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Inhibitors of Aminopeptidase B Suppress the Development of Hypertension in Spontaneously Hypertensive Rats

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In order to confirm the pathophysiological significance of the dissociation between the plasma levels of aminopeptidases A and B in spontaneously hypertensive rats, we tested the effects of several enzyme inhibitors including inhibitors of aminopeptidase B on the course of hypertension in these rats. In addition to an inhibitor of angiotensin-converting enzyme, inhibitors of aminopeptidase B (arphamenine B and bestatin) significantly suppressed the development of hypertension in these animals. This finding may represent an important clue to the pathogenesis of essential hypertension.

Keywords—aminopeptidase B; inhibitor; hypertension; spontaneously hypertensive rat

Our previous study showed that there is a dissociation between the plasma levels of aminopeptidases A (AP-A) and B (AP-B) in spontaneously hypertensive rats (SHR).¹⁾ Although the plasma level of AP-A showed an age-dependent decrease, such was not the case for AP-B in these animals. This observation suggested that some derangement of peptide metabolism in SHR may be related to the development of hypertension in this animal model. In order to examine this possibility, we tested the effects of chronic administration of inhibitors of AP-B (arphamenine B and bestatin) on the development of age-dependent hypertension in SHR. These agents do not show any hypotensive effect when administered to normal rats.

Materials and Methods

Experimental Animals—SHRs (male, 6 weeks of age) were obtained from Charles River Japan Inc., Atsugi, Kanagawa, Japan. They were kept on a pellet diet *ad libitum*. Three hours after the administration of inhibitors, the systolic blood pressure was measured indirectly from the caudal artery and recorded with an automatic blood pressure recording device (model USM-105-R, Ueda Electric Works Ltd., Tokyo, Japan) by the tail-pulse-pickup method. Rats were warmed at 38 °C for about five min prior to the measurement of blood pressure in order to induce sufficient blood flow in the tail artery.

Inhibitors—The inhibitors and their targets used in this study were arphamenine B for AP-B²⁾; bestatin for AP-B, Leu-AP, and triaminopeptidase³⁾; pepstatin for pepsin, cathepsin D, chymosin, and renin,^{4,5)} and foroxymithine for angiotensin-converting enzyme (ACE).⁶⁾ The structures and inhibitory activities of these inhibitors are shown in Table I. Arphamenine B, bestatin and foroxymithine were dissolved in 1% Gum Arabic solution, and given daily orally, while pepstatin was dissolved in the same solution, and given daily intraperitoneally at the following dose levels; arphamenine B 30 mg/kg, bestatin 30 mg/kg, pepstatin 3 mg/kg, and foroxymithine 30 mg/kg. The control animals were given the same volume of 1% Gum Arabic solution daily for 15 d. The doses of the inhibitors used were less than 1/20 of LD₅₀, and no apparent toxicity was seen at these doses.

Determination of Enzyme Activities—For the assay of AP-B and Leu-AP, the reaction mixture was prepared by mixing 0.25 ml of 2 mM L-glutamic acid β -naphthylamide hydrochloride (Glu·NA) or Leu·NA, respectively,

0.65 ml of phosphate-buffered saline (PBS, pH 7.2) and 0.1 ml of AP-B or Leu-AP in a series of test tubes. After the reaction, the solutions were processed as described previously,^{2,3)} and the absorbances at 525 nm were determined. For the assay of renin, the reaction mixture was prepared by mixing 0.5 ml of 0.06% ³H-Val nonapeptide, 0.3 ml of 0.05 M phosphate buffer containing 0.05% polyvinylpyrrolidone, (pH 7.5), 0.1 ml of distilled water and 0.1 ml of hog kidney renin. After the reaction, the solutions were processed as described previously.⁵⁾

