SOME PHARMACOLOGICAL ACTIONS OF SYNTHETIC ANALOGUES OF ANGIOTENSINAMIDE

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In order to evaluate the importance of some structural features of asparagyl¹-valyl⁵-angiotensin II (angiotensinamide) for its pharmacological actions, the relative potencies of angiotensinamide and five peptide analogues were studied on the blood pressure of the rat, the isolated rat uterus and the isolated guinea-pig ileum. All the modifications of the angiotensinamide structure that were studied led to a decrease of potency which, however, was not the same on all three preparations. The importance of the guanido group, the phenolic group and the length of the peptide chain for the pharmacological activities of these peptides is discussed.

The name *angiotensin* has been proposed by Braun-Menéndez and Page (1958) to replace the two trivial names, hypertensin and angiotonin, previously given to the product of renin action on the plasma α_2 -globulins.

The knowledge of the structure of angiotensin peptides (Skeggs, Lentz, Kahn, Shumway and Woods, 1956; Elliott and Peart, 1957) and the development of methods for their synthesis (Rittel, Iselin, Kappeler, Riniker, and Schwyzer, 1957), as well as that of structural analogues, permitted the study of the relation of peptide structure to pharmacological activity. In a previous paper (Paiva and Paiva, 1960a) we have investigated the effect of modifications of the *N*-terminal grouping

of valyl⁵-angiotensin II on its pressor and oxytocic activities, and concluded that a free β -carboxyl group in the *N*-terminal aspartyl residue, though not essential, is important for these two properties. A further study of the relation of angiotensin structure to activity was made possible by the availability of synthetic analogues prepared in the Ciba Laboratories. In the present paper we report a study of 6 such analogues, having in common an asparagyl instead of the aspartyl residue at the *N*-terminal end. Table I shows the amino acid sequences of valyl⁵-angiotensin II and of the 6 synthetic peptides studied. In order to simplify the reference to the different analogues, in this paper asparagyl¹-valyl⁵-angiotensin II will be

TABLE I
THE SEQUENCE OF AMINO ACIDS IN VALYL⁵-ANGIOTENSIN II AND IN SIX SYNTHETIC ANALOGUES

The nomenclature used was derived from the name angiotensinamide (asparagyl¹, valyl⁵-angiotensin II) according to the rules proposed by Konzett and Berde (1959).

Peptide	Amino Acid Sequence								
	1	2	3	4	5	6	7	8	9
Valyl ⁵ -angiotensin II	Asp	Arg	Val	Tyr	Val	His	Pro	Phe	
Angiotensinamide	Asp(NH ₂)	Arg	Val	Tyr	Val	His	Pro	Phe	
Isoleucyl ⁵ -angiotensinamide	Asp(NH ₂)	Arg	Val	Tyr	iLeu	His	Pro	Phe	
Nitroarginyl ² -angiotensinamide	Asp(NH ₂)	Arg	(NO ₂)	Val	Tyr	Val	His	Pro	Phe
Ornithyl ² -angiotensinamide	Asp(NH ₂)	Orn	Val	Tyr	Val	His	Pro	Phe	
Phenylalanyl ⁴ -angiotensinamide	Asp(NH ₂)	Arg	Val	Phe	Val	His	Pro	Phe	
Homotyrosyl ^{4,5} -angiotensinamide	Asp(NH ₂)	Arg	Val	Tyr	Tyr	Val	His	Pro	Phe

referred to as *angiotensinamide*, and the name of the other peptides will be derived from this in the manner proposed by Konzett and Berde (1959) for the nomenclature of oxytocin analogues.

The pharmacological preparations used in the present study were the blood pressure of the rat, the isolated rat uterus and the isolated guinea-pig ileum. The first preparation was chosen because the pressor action is the most characteristic pharmacological action of angiotensin *in vivo*, and the two other preparations were chosen for being the most sensitive to angiotensin yet described (Ludueña, 1940; Picarelli, Kupper, Prado, Prado, and Valle, 1954; Schwartz, Masson, and Page, 1955).

