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14. Supported by PHS grant MH-07230 to J.C. and predoctoral fellowship F-21,996 to G.F. The work, from a dissertation submitted by G.F., was carried out in the Department of Pharmacology, Boston University School of Medicine, Boston, Mass. G.F. thanks Conan Kornetsky for advice on experimental design and for invaluable discussions in the course of the studies.

2 August 1971


## Inhibition of the Renin-Angiotensinogen Reaction by Pepstatin


Abstract. *Pepstatin, an* N*-acylated pentapeptide obtained from culture filtrates of actinomycetes and first characterized as an inhibitor of pepsin, produces inhibition of the renin-substrate reaction both in vitro and in vivo.*


Pepstatin has recently been demonstrated to be a specific inhibitor of acid proteases such as pepsin, casein proctase, and hemoglobin proctase (*1*). The compound has been isolated from culture filtrates of various species of streptomycetes and has been chemically characterized as a pentapeptide, whose structure is (*1, 2*): isovaleryl-L-valyl-L-valyl-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid.

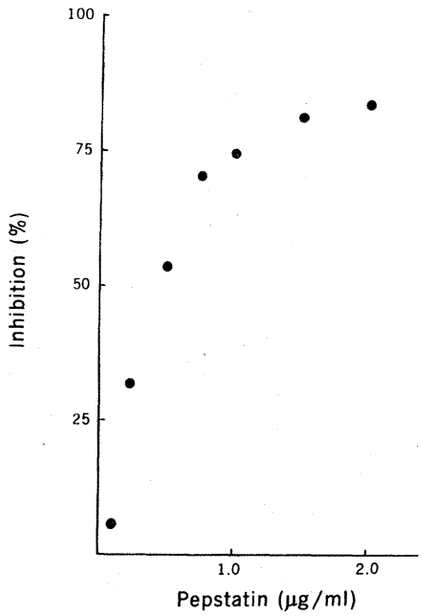


Fig. 1. Inhibition of the renin-angiotensinogen reaction by varying concentrations of pepstatin.

We have observed that pepstatin inhibits the action of the renal enzyme renin (a "neutral" protease) on its substrate (angiotensinogen, found chiefly in the $\alpha_2$-globulin fraction of plasma) in vitro. The following protocol was used for determining the inhibition. Lyophilized substrate—prepared from nephrectomized rats according to Boucher *et al.* (*3*)—with a specific activity of 65 ng/mg was diluted to 4000 ng/ml with Sörensen phosphate buffer (0.067*M*) *p*H 7.4 (*4*). The actual incubation mixture consisted of 1 ml of substrate solution (4000 ng), 0.01 dog unit of hog renin (Nutritional Biochemical), and either 0.1 ml of 0.9 percent NaCl or 0.1 ml of Sörensen buffer containing varying amounts of pepstatin (0.1 to 2.0 μg). After 20 minutes of incubation at 37°C, the reaction was stopped by the addition of 0.2 ml of 1*N* HCl, and the tubes were placed in boiling water for 5 minutes. Finally, 0.7 ml of 0.067*M* $Na_2HPO_4$ was added to correct the *p*H. Pressor activity was assayed in the urethan-anesthetized, ganglion-blocked rat nephrectomized 6 to 18 hours previously [see (*4*) for further details].

Incubation without pepstatin yielded 320 ng of angiotensin after 20 minutes. Values for the percentage of inhibition (Fig. 1) were obtained in comparison with this figure (nanograms of angiotensin formed in the presence of pepstatin/320 × 100 percent). As seen in Fig. 1, approximately 0.4 μg of pepstatin per milliliter of the incubation mixture inhibited the renin-substrate reaction by 50 percent. Under these conditions, maximum inhibition asymptotically approached 90 percent.

In the bilaterally nephrectomized rat, the intravenous injection of hog renin produces a sustained increase in blood pressure that is due to the continuous reaction of renin with its substrate (*5*). Our studies indicate that the intravenous injection of pepstatin, in doses of 50 to 200 μg, produces a reduction in the elevation of blood pressure caused by the injection of 0.005 dog unit of renin. This effect is relatively brief, with the blood pressure returning to preinjection levels within 10 to 20 minutes.

Pepstatin apparently has a very low toxicity; $LD_{50}$ (lethal dose, 50 percent effective) values obtained by intraperitoneal injection ranged from 1090 mg/kg for mice to 450 mg/kg for dogs, with the values for rats and rabbits being intermediate (*1*). As such, pepstatin could become a valuable tool for investigating the role of renin in various forms of experimental hypertension. It also has theoretically important applications in the treatment of acute renal failure, in which the intrarenal release of renin is thought to be a principal agent in the production of renal damage. The kinetic characteristics of this unique renin inhibitor and its possible in vivo applications remain to be studied.


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6. We thank Professor H. Umezawa for the pepstatin and H. Malorny and U. Miksche for technical assistance.

4 October 1971