regions of the brain. *J. Neurochem.* **13**, 655-669 (1966); 6) HOLZBAUER, M. AND SHARMAN, D.F.: The distribution of catecholamines in vertebrates. Handbook of Experimental Pharmacology, Edited by BLASCHKO, H. AND MASCHOLL, E., Vol. XXXIII, p. 110-133, Springer-Verlag, New York (1972); 7) CATTABENI, F., KOSLOW, S.H. AND COSTA, E.: Gas Chromatography-Mass Fragmentography: A new approach to the estimation of amines and amine turnover. Advances in Biochemical Psychopharmacology, Edited by COSTA, E., IVERSEN, L.L. AND PAOLETTI, R., Vol. 6, p. 37-59, Raven Press, New York (1972); 8) ENGELMAN, K. AND PORTNOY, B.: A sensitive double isotope derivative assay for norepinephrine and epinephrine: Normal resting human plasma levels. *Circulation Res.* **26**, 53-57 (1970); 9) KOSLOW, S.H., CATTABENI, F. AND COSTA, E.: Norepinephrine and dopamine: assay by mass fragmentography in the picomole range. *Science* **176**, 177-180 (1972)

RENIN INHIBITORY EFFECT OF N-ACETYL-PEPSTATIN

Mizuo MIYAZAKI, Hideki OKUNISHI, Tadamitsu KOMORI*
and Kenjiro YAMAMOTO*

*Department of Pharmacology, Shiga University of Medical Sciences, Seta,
Ohtsu, Shiga 520-21, and *Department of Pharmacology, Osaka City
University Medical School, Abeno, Osaka 545, Japan*

Accepted November 20, 1977

It has been demonstrated that pepstatins isolated from streptomyces inhibit the renin activity and among pepstatins and other renin inhibitory substances, pepstatin A, isovaleryl pentapeptide is known as a most potent renin inhibitor (1, 2, 3). The solubility of this compound limits application in many studies. In the present work, the renin inhibitory activity of a soluble acid protease inhibitor, N-acetyl-pepstatin, was demonstrated both *in vitro* and *in vivo*. N-acetyl-pepstatin was found in a culture filtrate of streptomyces naniwaensis by Murao and Satoi (4). The chemical structure is N-acetyl-valyl-valyl-4-amino-3-amino-hydroxy-6-methylheptanyl-alanyl-4-amino-3-amino-hydroxy-6-methylheptanic acid; M.W. 644 (5). The same acetyl pentapeptide was also found from strain MD494-A1 of streptomyces parvisporogenes by Aoyagi et al (6). N-acetyl-pepstatin is soluble in phosphate buffer at pH 7.4 in concentrations higher than 20 mg/ml.

In vitro experiments were carried out using partially purified dog renin and dog renin substrate as described in previous papers (7, 8). One tenth ml of 50 ng angiotensin I equivalent per hour (Ang-I Eq/hr) of renin and 0.2 ml of 200 ng Ang-I Eq of renin substrate were incubated for 1 hr at 37°C with N-acetyl-pepstatin dissolved in 1/3M phosphate buffer (0.2 ml, pH 7.4) containing 8 mM EDTA and 0.02% Neomycin. The angiotensin I formed in this mixture was determined by radioimmunoassay. Inhibitory activity of N-acetyl-pepstatin was expressed as percentage reduction in the production rate of angiotensin I. Average inhibitions of the renin activity by N-acetyl-pepstatin in a concentration of 10^{-6} and 10^{-5} M were $30.9 \pm 7.2\%$ and $64.8 \pm 3.2\%$, respectively ($n=8$), while those with the same concentration of pepstatin A were $49.7 \pm 7.8\%$ and $89.2 \pm 3.7\%$, respectively ($n=6$).

Renin inhibitory effect of N-acetyl-pepstatin *in vivo* was determined by inhibition of

hypertension induced by infusion of renin. Thirty-six male Wistar rats, weighing 481 ± 17 g were anesthetized with pentobarbital sodium (Nembutal, 40 mg/kg, i.p.) and treated with pentolinium tartrate (5 mg/kg, i.v.). Dog renin was infused at a constant rate of 502 ± 61 ng Ang-I Eq·hr/kg·min into right femoral vein. Systemic blood pressure measured from left femoral artery increased by 22.9 ± 1.7 mmHg and was maintained at this level during infusion. N-acetyl-pepstatin was injected i.v. in bolus. The hypotensive effect of N-acetyl-pepstatin is shown in Fig. 1. The systemic blood pressure decreased by 3 mmHg at a dose of 100 μ g/kg ($n=2$), 4.2 ± 1.28 mmHg at 400 μ g/kg ($n=5$), 9.2 ± 1.0 mmHg at 1 mg/kg ($n=10$), 13.5 ± 1.0 mmHg at 2 mg/kg ($n=6$), 17.5 mmHg at 4 mg/kg ($n=2$) and 18.5 mmHg at 6 mg/kg ($n=2$). The maximum hypotensive effect was attained 2 min after injection of a dose of 6 mg/kg and the blood pressure gradually returned to the pre-drug level. The induced hypotension persisted for approximately 10 min.

