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A Comparative Study of Pepsitensin and Hypertensin

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Croxatto and Croxatto[1] demonstrated that digestion of hypertensinogen (blood plasma globulin) and of other proteins with pepsin (pH 2-6) leads to the formation at the end of a few minutes of a substance having an intense vasoconstrictor and hypertensive action. This substance, pepsitensin, may be considered homologous with hypertensin which is produced by the interaction of hypertensinogen and renin. The notable similarity established for the two substances and the light which they may throw on the rôle of the kidneys and on the nature of hypertension led us to a more detailed comparative study, both chemical and pharmacological, of hypertensin and pepsitensin, prepared as previously described (Croxatto and Croxatto[2]).

This work presents some new properties of pepsitensin and some conditions for its formation.

Experimental investigations. In most of our experiments, hypertensinogen was used as substrate (prepared according to the method of Braun Menendez and coworkers[3]) in the preparation of pepsitensin, but lactoglobulin, lactalbumin and casein were also used. Since it was difficult to separate some hypotensive impurities from hydrolysates of the latter materials, hypertensinogen was preferred. After acidification of the substrate to pH 3-3.5, either Parke Davis or Northrop's crystalline pepsin was added in the proportion of 0.1-1 mg per cc. The mixture was incubated for a few hours and the digestion was ended at the end of a period (15 min to 120 hr) by the addition of 3-4 volumes of boiling alcohol. The concentration and further purification followed the method proposed by Braun Menendez and

1 Croxatto, H., and Croxatto, R., *Science*, 1942, **95**, 101.

2 Croxatto, H., and Croxatto, R., *Rev. Soc. Argent. Biol.*, 1941, **17**, 439.

3 Braun Menendez, E., Fasciolo, J. C., Leloir, L. F., and Muñoz, J. M., *J. Physiol.*, 1940, **98**, 283.

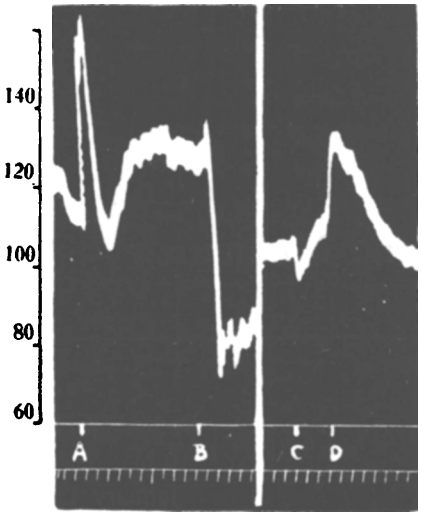

FIG. 1.

Arterial pressure (cat). Intravenous injection.
A. 25 μg of epinephrine.
B. 8 mg Fourneau.
C. 25 μg epinephrine.
D. Pepsitensin, dose equivalent to 15 μg epinephrine.

coworkers for hypertensin, with the difference that the final product was subjected to repeated dialysis. The vasoconstrictor action of the preparation was assayed with the Loewen Trendelenburg preparation upon the arterial pressure of cats anesthetized with

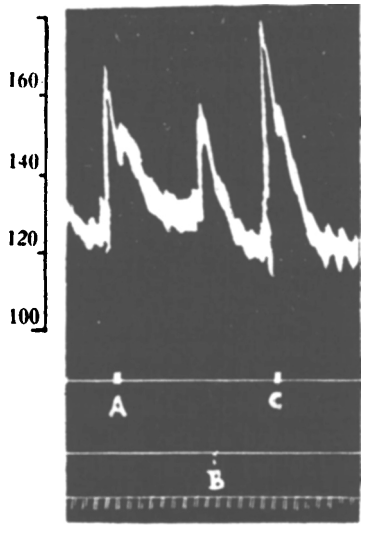

FIG. 2.

Arterial pressure (cat). Intravenous injection.
A. 2 cc pepsitensin solution.
B. 150 μg Veritol.
C. 2 cc pepsitensin solution.

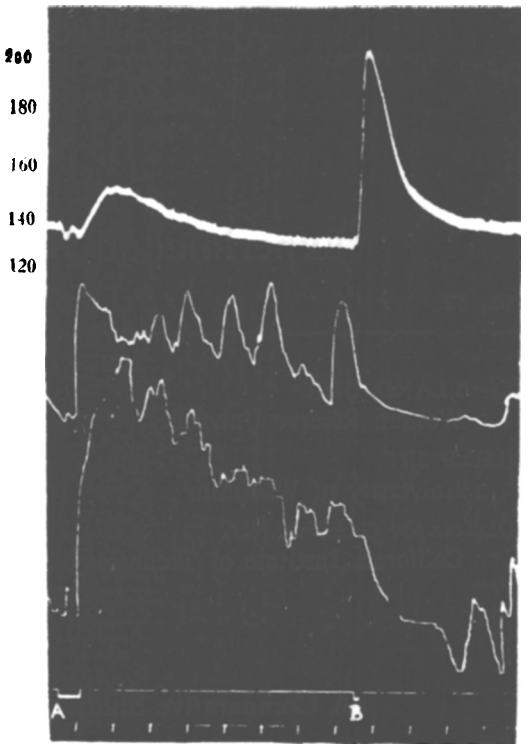

FIG. 3.