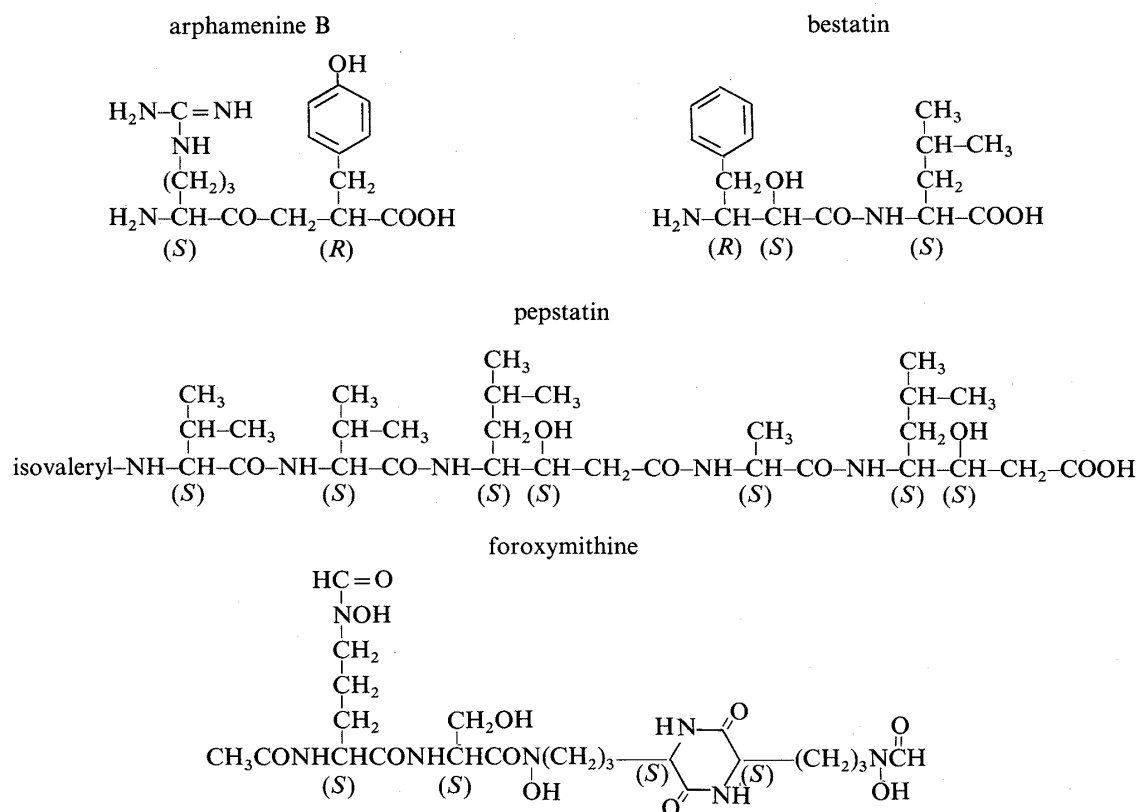
For the assay of carboxypeptidase A (CP-A)⁷⁾ or ACE,⁶⁾ the reaction mixture was prepared by mixing 0.05 ml of 10 mM hippuryl-L-phenylalanine or 12 mM hippuryl-L-histidyl-L-leucine with 0.25 ml of 0.05 M Tris-HCl buffer containing 0.9 M NaCl (pH 8.0), 0.15 ml of distilled water and 0.05 ml of CP-A or ACE. After the reaction, the solutions were processed as described previously, and the absorbances at 382 nm were determined.

Results and Discussion

Table I shows the *in vitro* actions of arphamenine B, bestatin, pepstatin and foroxymithine. Arphamenine B inhibits AP-B strongly and CP-A does so moderately, while bestatin inhibits AP-B and Leu-AP strongly. Pepstatin and foroxymithine inhibit renin and ACE, respectively.

Figure 1 shows the effects of daily injection of these inhibitors on age-related changes in

TABLE I. Inhibitory Activities of Arphamenine B, Bestatin, Pepstatin and Foroxymithine against Various Enzymes



Enzyme	IC ₅₀ (μg/ml)			
	Arphamenine B	Bestatin	Pepstatin	Foroxymithine
AP-B	0.002	0.05	> 100	> 100
Leu-AP	> 100	0.01	> 100	> 100
Renin	> 100	> 100	4.5	> 100
CP-A	5.2	> 100	> 100	> 100
ACE	> 100	> 100	> 100	7.0

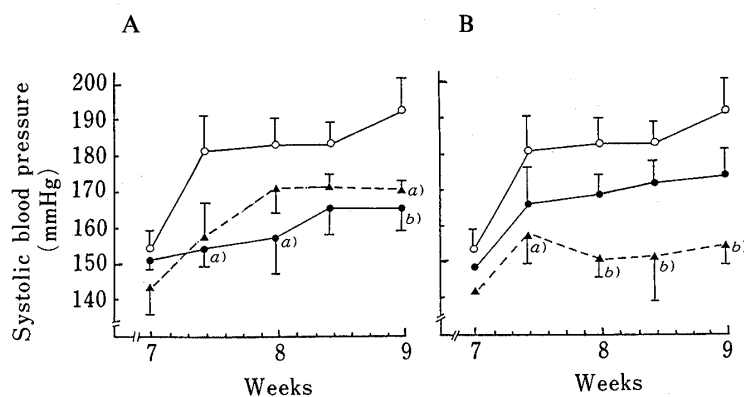


Fig. 1. Suppressive Effects of Several Enzyme Inhibitors on the Blood Pressure in SHR

A. ○—○, control; ●—●, bestatin (30 mg/kg); ▲—▲, arphamenine B (30 mg/kg).
 B. ○—○, control; ●—●, pepstatin (3 mg/kg); ▲—▲, foroxymithine (30 mg/kg).

The statistical significance of the difference between the controls and the treated animals is indicated as follows. a) $p < 0.05$, b) $p < 0.01$.

blood pressure in SHR. As can be seen, the blood pressure in the control animals gradually increased with age, showing an increase of 40 mmHg at 9 weeks of age. It is a matter of course that foroxymithine, an ACE inhibitor, showed the most striking hypotensive effect. However, it is noteworthy that bestatin also significantly suppressed the development of hypertension. Arphamenine B also tended to suppress the blood pressure, but a statistically significant effect was seen only at the age of 9 weeks. The effect of pepstatin, which is an inhibitor of renin, was not significant. This was possibly due to the fact that the absorption of this agent was not complete because of its insufficient solubility.

The observation that bestatin and arphamenine B showed significant hypotensive effects in SHR favors the possibility that the increase in the plasma level of AP-B, which was found in SHR in our previous study, really plays a pathogenetic role.¹⁾ The stronger effect of bestatin, as compared with that of arphamenine B may be taken to indicate that not only AP-B but also Leu-AP and triaminopeptidase are somehow associated with the development of hypertension in SHR. Regardless of the precise mechanisms involved, the present results may warrant further investigation of the peptide metabolism in this model of hypertension.

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