The effect of N-acetyl-pepstatin was also studied in binephrectomized male Wistar rats ($n=6$). Experiments were commenced 18 to 20 hr after binephrectomy. After pentobarbital sodium and pentolinium tartrate treatment, 400 ng Ang-I Eq·hr/kg of dog renin was injected. Increase in the blood pressure averaged 11.4 ± 0.4 mmHg. After the pressure had returned to the original level, N-acetyl-pepstatin infusion was started. No change of systemic pressure was observed during N-acetyl-pepstatin infusion. During the infusion, the same amount of renin (400 ng Ang-I Eq·hr/kg) was injected. Infusion rate was increased stepwise from 100 to 1000 μ g/kg·min. At a rate of 1000 μ g/kg·min N-acetyl-pepstatin, the pressure increase by renin was 3.6 ± 1.1 mmHg and such was significantly less than the control value of 11.4 ± 0.4 mmHg ($p < 0.001$). The pressor response of renin was restored 30 min after cessation of the N-acetyl-pepstatin infusion. The increase of pressure by the same amount of renin was 9.8 ± 1.2 mmHg. Typical recordings are shown in Fig. 2.

The inhibitory activity of soluble pepstatin, N-acetyl-pepstatin to renin was clearly demonstrated in this study. This soluble renin inhibitor is a valuable compound for the investigation of physiological and pathological roles of the renin angiotensin system. We also have investigated synthetic renin inhibitors of phosphatidylethanolamine and phosphorylethanolamine (8, 9, 10, 11). One of our derivatives, PE-104 was water soluble and

FIG. 1. Dose response relationship of N-acetyl-pepstatin in hypertensive rats induced by infusion of dog renin (502 ± 61 ng Ang-I Eq·hr/kg·min). Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and treated with pentolinium tartrate (5 mg/kg, i.v.). N-acetyl-pepstatin was given i.v. in bolus. Each point is the maximum hypotensive value of the response in different rats.

FIG. 2. Effect of injection of dog renin (400 ng angiotensin I equivalent per hr, i.v.) on systemic pressure in an anesthetized rat treated with pentolinium tartrate (5 mg/kg, i.v.). Pressor responses to renin before and during infusion of different doses of N-acetyl-pepstatin and 30 min after cessation of infusion are presented. Arrows indicate renin injection points. N.A.P.: N-acetyl-pepstatin.

did not inhibit pepsin activity while pepstatins inhibited pepsin and other acid proteases (1). The secondary effect of simultaneously occurring inhibition to other enzymes except renin, *in vivo*, is not clear. Studies with these renin inhibitors of different characteristics in combination are being done in attempts at elucidation.

Acknowledgements: We are grateful to Drs. H. Umezawa and T. Aoyagi, Institute of Microbial Chemistry for kindly providing pepstatin A. Thanks are also due to Drs. S. Igarashi and N. Kuwana, Eisai Co. Ltd. for the gift of N-acetyl-pepstatin.

REFERENCES

- 1) AOYAGI, T., MORISHIMA, H., NISHIZAWA, R., KUNIMOTO, S., TAKEUCHI, T. AND UMEZAWA, H.: Biological activity of pepstatins, pepstanone A and partial peptides on pepsin, cathepsin D and renin. *J. Antibiotics* **25**, 689-694 (1972);
- 2) GROSS, F., LAZAR, J. AND ORTH, H.: Inhibition of the renin-angiotensinogen reaction by pepstatin. *Science* **175**, 656 (1972);
- 3) MILLER, R.P., POPER, C.J., WILSON, C.W. AND DeVITO, E.: Renin inhibition by pepstatin. *Biochem. Pharmacol.*

- 21**, 2941-2944 (1972); 4) MURAO, S. AND SATOI, S.: New pepsin inhibitors (S-PI) from streptomyces EF-44-201. *Agr. Biol. Chem.* **34**, 1265-1267 (1970); 5) FUKUMURA, M., SATOI, S., KUWANA, N. AND MURAO, S.: Structure elucidation of a new pepsin inhibitor (S-PI). *Agr. Biol. Chem.* **35**, 1310-1312 (1970); 6) AOYAGI, T., YAGISAWA, Y., KUMAGAI, M., HAMADA, M., TAKEUCHI, T. AND UMEZAWA, H.: New pepstatins, pepstatins BU, PR and AC produced by streptomyces. *J. Antibiotics* **26**, 539-541 (1973); 7) MORIMOTO, S., YAMAMOTO, K., HORIUCHI, K., TANAKA, H. AND UEDA, J.: A release of renin from dog kidney cortex slices. *Japan. J. Pharmacol.* **20**, 536-545 (1970); 8) HOSOKI, K., MIYAZAKI, M. AND YAMAMOTO, K.: Renin inhibitory effect of 2-[4-(4'-chlorophenoxy)-phenoxyacetylaminol-ethylphosphorylethanolamine (PE-104) in vitro and in vivo, *J. Pharmacol. exp. Ther.* (1977) (in press); 9) MIYAZAKI, M., HOSOKI, K. AND YAMAMOTO, K.: Renin inhibition by synthetic phosphatidyl and phosphorylethanolamines, *Japan. Circulation J.* **40**, 901-910 (1976); 10) MIYAZAKI, M., YAMAMOTO, K. AND HOSOKI, K.: Renin inhibitory effect of synthetic phosphorylethanolamine (PE-104) in the rat. *Clin. Sci. Mol. Med.* **51**, 89s-91s (1976); 11) MIYAZAKI, M. AND YAMAMOTO, K.: Synthetic phosphatidylethanolamines as renin inhibitors. *Proc. Soc. exp. Biol. Med.* **155**, 468-473 (1977)