Upper line: arterial pressure (cat). Middle line: uterine contractions. Lower line: bladder contractions. Intravenous injection.
A. 2 cc pepsitensin. B. 25 μg epinephrine.

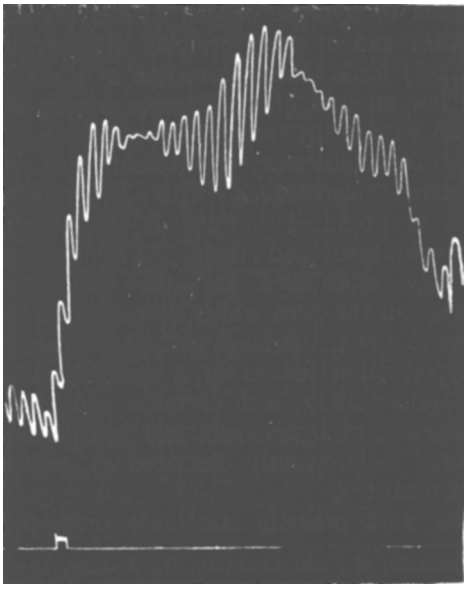

FIG. 4.

Recording of intestinal contractions *in vitro* after administration of 1.75 mg atropine. The mark shows the addition of 1 cc of pepsitensin.

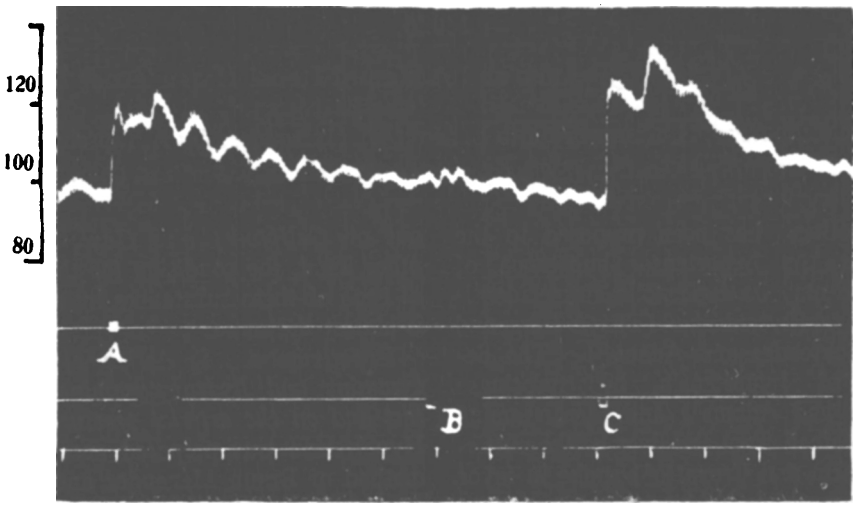

FIG. 5.
Arterial pressure (cat). Intravenous injection.
A. 0.12 cc of pepsitensin incubated at 37° with crystalline pepsin at pH 7.
B. 0.25 cc pepsitensin incubated for 6 hours at pH 1.8.
C. Like B, at pH 1.8 without pepsin.

Dial. After 15 min of digestion there was an appreciable formation of hypertensive material. The yield of pepsitensin was high after 1-4 hr, and a more prolonged incubation decreased the vasoconstrictor action instead of increasing it. A pharmacological study revealed that the pepsitensin has above all the properties of hypertensin as described by Braun Menendez and coworkers. Neither produced tachyphylaxis when sufficiently purified; removal of the kidneys and adrenals did not influence the effect. The previous injection of Fourneau 933 (4 mg per kilo) did not invert the hypertensive effect of hypertensin and pepsitensin, a property differentiating them from epinephrine. (Fig. 1). Veritol (80 μg per kilo) potentiated the vasoconstrictor effect of both substances (Fig. 2); atropine, in contrast, did not change it. Recording of the tonus of the intestine, uterus and bladder of the cat and rat shows that pepsitensin also strongly stimulates the smooth musculature of each of these organs (Fig. 3). This effect is not abolished by atropine in a dosage which totally inhibited acetylcholine (Fig. 4).

We have been able to demonstrate that pepsitensin is insoluble in acetone; and is thermostable although it is destroyed by boiling with 0.15 N NaOH for 15 min. As it had been demonstrated (Croxatto and Croxatto[2]) that both substances lose their vasoconstrictor when treated with enzymes: trypsin, tryosinase, aminoöxidase and renal hypertensinase (prepared by various procedures), it was very important to determine whether pepsitensin, like hypertensin, was destroyed by the hydrolytic action of pepsin. Purified preparations of pepsitensin were subjected to 3-6 hr digestion in acid media with crystalline pepsin, and it could thus be shown that under these conditions pepsitensin completely loses its vasoconstrictor effect (Fig. 5). It could also be demonstrated that simple acid hydrolysis of hypertensinogen, by the addition of HCl to pH 3, may lead to the formation of a substance similar to pepsinogen when the substrate is incubated for several hours at 37°. However, the yield is very much lower than that obtained from the action of pepsin.

Summary. A comparative study of pepsitensin and hypertensin agree in repect to all their known chemical and pharmacological characteristics.

We wish to thank Professor B. Houssay for his kindness in providing us with part of the hypertensin used in these experiments, and to thank Professor E. Cruz Coke for his gift of Northrop pepsin.