Preliminary Notes

PN 1263

Potentiation of the pressor effects of angiotensin in alkaline solution

One of the methods usually employed to assay the pressor action of the angiotensins consists in injecting their solutions intravenously into anesthetized rats and registering the induced increase in blood pressure.

We have accidentally found that the pressor response is considerably enhanced when the polypeptide solutions have a pH $\geqslant$ 11. This effect, originally observed with impure pepsitensin[1], could be reproduced with synthetic Val_5angiotensin II β-aspartyl amide, and the corresponding free acid (kindly provided by Drs. F. GROSS, R. SCHWYZER AND B. ISELIN from Ciba Ltd., Basel, Switzerland); with a mixture of $Ileu_5$angiotensin I and II (obtained from Dr. L. T. SKEGGS); and with impure bovine angiotensin[2].

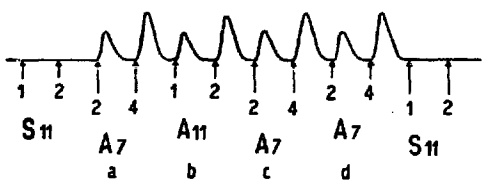

Fig. 1. Programme of injections which was followed to compare the pressor effect of neutral and alkaline solutions of angiotensin. S 11: saline brought to pH 11.7 with 1 N sodium hydroxide; A 7: solution of angiotensin (Val_5-angiotensin II, β-aspartyl amide, synthetic, Ciba), in saline at pH 7 (0.4 μg/ml); A 11: A 7 adjusted to pH 11.7 with 1 N sodium hydroxide. Volume injected: 1, 0.008 ml; 2, 0.016 ml; 4, 0.032 ml[3].

Male, or female, white rats from the Institute of Physiology, Buenos Aires Medical School, 21–28 days old and of 43–60 g in body weight, were used throughout. They were nephrectomized under urethane anesthesia (100 mg/100 g of body weight), 1 h beforehand and prepared for the pressor assay as already described[3].

Experiments have been performed accordingly with the procedure outlined in Fig. 1. It was found that the injection of saline (0.15 M NaCl) adjusted to pH 11.7 is sometimes followed by a pressor response but the rat becomes rapidly insensitive when submitted to repeated injections of this solution. The pressor activities of angiotensin at pH 11.7, or pH 7, have been calculated by comparing the responses *b* and *c*, respectively, with *a* and *d* taken as standards (see Fig. 1)[3]. The results in Table I, Expt. A, are thus expressed relatively and in each experiment the average of *a* and *d* is considered as 100% activity.

The results show that angiotensin, when injected at pH 11.7, is 66 $\pm$ 11% more active than at pH 7, and this difference is highly significant ($t = 6.05$; $P \ll 0.001$).

In order to explain this fact several possibilities should be explored:

(a) The high pH of the solvent may affect the sensitivity of the rat to the drug. This hypothesis was tested using rats prepared in the usual way but with both jugular veins cannulated. It was then possible to compare the effect of a single injection of

alkaline angiotensin with that of a simultaneous double injection: neutral angiotensin into one jugular vein and alkaline saline into the other. Table I, Expt. B, shows the results of one set of experiments of this type. In all cases single injections of saline at pH 11.7 and angiotensin at pH 7 were made before and after the double injections.

TABLE I

POTENTIATION OF THE PRESSOR EFFECT OF ANGIOTENSIN IN ALKALINE SOLUTION

Control value in Expt. A corresponds to the average pressor response to angiotensin injected at pH 7. In Expt. B this value is the average pressor response to the simultaneous injections, through separate jugular veins of neutral angiotensin and alkaline saline. A 11 are single injections of alkaline angiotensin. All values ± standard error. See text and Fig. 1.

