COMPARISON OF RED CELL ANGIOTENSINASE ACTIVITY IN MAN AND ANIMALS

AMINKHAN ALADIN, HIROSHI AKUTSU, EINOSUKE UEDA,
TATSUO KOKUBU AND YUICHI YAMAMURA

Various mamalian red cell angiotensinase activities and their apparent K_m values were compared. The strongest angiotensinase activity was found in the human red cells. Aminopeptidase activity and the angiotensinase activity not being the same suggested the presence of one other angiotensin inactivating enzyme besides aminopeptidase.

THE PATHOGENESIS of hypertension, especially of renal hypertension, on the basis of the renin-angiotensin system has been studied by many investigators in recent years. Angiotensin II which has a pressor activity is inactivated by various polypeptidases named angiotensinases.

Kokubu and co-workers from this laboratory previously reported the presence of an angiotensin II inactivating enzyme, so called angiotensinase, in the human and rabbit red cells.¹ The present studies were conducted to compare the activities of the enzymes, angiotensinases, in the human red cells with those of the rabbit, dog and pig red cells. The apparent K_m of the enzymes from these red cells and also their aminopeptidase activities using L-leucyl- β -naphthylamide as a substrate, so called arylamidase activity, were compared.

METHOD

The angiotensinase activity was determined by bioassay. In each case the enzyme material was incubated with a constant amount of (α -L-Asp¹, Ile⁵)-angiotensin II [Inst. of Protein Research, Osaka University] in vitro under the conditions mentioned below. Following incubation the enzyme reaction was stopped by placing the

sample in a boiling water bath for ten minutes. Pressor activity of the supernatant was tested on the blood pressure of a rat. A rat weighing 170–180 gms. was vagotamized, anesthetized by injecting pentobarbitone sodium 50 mg/Kg., intraperitoneally, and given pentolinium tartrate 10 mg/Kg. subcutaneously. The pressor response of the unknown solution was bracketed between known amounts of synthetic angiotensin II used as a substrate. The amount of angiotensin inactivated (ng.) was used as a indicator for the

Fig.1. Lineweaver-Burk plot of the relationship between the reciprocals of substrate concentrations and reaction velocity. Human Red Cell hemolysate (1 : 10) $\times$ 2 was incubated with different concentrations of substrate, (10 μ g, 20 μ g, 30 μ g, 50 μ g) at 37°C for 30 mins (pH 6.8).

Key Words:

Red cell angiotensinase activity-
Aminopeptidase activity-renin
angiotensin system

(Received for publication: May 18, 1973)

3rd Department of Internal Medicine, Osaka University Medical School, Fukushima-ku,
Osaka Japan

TABLE I ANGIOTENSINASE AND ARYLAMIDASE ACTIVITIES IN HUMAN, RABBIT, DOG AND PIG RED CELLS

Material	Protein amount mg/ml	Specific activity of L-leucyl- β -naphthylamidase $\mu\text{g}/\text{mg protein/h}$	Specific activity of angiotensinase $\mu\text{g}/\text{mg protein/h}$	Apparent Km
Human red cells	359	1.96	1.002	$4.3 \times 10^{-5} \text{M}$
Rabbit	603	5.4	0.7	$2 \times 10^{-4} \text{M}$
Dog	373.5	1.6	0.39	$4 \times 10^{-4} \text{M}$
Pig	390	1.7	0.42	$2.2 \times 10^{-4} \text{M}$

angiotensinase activity.

The enzyme material was usually incubated, with 1.0 μg of synthetic angiotensin II and 0.5 ml of 0.01 M phosphate buffer (pH 6.8) plus saline to bring the final volume to 2 ml, for 30 min. at 37°C.

The enzyme material was prepared by washing normal red cells of the animals mentioned, including human red cells, with saline, hemolysis by freezing and dilution (1:10) with distilled water. The preparations were centrifuged for 15 minutes at 15000 $\times$ g and each supernatant was used for the experiment.

The arylamidase activity was determined by Goldbarg's method² using L-leucyl- β -naphthylamide as a substrate.

Protein concentration was measured according to Lowry et al.³

The Km was determined, in each case, by incubating a constant amount of the enzyme material with varying substrate amounts, (10 μg , 20 μg , 30 μg , 50 μg .) in vitro under the conditions mentioned below. Following incubation the enzyme reaction was stopped by placing the sample in a boiling water bath for 10 minutes. The pressor activity of the remaining angiotensin II was tested on the blood pressure of a rat using exactly the same method as the one used in the determination of the angiotensinase activity. Varying concentrations of angiotensin II inactivated by a constant amount of the enzyme material were used in determining the Km by the Lineweaver-Burk⁴ plotting method.

The enzyme material was usually incubated

with varying amounts (10 μg , 20 μg , 30 μg , 50 μg .) of the substrate and 0.4 ml of the 0.01 M phosphate buffer (pH 6.8) plus saline to bring the final volume to 2 ml, for 15 minutes at 37°C.

RESULTS AND DISCUSSION

The results show (Table I) that the specific activity of human red cell angiotensinase is 30% higher than that of the rabbit red cell angiotensinase and 50% higher than either those of the pig or dog red cell angiotensinases.

The fact that the specific activities of the enzymes, angiotensinase and L-leucyl- β -naphthylamidase are not same suggests the possibility of the presence of at least one other angiotensin inactivating enzyme in the red cells besides the aminopeptidase. Table 1 gives the apparent Km of angiotensinases from different red cells while Fig. 1 shows the Km of human red cell angiotensinase. The Km of the human red cell angiotensinase being the smallest suggests that the red cell angiotensinase has the highest affinity for angiotensin.

REFERENCES

1. KOKUBU, T., AKUTSU, H., FUJIMOTO, S., UEDA, E., HIWADA, K., & YAMAMURA, Y.: *Biochem. Biophys. Acta.* **191**: 668, 1969.
2. GOLDBARG, J. A., PINEDA, E. P., & RUTENBURG, A. M.: *Am. J. Clin. Path.* **32**: 571, 1959.
3. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. W., & RANDALL, R. J.: *J. Biol. Chem.* **193**: 265, 1951.
4. LINEWEAVER, H. & BURK, D.: *J. Am. Chem. Soc.* **56**: 658, 1934.