●) and humans (○—○). Samples were incubated at optimal concentrations of chloride ions, i.e. 0.2, 1.2 and 0.6 M for the rice eel, rats and humans, respectively.

### Effects of Chloride and Cobalt Ions on ACE Activity

Plasma samples from rice eel and mammals showed the dependence of ACE activity on chloride ions (Fig. 2). With increasing chloride concentrations, the enzyme activities were increased and reached a maximum at 0.2 M chloride in the rice eel, at 0.6 M in human, and at 1.2 M in rats. Higher chloride concentrations were required for the optimal ACE activity for the samples from the human and rat than those from the rice eel (fig. 2). Influence of the cobalt ion on ACE activity in the plasma of the rice eel was different from that

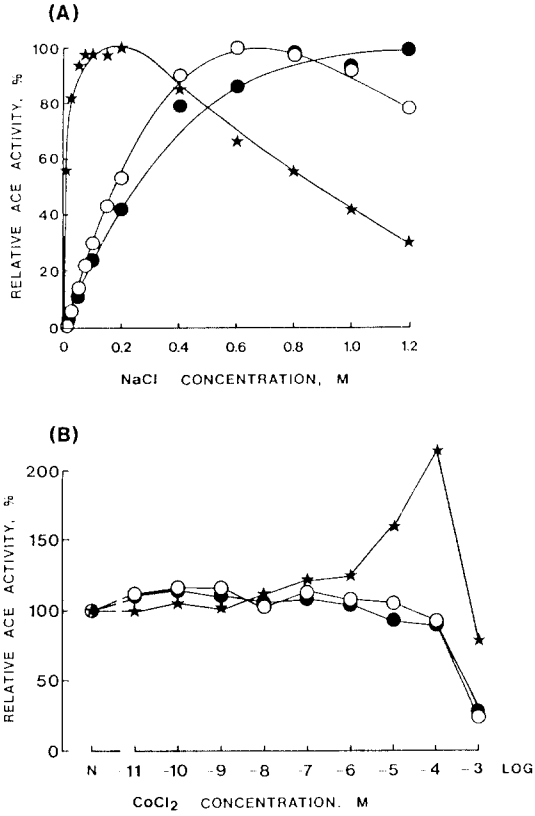


**Fig. 2.** Effects of chloride and cobalt ions on ACE activity in plasma samples from the rice eel (★—★), rats (●—●) and humans (○—○). Samples were incubated at the optimal buffer pH, i.e. 10, 8.5 and 8.3 for the rice eel, rats and humans, respectively. Panel A, effect of NaCl on enzyme activity; Panel B, effect of $CoCl_2$ on enzyme activity.

