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Renin inhibition by pepstatin

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PEPSTATIN is a small molecular weight fermentation product reported to be a good inhibitor of pepsin.[1] Pepsin is known to have renin-like activity. At pH values near neutrality, where pepsin has little or no proteolytic activity, it acts on renin substrate to produce angiotensin I.[2] Thus, in the enzymatic assay system for renin *in vitro*, pepsin must cleave the same leucyl–leucyl bond as does renin. It occurred to us, therefore, that pepstatin might also be an inhibitor of renin. This hypothesis was tested by adding pepstatin to the incubation mixture in our assay system for renin *in vitro*.

The renin preparation* was incubated with an excess of crude bovine substrate[3] at pH 6·5 and 37°. The reaction was stopped by immersion in a boiling water bath for 10 min and the denatured proteins were removed by centrifugation. The supernatant solution was then assayed for pressor activity in the nephrectomized, pentolinium-treated rat, using Hypertensin (Ciba) as the standard. When pepstatin† was added to the incubation, the inhibition curve shown in Fig. 1 was obtained. The concentration of

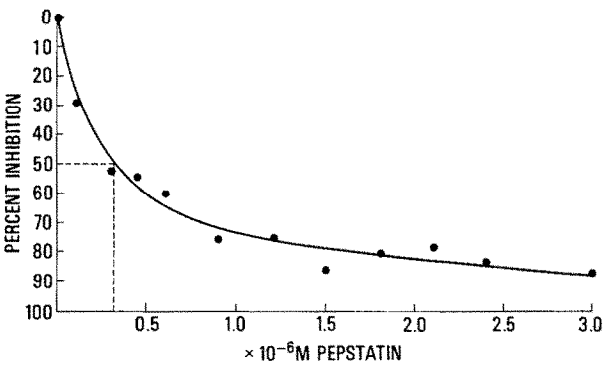

FIG. 1. Pepstatin inhibition curve for renin. Incubation mixture contained 16 mg/ml substrate and 1 μg/ml renin in total volume of 3·3 ml. Control without inhibitor produced 67 ng/ml angiotensin in 2 hr. Point of 50 per cent inhibition = 0·32 × 10^{-6} M pepstatin.

pepstatin giving 50 per cent inhibition was 0·32 × 10^{-6} M. The reaction followed Michaelis–Menton kinetics and a Lineweaver–Burke plot indicated that the inhibition obtained was of the competitive type. This was substantiated by the finding that the enzyme-inhibitor complex could be dissociated by dialysis. Pepstatin was also found to be active in the presence of plasma. When dialyzed, nephrectomized cat plasma was used as the substrate, inhibition of renin was obtained even when the pepstatin was added as late as 40 min after the start of a 1-hr incubation.

Pepstatin was then tested for its ability to inhibit renin *in vivo*. It was found that 200 μg/kg reduced the response to 0·1 GU of renin in a pithed, nephrectomized cat from 21 to 10 mm of mercury. However, in order to obtain such an effect, the renin had to be injected within two circulation times after the injection of the pepstatin (both given intravenously). This apparent rapid clearing of pepstatin from the blood was also seen in infusion experiments in rats. When the blood pressure of a normal rat was

* The renin used in these studies was a commercial preparation of purified hog renin from the Pentex Division of Miles Laboratories, Inc., assaying 7·0–8·0 GU/mg.

† We thank Dr. H. Umezawa, Institute of Microbial Chemistry, Tokyo, Japan, for the generous gifts of pepstatin used in this work.

elevated by the continuous infusion of renin, the simultaneous infusion of pepstatin reduced the blood pressure by only a small amount. However, when given as a single intravenous injection, pepstatin caused a significant reduction in blood pressure.

A series of experiments were, therefore, designed using anesthetized rats prepared so that their blood pressure could be continuously recorded from a transducer through the carotid artery. The animals were maintained with a positive pressure pump, with the right femoral vein cannulated for infusion, and the left femoral vein cannulated for injections. Pepstatin injected into normal rats prepared in this manner at dosage levels up to 260 μg/kg had no effect on the blood pressure (Fig. 2). However, when the blood pressure of a normal rat was elevated 15–25 mm of mercury by the continuous infusion of renin, the injection of 200 μg/kg of pepstatin reduced the blood pressure to the normal baseline pressure in less than 1 min. If the infusion of renin was continued, the blood pressure returned to the elevated baseline level in about 4 min. If the infusion of renin was stopped immediately after the injection of pepstatin, the elevated blood pressure returned to normal four times faster than it did in the absence of pepstatin (Fig. 2). These results were verified in four normal rats.

