

Renin-like Activity in the Plasma and Kidney of a Snake, *Elaphe quadrivirgata*

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Plasma of *Elaphe quadrivirgata* was incubated *in vitro* under various conditions, and angiotensin I produced was estimated. Renin-like activity deduced from the amount of angiotensin I formation was higher at 37° than at 20° or 4°. The enzyme activity in the snake was higher at pH 4.5 than at 5.0, while that in mammals is highest at pH 5.0. The activity was markedly reduced in winter during hibernation, compared with that in summer. Among the organs renin-like activity was found only in the kidney.

Thus, the renin-like active enzyme present in the plasma of *Elaphe quadrivirgata* may originate mainly from the kidney. The enzyme activity depends on the incubation conditions, and the season of sample collection.

Keywords—renin-like; renin-angiotensin; radioimmunoassay; angiotensin I; snake body constituents

It is widely recognized that many hormone-like substances are derived from plasma proteins in animals, and these substances play important physiological and pathological roles. Renin-angiotensin is one of such systems. Recently, it was reported that angiotensin I-converting enzyme (converting angiotensin I to angiotensin II) is the same enzyme as kininase II (inactivating kinin).²⁾ These systems may be involved in maintaining homeostasis in the animal body.

Potent kinin-releasing enzymes are known to be present in snake venoms, but the renin-angiotensin system has not yet been detected. However, Nakajima and co-workers have recently carried out structure determination of non-mammalian angiotensin, and a new angiotensin-like substance was reported to be present in *Elaphe climacophora*, a non-poisonous snake.³⁾

We therefore investigated whether a renin-angiotensin system is functioning in the snake body.

Methods

Non-poisonous snakes, *Elaphe quadrivirgata*, were obtained from a snake farm. Angiotensin I, produced by renin-like enzymes when angiotensinase and angiotensin I converting enzyme are inactivated,^{4,5)} was estimated by radioimmunoassay, and the amount of renin-like substances in the plasma and in various organs was determined.

Blood was collected from the snake neck into a vacuum tube (Thermo Co.) containing EDTA-2Na, and the liver, kidney, seminal vesicle and ovarium were obtained at the same time. All the samples collected were immediately ice-cooled. Blood was centrifuged at 3000 rpm for 30 min in a refrigerated centrifuge. Inhibitor (1 ml) to angiotensinase and angiotensin I-converting enzyme was added to 1 ml of the plasma supernatant.^{4,5)} The inhibitor consisted of 8-hydroxyquinoline, dimercaprol and Tween 20 dissolved in 0.2 M acetate buffer, pH 5.0. In control experiments, 0.2 M acetate buffer, pH 4.5, was used instead of the inhibitor solution.

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The excised organs were minced, and 5 ml of the inhibitor solution was added per 1 g wet weight of each organ.⁶⁾ The organ samples and the plasma were incubated at the indicated temperatures for the indicated times. After incubation, the reaction mixture was heated in a boiling water bath for 10 min to remove protein, then after cooling, the mixture was centrifuged at 10000 rpm for 10 min under refrigeration. An aliquot of the supernatant solution, 0.1 ml, was taken for radioimmunoassay of angiotensin I. A control experiment (zero time value) was performed by incubating the reaction mixture at 0° under the same conditions (Fig. 1).

Estimation of angiotensin I was performed by radioimmunoassay with the Renin RIA Kit (Dainabott Labs.). The supernatant (0.1 ml) was treated with 0.5 ml of 0.1 M Tris-acetate buffer, pH 7.4, containing 1% lysozyme and 0.01% thimerosal, 0.1 ml of ¹²⁵I-angiotensin I solution and 0.1 ml of human angiotensin I antibody. The mixture was allowed to stand at 4° for 18 hr, and then 0.2 ml of dextran-charcoal suspension was added, and after shaking for 15 min, the mixture was again allowed to stand at 4° for 20 min. The fraction precipitated by centrifugation at 3000 rpm for 20 min was subjected to radioactivity determination in a well-type scintillation counter. In parallel experiments to obtain a calibration curve for angiotensin I assay, 0.1 ml of angiotensin I solution containing 8, 4, 2, 1, or 0.5 ng/ml was transferred into separate test tubes, then 0.1 ml of angiotensin I-free serum (for calibration), 0.4 ml of 0.1 M Tris-acetate buffer, pH 7.4, and 0.1 ml of ¹²⁵I-angiotensin I solution and 0.1 ml of angiotensin I antibody were added to each tube. The mixture was incubated at 4° for 18 hr, and treated as described above. In order to determine total radioactivity, 0.1 ml of ¹²⁵I-angiotensin I solution was mixed with 0.7 ml of 0.1 M Tris-acetate buffer, pH 7.4, and the radioactivity was measured.