METHODS

The angiotensin analogues were all synthetic products prepared at Ciba Laboratories, Basle, and purified as free peptides in the final stage of the synthesis by counter-current distribution. The purity of the products was checked by paper chromatography and they were found to be either chromatographically pure or to contain small amounts (not more than 1%) of the corresponding aspartyl-peptides (formed by hydrolysis of the *N*-terminal asparagine amide group). All preparations contained 1 to 3 molecules of water and 1 to 2 molecules of acetic acid per molecule of peptide (Schwyzer, 1960).

Pharmacological Assays

Rat Blood Pressure.—Albino rats, weighing between 200 and 250 g., were anaesthetized with urethane 150 mg./100 g. body weight, and were kept on artificial respiration. The blood pressure was recorded with a mercury manometer connected to the carotid artery. Injections were made through a plastic cannula in a femoral vein. The rats were heparinized, and received an intravenous injection of hexamethonium bromide 5 mg./100 g. body weight. For the assay of *pressor activity* 0.1 or 0.2 ml. of the peptides, in isotonic solution, was injected.

Rat Uterus.—Virgin rats weighing 150 to 200 g. received subcutaneous injections of 10 µg. of stilboestrol per 100 g. body weight and were killed 16 to 20 hr. later by a blow on the head. The uterine horns were suspended in aerated de Jalon solution in a 2 ml. bath at 30°. The contractions were recorded with an isotonic lever with a six-fold magnification and a load of 1.0 to 1.5 g. In this paper, the activity of the peptides on this preparation is called *oxytocic activity*.

Guinea-pig Ileum.—Terminal segments of the ileum of guinea-pigs weighing 150 to 200 g. were suspended in a 2 ml. bath of aerated Tyrode solution at 35°. The contractions were recorded as described above for the rat uterus. The effect on the isolated guinea-pig ileum is called *gut-stimulating activity*.

The pressor, oxytocic and gut-stimulating activities of the 5 analogues of angiotensinamide were estimated

by assays against standard solutions of angiotensinamide. For each analogue, a rough preliminary estimate of activity was made, and then approximately equipotent solutions of the peptide and of angiotensinamide were compared using a four-point design. The evaluation of potency was made by measuring the height of the recorded increases in blood pressure or contractions of the isolated smooth muscles, and submitting the data to the statistical treatment described by Schild (1942) for obtaining a potency ratio and fiducial limits. The relative potency of each peptide was defined as the ratio of the activity of one mole of peptide to the activity of one mole of angiotensinamide, which was arbitrarily taken as 100.

RESULTS

All the peptides had detectable activity on the three pharmacological preparations. Qualitatively, no difference was detected between the pressor response to angiotensinamide on the rat and that of the other analogues. Although the oxytocic and gut-stimulating activities of isoleucyl⁵- and homotyrosyl^{4:5}-angiotensinamides were qualitatively indistinguishable from angiotensinamide, the other 3 analogues produced a different type of response. On the isolated rat uterus, addition of nitroarginyl²-, ornithyl²- or phenylalanyl⁴-angiotensinamide to the bath caused only a single contraction, instead of the rhythmic response characteristic of angiotensinamide (Paiva and Paiva, 1960b). On the isolated guinea-pig gut, the three last-mentioned analogues produced a tonus of shorter duration than that produced by

FIG. 1.—Isolated rat uterus suspended in a 2 ml. bath containing de Jalon solution at 30°. Responses to additions of phenylalanyl⁴-angiotensinamide (P), angiotensinamide (A) and homotyrosyl^{4:5}-angiotensinamide (H). At W the preparation was washed three times. Time, min.

FIG. 2.—Isolated guinea-pig ileum suspended in a 2 ml. bath containing Tyrode solution at 35°. Contractions produced by angiotensinamide (A), homotirosyl^{4:5}-angiotensinamide (H), and phenylalanyl⁴-angiotensinamide (P).

angiotensinamide. To illustrate these qualitative differences, Figs. 1 and 2 show the response of the rat uterus and of the guinea-pig gut to angiotensinamide and to homotirosyl^{4:5}- and phenylalanyl⁴-angiotensinamide.