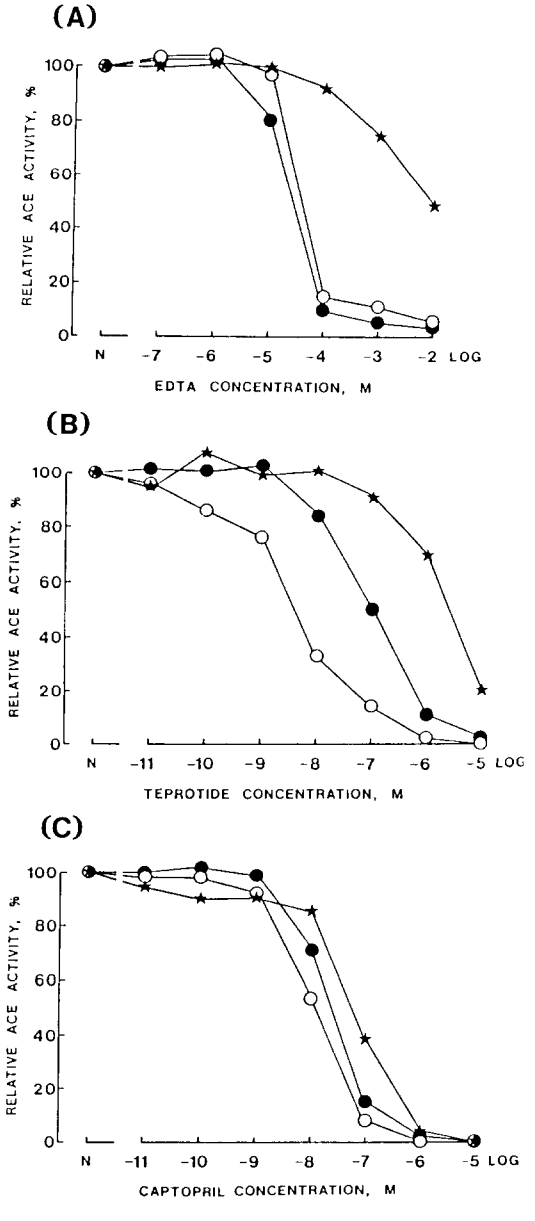


**Fig. 3.** Effects of EDTA, teprotide and captopril on ACE activity in plasma samples from the rice eel (★—★), rats (●—●) and humans (○—○). Samples were incubated under optimal conditions of buffer as described in Figs. 1 and 2. Panel A, effect of EDTA on enzyme activity; Panel B, effect of teprotide on enzyme activity; Panel C, effect of captopril on enzyme activity.

in the two mammalian subjects. Plasma samples from the human and rats showed little change in ACE activity with low concentrations of cobalt ion, and a sudden decrease of the activity at $10^{-3}$ molar concentration. Plasma samples from the rice eel, however, showed a 100% increase in ACE activity over the control value at $10^{-4}$ molar concentrations of cobalt ion, and a decease at higher concentrations ($10^{-3}$ M).

### Effects of EDTA, Captopril and Teprotide on ACE Activity

Ethylene diamine tetraacetic acid (EDTA), as a chelating agent, and captopril (SQ 14,225) and teprotide (SQ 20,881), as well known site-directed ACE inhibitors, showed dose-dependent inhibition of ACE activity in plasma samples from the rice eel as well as rats and human subjects (Fig. 3). Even though the dose dependencies for inhibition were similar, the susceptibility was less in plasma samples from the rice eel than from the mammals.

### Thermal Stability on ACE Activity

When plasma samples were denatured at high temperature to compare the thermal stability, all samples were relatively stable at 45°C. With increasing temperature and time of incubation, however, ACE activity in the rice eel was less stable than in mammals (Fig. 4).

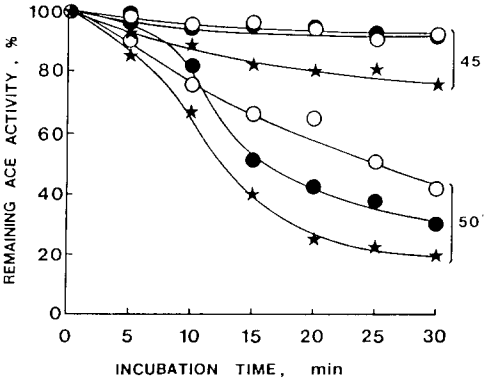


**Fig. 4.** Comparison of thermal stability of ACE activity in plasma samples from the rice eel (★—★), rats (●—●) and humans (○—○). Preincubations at 45°C and 50°C were terminated by placing test tubes in an ice water bath. Enzyme reactions were then initiated by the addition of NaCl and the substrate under optimal conditions as described Figs. 1 and 2.

**Distribution of ACE Activity in Various Tissues of the Rice Eel**

The ACE activities in plasma and crude homogenates of various tissues are represented in Figures 5 and 6. The plasma ACE activity in the rice eel was calculated to be 113.24 ± 11.54 nM His-Leu/min/ml. The highest ACE activity among the various tissues in the rice eel was detected in the brain as 2.39 ± 1.05 nM His-Leu/min/mg of wet tissue.

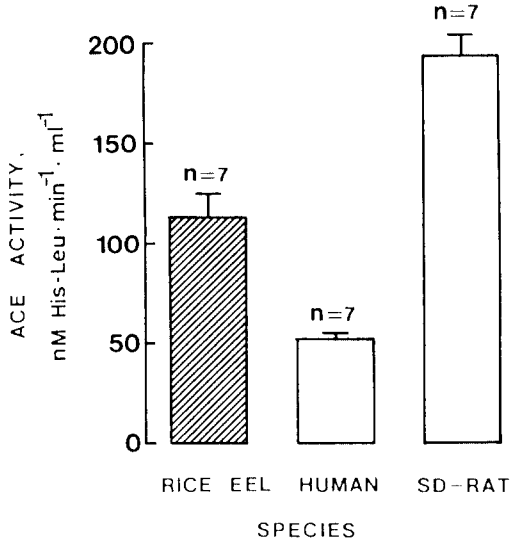


**Fig. 5.** Comparison of ACE activity in plasma samples from the rice eel, rats and humans. Samples were incubated under optimal conditions of buffer as described in Figs. 1 and 2.

