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Demonstration of the *in vivo* action of pepstatin: effects on plasma angiotensin II concentration*

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PEPSTATIN, an *N*-acylated pentapeptide obtained from culture filtrates of actinomycetes, was originally characterized as an inhibitor of pepsin and other acid proteases.[1] Subsequently, we[2,3] and others[4] have demonstrated that pepstatin inhibits the reaction of the "neutral" protease renin with its plasma substrate. The fall in blood pressure induced by pepstatin in certain experimental conditions in the rat may be a consequence of such an inhibition. While it has been clearly demonstrated that pepstatin is a true enzymatic inhibitor of renin *in vitro*,[4]† no studies to more precisely define its mechanism of action *in vivo* have been undertaken. In the present paper, we report on experiments which show that, in the rat, pepstatin diminished the formation of angiotensin during the infusion of renin.

TABLE 1. EFFECTS OF PEPSTATIN ON PLASMA ANGIOTENSIN II CONCENTRATION IN NEPHRECTOMIZED RATS GIVEN A SINGLE INJECTION OF 0·05 DOG UNITS HOG RENIN

		Blood pressure (mm Hg)				
	n	Before renin	At 1st blood sampling	Before pepstatin or saline	After pepstatin or saline	BP
Pepstatin	10	113 ± 4	136 ± 4	118 ± 4	109 ± 4	−9* ± 31
		N.S.	N.S.	N.S.	$P < 0{\cdot}03$	$P < 0{\cdot}01$
Control	10	112 ± 5	139 ± 5	120 ± 4	120 ± 4	
		Angiotensin II plasma concentration (pg/ml)				
Pepstatin	10		190 ± 8		63 ± 7	
			N.S.		$P < 0{\cdot}0005$	
Control	10		208 ± 11		124 ± 12	

Values are means ± S.E.M.
* $P < 0{\cdot}001$ as compared with BP values before pepstatin injection, paired data.
N.S. = not significant.

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TABLE 2. EFFECTS OF PEPSTATIN ON PLASMA ANGIOTENSIN II CONCENTRATION IN NEPHRECTOMIZED RATS INFUSED WITH 0·05 DOG UNITS HOG RENIN/min

		Blood pressure (mm Hg)							
	n	Before renin	At 1st blood sampling	Before 1st pepstatin injection	At blood sampling after 1st pepstatin (1 mg/kg)	Δ BP due to pepstatin or saline	Before 2nd pepstatin injection	At blood sampling after 2nd pepstatin (2 mg/kg)	Δ BP due to pepstatin or saline
Pepstatin	6	104 ±3	125 ±3	126 ±4	115 ±4	−11* ±1	127 ±4	108 ±6	−19* ±2
					N.S.		N.S.	P < 0·05	
Control	6	102 ±4	122 ±4	122 ±4	122 ±4		122 ±4	122 ±4	
		Angiotensin II plasma concentration (pg/ml)							
Pepstatin	6		276 ±37	P < 0·0025	135 ±14	P < 0·025	107 ±12		
			N.S.		P < 0·005		P < 0·0025		
Control	6		218 ±12	N.S.	207 ±17	N.S.	227 ±25		

Values are means ± S.E.M.

* P < 0·001 as compared with BP values before pepstatin injection, paired data.

N.S. = not significant.

Two series of experiments were performed using male Sprague–Dawley rats weighing 190–220 g. All animals were bilaterally nephrectomized under ether anesthesia 18–24 hr before the actual experiments. Subsequently, they were anesthetized with the sodium salt of 5-ethyl-5-(1′-methyl-propyl)-2-thiobarbituric acid (Inactin®), 50 mg/kg intraperitoneally and 50 mg/kg subcutaneously. A tracheotomy was performed, and the right jugular vein, left carotid artery, and right femoral artery were cannulated. Blood pressure was measured from the carotid artery via a Statham P23 pressure transducer connected to a Hitachi Perkin-Elmer servo recorder. In the first series of experiments, 20 animals received 0·05 Dog Units (DU) hog renin intravenously. When the blood pressure reached the plateau (after 1–2 min), 1 ml of blood was rapidly withdrawn from the femoral artery and placed into a cooled tube containing 50 μl 0·125 **M** Na_2EDTA and 0·025 **M** *o*-phenanthroline. To compensate for volume loss, 1 ml of 2% polyvinylpyrollidone (Periston-N®) in 0·9% NaCl was injected slowly via the jugular vein. Six minutes later, when the blood pressure had again stabilized, 10 animals were given 1 mg pepstatin/kg body wt i.v. Blood pressure began to decline within 30 sec and reached a minimum after 1–2 min, at which time an additional 1 ml blood sample was taken. The other ten animals received 0·3 ml 0·9% NaCl i.v. Blood samples were similarly obtained from the femoral artery.

In the second series of experiments, the animals were infused with hog renin via a catheter in the femoral vein at a rate of 0·005 DU/min (0·01 ml/min). This rate of infusion, in preliminary experiments, was found to increase blood pressure by approximately 20 mm Hg above normal (comparable with the increase in the first experimental series) and to produce plasma angiotensin concentrations similar to those seen in intact anesthetized rats. After the blood pressure had reached a plateau and stabilized, the renin infusion was continued for 10 min. Then, 1 ml of blood was withdrawn from the femoral artery and placed into cooled tubes containing the EDTA–phenanthroline solution, as above. To compensate for volume loss, 1 ml of heparinized whole blood collected from a nephrectomized donor rat was slowly injected via the jugular vein. Again, after the blood pressure had stabilized, the infusion was continued for 10 min. Subsequently, pepstatin (1 mg/kg body wt) was given intravenously. At the point of maximum pepstatin response, generally 1–3 min after administration of the substance, a 1 ml blood sample was taken, and 1 ml of donor blood was administered. Finally, an additional 10 min stabilization period was allowed, after which 2 mg pepstatin/kg body wt were administered and another 1 ml blood sample taken.

All blood samples were kept on ice, and centrifuged at 0° within 40 min of collection. Plasma samples were stored at −25°. Plasma angiotensin II concentration was determined by radioimmunoassay.[5] Statistical analyses were performed using Student's *t*-test for paired values. Results are expressed in the tables and text as means ± S.E.M.

Blood pressures and plasma angiotensin II concentrations (PAC) from the first experimental series are shown in Table 1. As may be seen, the administration of pepstatin resulted in a significant decrease in PAC as compared with control animals. However, since PAC fell spontaneously in the control group, the second series of experiments was done, in which renin was continuously infused and blood replaced (Table 2). Blood pressure of the animals receiving the renin infusion remained essentially constant throughout the experiment. Similarly, in the control group, no significant changes in PAC occurred. However, the administration of two doses of pepstatin (1 and 2 mg/kg body wt) resulted in a dose-dependent decrease in PAC.

We have previously suggested that pepstatin is an inhibitor of the reaction between renin and its plasma substrate both *in vitro* and *in vivo*.[2,3] Subsequently, we[2,*] and others[4] have shown that pepstatin exerts a specific inhibition of renin activity *in vitro*. As we have commented earlier,[3] the measurement of plasma angiotensin I or II after the administration of pepstatin is the only means of providing proof that pepstatin also interferes with the renin-substrate reaction *in vivo*. In the present communication, we have demonstrated that the administration of pepstatin to renin-infused nephrectomized rats causes a dose-dependent decrease in plasma angiotensin II concentration.

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