Experiment	*Number of rats*	*Relative pressor activity (%)*	
		Control	*A 11*
A	14	105 ± 2	171 ± 10
B	8	98 ± 5	177 ± 7

The average of the pressor response of the rat to angiotensin at pH 7 during these controls is considered 100% of activity. In five animals the injection of 1 vol. of alkaline angiotensin was compared with the injection of 1 vol. of neutral angiotensin plus the simultaneous injection of one volume of alkaline saline through the other vein. With the other three rats the neutral angiotensin solution used was four times more concentrated than the corresponding alkaline angiotensin. Accordingly 0.25 vol. of neutral angiotensin were injected simultaneously (although slightly delayed) with 0.75 vol. of alkaline saline thus ensuring a previous flooding of the receptors by alkali. Independently of such minor variations in the experimental procedure the results were very similar in all instances indicating that potentiation of the pressor effect occurs only when the angiotensin solution is at a high pH prior to its injection. Hypothesis (a) is thus discarded[4].

(b) Angiotensin in solution may be polydisperse: On this basis an equilibrium (monomer $\rightleftarrows$ polymers), which is sensitive to pH, could be postulated and it is conceivable that the biological activity of each molecular species might not be identical. To test this hypothesis the molecular weight of angiotensin was measured by ultracentrifugation* at pH 3, 5.6 and 11. The values found, gave no evidence of polymerization.

(c) Rearrangements within the polypeptide chain: RINIKER, BRUNNER AND SCHWYZER[5] have reported that an unnatural isomer of the Val_5angiotensin II free acid is 50% more active than the natural product. This isomer arises during hydrolysis of the β-amide group on the aspartic acid in the synthetic molecule. Under relatively mild conditions the aspartic acid is rearranged and replaces by its β-carboxyl group the α-carboxyl group involved in the normal peptide link with the next amino acid of the chain. The occurrence of a similar phenomenon in our experiments is improbable since the potentiation effect is freely reversible: a neutral solution of angiotensin brought to pH 11.7 and injected within 1 min of the alkalization gives rise to an increased pressor response. The same solution when neutralized again gives

* We acknowledge the kind collaboration of Drs. L. C. CRAIG and D. A. YPHANTIS, from the Rockefeller Institute, New York, in the performance of these measurements.

the original pressor potency in the assay and the whole process can be repeated several times.

(d) Changes in conformation of the polypeptide molecule induced by high pH: It is already known that substances suddenly injected into the bloodstream do not readily mix and equalization is attained only after several recirculations. If such is also true for alkaline angiotensin it is possible that a substantial proportion of the injected angiotensin molecules could reach the receptors at a high pH, as *in vitro*, and thus act in a different, and more active, conformation.

It is very difficult to devise a direct biological experiment to test this hypothesis but an independent piece of evidence in favour of a change in conformation of the angiotensin molecule with rising pH of the solvent has been obtained by differential ultraviolet spectroscopy against an equimolar mixture of its constituent amino acids, at similar pH's. It has been observed that, when the pH is increased, a pronounced peak of absorption appears at 250 mμ when the pH is 10 or higher. Similar changes have been usually ascribed to alterations in the conformation of proteins and polypeptides.

In summary, a potentiating effect of high pH on the pressor activity of angiotensin has been found. Different possible explanations have been discussed and a change of the angiotensin molecule conformation seems to fit best the facts described. Differential ultraviolet spectroscopic studies appear to support this conclusion.

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[1] E. BRAUN MENÉNDEZ AND A. C. PALADINI, *Circulation*, 17 (1958) 668.
[2] A. C. PALADINI AND E. BRAUN MENÉNDEZ, *Biochim. Biophys. Acta*, 18 (1955) 580.
[3] A. C. PALADINI, E. BRAUN MENÉNDEZ, I. S. DEL FRADE AND Z. M. MASSANI, *J. Lab. Clin. Med.*, 53 (1959) 264.
[4] D. A. YPHANTIS, *Ann. N.Y. Acad. Sci.*, 88 (1960) 586.
[5] B. RINIKER, H. BRUNNER AND R. SCHWYZER, *Angew. Chem. Intern. Ed. Engl.*, 1 (1962) 405.

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PN 1271

A role of the cell wall as a "primary reservoir" of vitamin B_{12} in a B_{12}-requiring Lactobacillus

The cells of *Lactobacillus delbrueckii*, an organism which requires vitamin B_{12} as an essential growth factor, elongate abnormally when the organism is grown in a B_{12}-deficient medium[1]. In a B_{12}-rich medium, however, they accumulate this vitamin