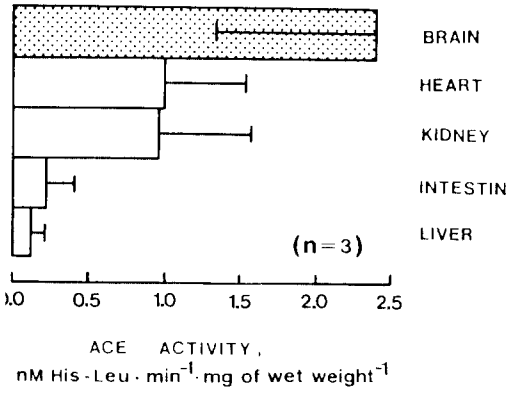


**Fig. 6.** ACE activity in crude homogenates of various tissues of the rice eel, *Monopterus albus*. Samples were incubated under optimal conditions of buffer as described in Figs. 1 and 2.

## Discussion

Our results provide the presence and comparative characteristics of plasma ACE activity and the uneven distribution of ACE activity in various tissues of the rice eel, *Monopterus albus*. The most striking fact of our results obtained is the very high ACE activity in the brain from the rice eel. A positive correlation has been reported to exist in some areas of the brain between ACE activity and other components of the renin-angiotensin system (Chevillard and Saavedra, 1982), implying a physiological role for ACE in the generation of angiotensin II in these areas. In the rice eel, the role of the high ACE activity in the brain for the renin-angiotensin system is still unknown.

We also obtained the interesting results in the physical properties of plasma ACE activity. The pH range for peak activity of ACE in plasma of mammals was similar to those reported by others (Bünning et al., 1983; Maguire and Price, 1984), but that was much more alkaline in the rice eel than in mammals. It was well known that ACE requires an anion, especially chloride, for the enzyme activity. Chloride activation is a characteristic feature of ACE. It was one of the earliest properties of the enzyme recognized, and served as a means to differentiate ACE from other peptidyldipeptide hydrolases (Gorenstein and Snyder, 1979). In spite of its obvious significance, chloride activation of ACE has not been the subject of extensive investigation. Bünning and Riordan (1983) reported that, in the alkaline pH range, increasing anion concentrations decreases the enzyme activity. It is of interest to note such a low optimal chloride concentration as in the present experiment.

Inhibition of the ACE activity by EDTA, teprotide and captopril was very similar to the results obtained by other worker (Maguire and Price, 1984). These findings suggest that the catalytic active site for the enzyme activity in plasma samples from the rice eel is very similar to that of mammals. Velletri et al. (1985) have reported that cobalt can stimulate ACE activity, and that this activation may be the result of an exchange of

cobalt with the naturally occurring zinc to form a more active holoenzyme. Over 100% of ACE activity in the rice eel was increased at 10 $^{4}$M $CoCl_2$, whereas the activity in mammals was scarcely increased. The data were consistent with the previous report (Cho et al., 1987b). Differences in the response of each enzyme activity to cobalt may be related to the specific interaction of zinc with the structures of the enzymes. Velletri et al. (1985) have reported that zinc may play an important role in the maintenance of the thermal stability of ACE. It was shown in the present experiment that the enzyme is unstable compared to that of mammals in thermal stability. The interpretation of this finding cannot be addressed in this paper, and is the subject of further investigation.

## Acknowledgements

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## 드렁허리(*Monopterus albus*)의 Angiotensin I-Converting Enzyme의 특성에 관하여

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드렁허리의 혈장에서 얻은 angiotensin I-converting enzyme(ACE)의 활성도를 포유동물과 비교하였다. 드렁허리의 혈장내 ACE는 pH 10에서 가장 높은 활성도를 나타냈으며 포유동물에서 보다 알칼리성이었다. 또한 최적활성도를 나타내는 데 필요한 Cl 이온의 요구성은 종에 따라 다르게 나타났다. 드렁허리의 ACE 활성도는 ACE의 활성억제제인 EDTA, teprotide 및 captopril에 의해서 이들의 농도에 따라 억제되었으며, 이 억제현상은 포유동물의 혈장 ACE와 매우 비슷하였다. 드렁허리의 ACE 활성도는 cobalt에 의하여 증가되었으며, 포유동물의 경우에 비해서 열에 불안정하였다. 또한 여러 조직 중에서는 뇌에서 가장 높은 ACE 활성도가 측정되었다.