In spite of these qualitative differences, we decided to make a quantitative comparison of the oxytocic and gut-stimulating potencies of angiotensinamide with those of all the other peptides. The oxytocic activity was assessed from the height of the first contraction of the rat uterus, and the gut-stimulating activity from the maximum height of contraction of the ileum during the 90 sec. of contact with the drug. The results of the assays are summarized in Table II. No significant deviation of parallelism was found in any of the assays ($P > 0.2$).

The possibility of competitive inhibition of angiotensinamide by homotirosyl^{4:5}-angiotensinamide was investigated in the three pharmacological preparations. The administration of mixtures of angiotensinamide with 100-fold concentrations of the homotirosyl analogue resulted in simple summation of effects, without any sign of inhibition. Similar summation of effects was observed with mixtures of equipotent amounts of angiotensinamide with each of the other analogues; in these mixtures, the ratio between the molar concentration of the analogues and that of angiotensinamide ranged from 2, in the case of the pressor action of the mixture with isoleucyl⁵-angiotensinamide, to 350 in the case of the gut-stimulating action of the mixture with nitroarginyl²-angiotensinamide.

DISCUSSION

All the analogues studied were less active than angiotensinamide on the three pharmacological preparations. It is remarkable that relatively small alterations in the structure of angiotensinamide resulted in marked differences in the three pharmacological activities studied. One example of this is seen in Table II, where the ratios between the potencies of angiotensinamide and nitroarginyl²-angiotensinamide were found to be 4 for the pressor, 32 for the oxytocic, and 350 for the gut-stimulating activity. Another example illustrating that small changes in the structure of angiotensinamide resulted in marked alterations of pharmacological activity is where the presence of an extra methyl group, in isoleucyl⁵-angiotensinamide, resulted in a product that was about one-half to one-third as active as angiotensinamide. Furthermore, small activity was found for phenylalanyl⁴-angiotensinamide, which differs

TABLE II

RELATIVE POTENCIES OF SOME ANGIOTENSIN ANALOGUES ON THE RAT BLOOD PRESSURE, THE ISOLATED RAT UTERUS AND THE ISOLATED GUINEA-PIG ILEUM

Relative potencies were calculated on a molar basis, the value 100 being arbitrarily assigned to angiotensinamide. Figures in parentheses indicate the 95% fiducial limits.

Peptide	Rat Blood Pressure	Isolated Rat Uterus	Isolated Guinea-pig Ileum
Angiotensinamide	100	100	100
Isoleucyl ⁵ -angiotensinamide	52 (± 8)	33 (± 3)	35 (± 5)
Nitroarginyl ² -angiotensinamide	25 (± 3)	3.1 (± 0.2)	0.29 (± 0.05)
Ornithyl ² -angiotensinamide	8.9 (± 0.9)	1.6 (± 0.2)	0.73 (± 0.08)
Phenylalanyl ⁴ -angiotensinamide	4.9 (± 0.8)	2.5 (± 0.4)	3.2 (± 0.2)
Homotirosyl ^{4:5} -angiotensinamide	0.33 (± 0.05)	0.10 (± 0.02)	0.10 (± 0.02)

from angiotensinamide only by the absence of one phenolic hydroxyl group. These differences, however, are not so surprising, in view of the reported discrepancies (Berde, Doepfner, and Konzett, 1957; van Dyke, 1959) in the potencies of oxytocin and vasopressin analogues towards different biological tests.