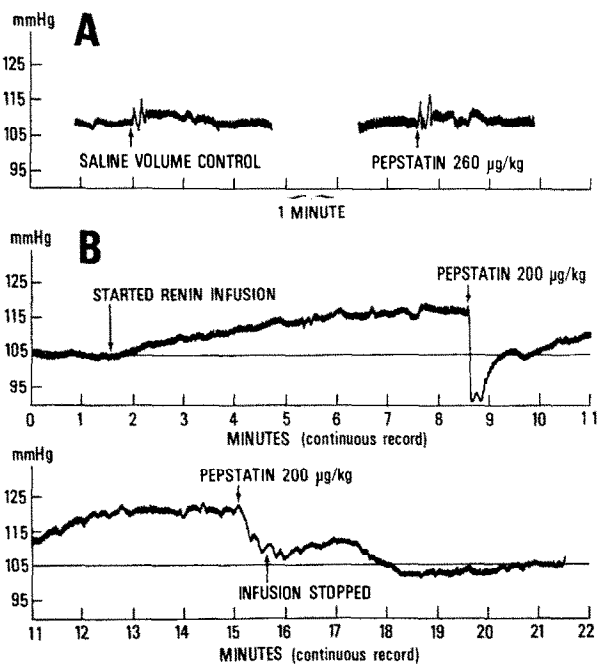

FIG. 2. Blood pressure record of normal rat. Renin infused at rate of 0·0082 GU/min. Pepstatin sample concentration was 124 μg/ml in phosphate buffered saline, pH 7·3.

A similar experiment was used to demonstrate that the inhibition of the pressor response to renin was specific for renin and not just a nonspecific depressor effect. Paredrine,* a pressor agent completely unrelated to renin, was infused in another normal rat in the same manner as renin was administered to previous rats. Pepstatin had no effect on the elevated pressure due to Paredrine® infusion when injected i.v. at 200 μg/kg. The same dose of pepstatin did reduce to normal the same elevation of blood pressure due to renin infusion in the same rat. It was also shown that when pepstatin was incubated with angiotensin II, subsequent injection of the incubation mixture into a test rat gave the same pressor response as a control sample without pepstatin.

Pepstatin was then tested for hypotensive activity in three unilaterally nephrectomized, chronically hypertensive, Goldblatt rats prepared in the same manner as the normal rats. A definite hypotensive response was obtained by intravenous injection of pepstatin at dosage levels above 50 μg/kg in all three animals (Fig. 3). A dose–response curve obtained in one of these animals is shown in Fig. 4.

These results demonstrate that pepstatin is a good, competitive inhibitor of renin, both *in vitro*

* Hydroxyamphetamine hydrobromide, Smith Kline & French.

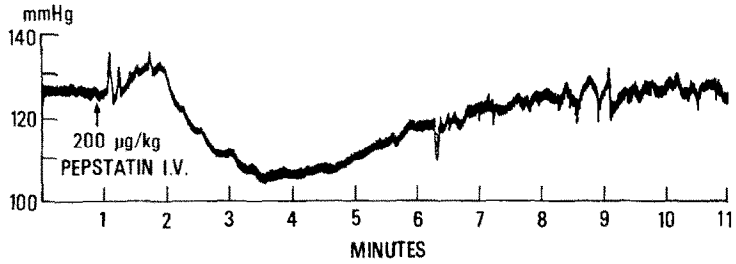

FIG. 3. Blood pressure record of Goldblatt rat. Blood pressure before anesthesia was 206 mm Hg. Pepstatin sample same as for Fig. 2. Time of recovery to elevated blood pressure level was dose related.

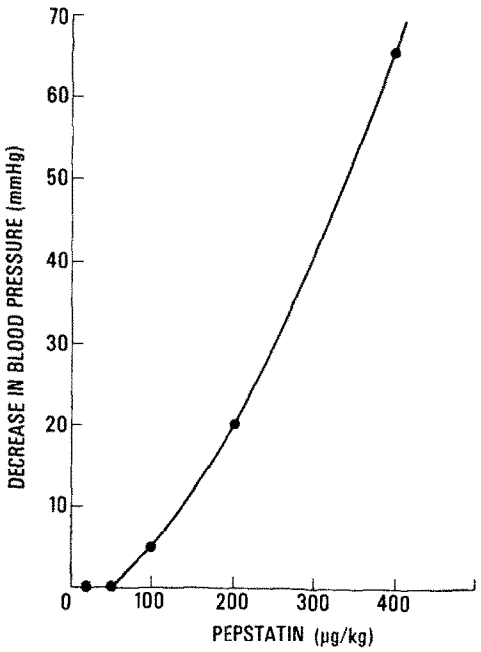

FIG. 4. Dose–response curve for pepstatin in a chronically hypertensive Goldblatt rat.

and *in vivo*. The reason for its failure to lower the blood pressure of normal rats in contrast to its hypotensive effect in Goldblatt rats is not known. However, similar results have been reported for a phospholipid pre-inhibitor of renin.[4] Perhaps, under normal conditions, mechanisms involved in maintaining homeostasis other than the renin–angiotensin system are sufficient to counteract the effect of pepstatin at these dosage levels. On the other hand, these results again seem to implicate the renin–angiotensin system in the one-kidney Goldblatt type of hypertension, even though plasma renin levels do not appear to be elevated in this type of chronic Goldblatt animal. Perhaps some of the recently discovered tissue renin systems[5–7] are involved. In any case, pepstatin should prove to be a useful tool in examining the problem of the relationship of the renin, renin-substrate reaction to the maintenance of normal homeostasis and to the various forms of hypertension.*

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* Since submitting this work for publication, we have become aware of a report by another group concerning the inhibition of renin by pepstatin.[8]

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