Calculation

Background radioactivity was subtracted from the total and sample radioactivities, and the sample radioactivity was divided by the total radioactivity to obtain the percentage of free fraction (precipitate). Using a calibration curve obtained by plotting the content of angiotensin I generation against radioactivity in the precipitate, angiotensin I contents in sample were determined. The amount of angiotensin I formed in 1 hr (and 2 and 3 hrs in the case of the kidney) per 1 ml of plasma or 1 g wet weight of organs was converted to renin-like activity in the plasma or in organs.

Results

The calibration curve of angiotensin I was linear in the range of 0.5 to 4 ng/ml of angiotensin I. The time courses of angiotensin I generation on incubation of the plasma are shown in Fig. 1. The physiological condition of the snake is one of the factors affecting the plasma level of renin-like activity; that is, when the snake blood was collected in the summer (June to July) the renin-like activity was higher than that in blood collected in the winter (November to December). Release of angiotensin I was measured at various pH's and at various temperatures. Renin-like activity was higher on incubation at pH 4.5 than at 5.0, and higher at 37° than at 20° or 4°. Renin-like activity was higher when the inhibitor solution containing inhibitors for angiotensinase and angiotensin-converting enzyme was added (Table I). Various organs were incubated in order to examine angiotensin I formation, if any. Renin-like activity was evident in the kidney (0 ng·g wet wt/1 hr, 1.5 ng/g wet wt/2 hr, and 5.7 ng/g wet wt/3

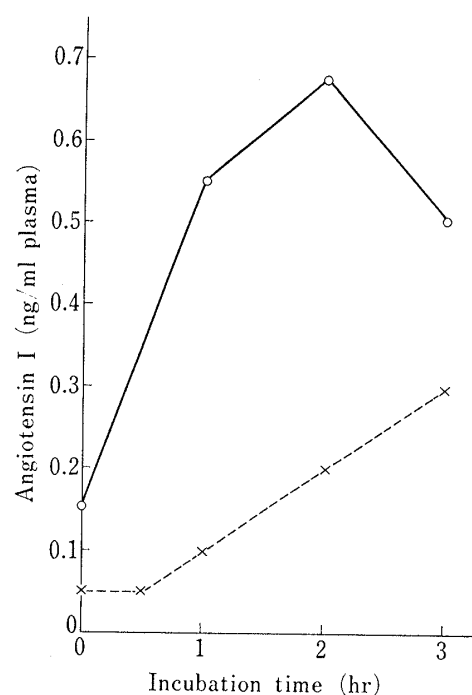


Fig. 1. Rate of Immunoreactive Angiotensin I Generation in the Snake Plasma

—○—: angiotensin I level in summer,
 ---×---: angiotensin I level in winter.
 Incubated at 37°, pH 5.0.

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TABLE I. Snake Plasma Renin-like Activity produced Under Various Conditions

Season of collection	Conditions of incubation		Angiotensin I ^{a)} (ng/ml/hr)
	Temp (C°)	pH	
Summer (June—July)	37	4.5	6.67
	37	5.0	0.50
	20	4.5	5.17
	20	5.0	0.90
Winter (November—December)	37	4.5	0.45
	37	5.0	0.10
	20	4.5	0.24
	20	5.0	0.05
	4	5.0	0.02
	37	4.5 ^{b)}	0.05

Blood was collected in summer and in winter to determine the seasonal change of the activity.

a) Renin-like activity in plasma expressed in terms of angiotensin I formed.

b) Incubated without inhibitor.

hr), while other organs were inactive. Thus, in the snake, renin-like activity is considered to originate from the kidney, as in the mammals.

Discussion

Renin-like activity was found in the plasma of a non-poisonous snake, *Elaphe quadrivirgata*. Release of angiotensin I was determined as an index of the renin-like activity. The activity depended on the incubation conditions and the season of sample collection. The renin-like activity in the snake plasma may cause an angiotensin-like substance to be generated on *in vitro* incubation; it is immunologically cross-reacting with human angiotensin I antibody. The amount of renin-like activity was reduced when incubation was performed without adding inhibitor solution. This suggests that the angiotensin I produced is converted to angiotensin II or decomposed.

It appears that the snake renin-like activity is very similar to that of man. The snake renin-like activity is higher at pH 4.5 than at pH 5.0, unlike the renin activity in human (pH 6.5 to 5.5).^{7,8)} The renin-like activity in the snake is considerably lower during the period of hibernation. The activity was found only in the kidney of the snake, as is the case in mammals.

The renin-angiotensin system or a similar system may have a homeostatic control function in the snake body.

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