Some conclusions regarding the importance of the guanido group of angiotensinamide for its pharmacological activities may be drawn from the results obtained with nitroarginyl²- and ornithyl²-angiotensinamide. The nitroarginyl analogue, in which the ionization of the guanido group is blocked, had a pressor activity that was one-quarter that of angiotensinamide, but still almost three times that of the ornithyl analogue, in which the guanido group was replaced by an amino group. This suggests that the importance of the guanido group for the pressor activity of angiotensinamide may not be attributed entirely to its basic character. The same is not so evident with regard to the oxytocic activity, since the two analogues have very low oxytocic potencies, nitroarginyl²-angiotensinamide being still twice as active as ornithyl²-angiotensinamide. The gut-stimulating activity, on the other hand, seems to be more dependent on the basicity of the guanido group, since this activity is negligible in nitroarginyl²-angiotensinamide, whereas the ornithyl analogue is 2.5 times as potent. The inability of ornithine to replace arginine, especially in the case of the gut-stimulating activity, may be explained by the shortening of the side-chain interfering with the interaction of the basic group with the receptor. This explanation seems better than the one offered by Katsoyannis and du Vigneaud (1958) for the similar case of arginine and lysine vasopressins, namely, that the differences in the pK'_3 of the two amino acid residues might be responsible for the difference in the pharmacological activity of the two analogues. Since the two pK'_3 values are well above the physiological pH, the arginine guanido group and the ornithine amino group should both be completely ionized at that pH and, therefore, equally charged.

Homotyrosyl⁴-angiotensinamide, although inducing the same type of response as angiotensinamide, was the least active of the analogues. The absence of competitive inhibition of angiotensinamide by this analogue suggests that its low activity

may be due to a disproportionate distance, introduced by the extra tyrosyl residue, between two points of attachment to the receptor.

After this paper had been submitted for publication we had knowledge of a paper by Gross and Turrian (1960), in which the pressor activity of the first four analogues of Table II were studied on the nephrectomized rat.

These authors found that the isoleucyl analogue was as active as angiotensinamide, and that the arginyl and ornithyl analogues were, respectively, one-half and one-quarter as active as angiotensinamide. However, no measure of the error involved in their assays is given, and it is impossible to determine the significance of the difference between our results.

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REFERENCES

- Berde, B., Doepfner, W., and Konzett, H. (1957). *Brit. J. Pharmacol.*, **12**, 209.
- Braun-Menéndez, E., and Page, I. H. (1958). *Nature*, **181**, 1061.
- Elliott, D. F., and Peart, W. S. (1957). *Biochem. J.*, **65**, 246.
- Gross, F., and Turrian, H. (1960). In *Polypeptides which Affect Smooth Muscle and Blood Vessels*, p. 137. Ed. M. Schachter. Oxford: Pergamon Press.
- Katsoyannis, P. G., and du Vigneaud, V. (1958). *Arch. Biochem.*, **78**, 555.
- Konzett, H., and Berde, B. (1959). *Brit. J. Pharmacol.*, **14**, 133.
- Ludueña, F. P. (1940). *Rev. Soc. argent. Biol.*, **16**, 358.
- Paiva, A. C. M., and Paiva, T. B. (1960a). *Biochem. Pharmacol.*, in the press.
- Paiva, T. B., and Paiva, A. C. M. (1960b). *J. Pharmacol. exp. Ther.*, **130**, 177.
- Picarelli, Z. P., Kupper, R., Prado, E. S., Prado, J. L., and Valle, J. R. (1954). *Circulat. Res.*, **2**, 354.
- Rittel, W., Iselin, B., Kappeler, H., Riniker, B., and Schwyzler, R. (1957). *Helv. chim. Acta*, **40**, 614.
- Schild, H. O. (1942). *J. Physiol. (Lond.)*, **101**, 115.
- Schwartz, H., Masson, G. M. C., and Page, I. H. (1955). *J. Pharmacol. exp. Ther.*, **114**, 418.
- Schwyzler, R. (1960). Personal communication.
- Skeggs, L. T., Lentz, K. E., Kahn, J. R., Shumway, N. P., and Woods, K. R. (1956). *J. exp. Med.*, **104**, 193.
- van Dyke, H. B. (1959). *Symposia and Special Lectures, XXIst Int. Congr. Physiol. Sciences*, p. 61. Buenos Aires.