TRYPTIC SPLITTING OF VASOPRESSIN ANALOGUES CONTAINING HOMOLOGUES OF LYSINE OR ARGININE IN POSITION 8

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Vasopressin analogues containing an amino acid with a shorter side chain in position 8 of the peptide chain were more resistant to tryptic splitting of the peptide bond formed by the basic amino acid and terminal glycine amide. In the lysine vasopressin series, analogues with ornithine or lower homologues of lysine in position 8 were not hydrolyzed. In the arginine vasopressin series, the analogue containing 3-guanidino-2-aminopropionic acid in position 8 was completely resistant to the action of trypsin. The vasopressin analogue with norarginine in position 8 was split at a lower rate than natural arginine vasopressin.

The presence of arginine of L-lysine in the molecule of natural vasopressin makes the peptides potential substrates of trypsin[1-4]. The basic character of the amino acid in position 8 is a prerequisite for the expression of the typical vasopressin-like biological effects. In order to study the dependence of the biological effect on the position of the basic group in the amino acid side chain in position 8, a series of vasopressin analogues was prepared. This series can also serve as a suitable model for studying the substrate specificity of trypsin. The hydrolysis of esters of N-protected homologues of arginine or lysine has already been investigated[5-10]; the hydrolysis of amides has not yet been studied. The degree to which the individual peptides are resistant to tryptic action was estimated by a biological assay. After the incubation of vasopressin analogues with trypsin, the remaining amount of substance with pressoric activity was determined and the hydrolytic rate was expressed as a rate constant of the first order.

EXPERIMENTAL

Materials. Bovine trypsin was purchased from Worthington (USA), [8-lysine]vasopressin (*I*) was prepared in the Department of Organic Synthesis of our Institute and purified according to ref.[11], [8-ornithine]vasopressin[12] (*II*) was purchased from Sandoz (Switzerland), [8-L-ornithine]deamino-6-carba-vasopressin[13] (*III*), [8-L-diaminobutyric acid]deamino-vasopresin[14] (*IV*), [8-L-diaminopropionic acid]vasopressin[15] (*V*), [8-L-diaminopropionic acid]deamino vasopressin[16] (*VI*), [8-L-homoarginine]deamino-vasopressin[17] (*VII*), [8-L-arginine]deamino-vasopressin[18] (*VIII*), [8-L-norarginine]deamino-vasopressin[19] (*IX*), [8-L-β-guanido-α-aminopropionic

acid]deamino-vasopressin[16] (*X*), were synthesized in the Department of Organic Synthesis of our Institute. [8-L-Arginine]vasopressin (*XI*) was isolated from the bovine neurophysin complex as described by Prusík and coworkers[20].

Methods: Vasopressins and their analogues were incubated at a concentration of $1 . 10^{-2}$ mg/ml in 20 mM Tris-HCl buffer, pH 7·5 with trypsin at 37°C. The total volume was 2 ml. The concentration of trypsin and the incubation time for the individual analogues was settled according to preliminary experiments. For compounds *I* and *XI*, the concentration of trypsin was $1 . 10^{-3}$ mg/ml, and the reaction time 15 min. In the case of the remaining analogues, the concentration of the enzyme was enhanced by one order and the incubation time was prolonged to 1 h. The enzymic reaction was stopped by 5 min boiling.

The concentration of the remaining hormone or vasopressin analogue was determined by the pressoric assay performed on the pithed rat[21]. Samples of hormone or corresponding analogue incubated in the absence of trypsin were used for reference. For the calculation of the results the four point test was applied or a calibration curve was constructed and the actual concentration of biologically active compound was read off.

RESULTS AND DISCUSSION

The problem of tryptic hydrolysis of vasopressin analogues appeared when we failed to prepare the desglycineamide derivative of [8-L-ornithine]deamino-carba-6-vasopressin by tryptic splitting, while under the same conditions the corresponding derivative of [8-arginine]deamino-carba-6-vasopressin was prepared by trypsin action. We solved the problem by studying the hydrolysis of vasopressin analogues by trypsin in dependence on the position of a positively charged group in the side chain of the amino acid in position 8 (*i.e.* on its distance from the backbone of the peptide molecule).

The tryptic hydrolysis of both vasopressins was studied in detail in the past[1-4]. It was found that [8-arginine]vasopressin was a better substrate of trypsin than [8-lysine]vasopressin, and that the product of hydrolysis was the desglycineamide derivative of vasopressin. Table I shows that if the NH_2 group of the diamino acid in position 8 is shifted from the ε carbon to the δ carbon, the peptide bond between the basic amino acid and glycineamide becomes completely resistant to tryptic cleavage. This finding was confirmed by studying vasopressin analogues containing the lower homologues of L-lysine, namely L-2,4 diaminobutyric acid or L-2,3 diaminopropionic acid, instead of L-lysine. No decrease of biological activity was observed after 1 h of incubation. This confirms the integrity of the C-terminal peptide bond. In this connection we should like to mention the data published by Lindenberg[22] on the preparation of [8-D-homolysine]deaminovasopressin by splitting the racemic mixture of the analogue with trypsin, which led to the inactivation of the analogue with L-homolysine in position 8.

Tryptic hydrolysis was affected to a lesser extent when the amino group was placed farther from the hydrolyzed bond when it was nearer.

The results we obtained in experiments with vasopressin analogues containing arginine and its homologues followed a somewhat different pattern, as was mentioned above, [8-L-arginine]vasopressin was rapidly hydrolyzed by trypsin. The series of analogues we had at our disposal were derived from deamino-vasopressin. Therefore we related the estimated experimental values to the hydrolysis of [L-arginine]-deamino-vasopressin. By comparing the rate constants in the homological series we can see that the highest value was found in the case of the arginine homologue; analogues of vasopressin containing 8-homoarginine and norarginine were hydrolyzed more slowly and the last of the series, [8-L-3-guanido-2-amino propionic acid]-deamino vasopressin was not split at all. In contrast to the lysine series, tryptic splitting of the arginine series was eliminated only after the bulkier positively charged guanido group was shifted to the β-carbon of the linear chain. Furthermore, data on the tryptic hydrolysis of some esters of protected arginine homologues show[5-7], that the methyl ester of α-N-toluene-sulfonyl-L-arginine was readily hydrolyzed whereas the derivative with homoarginine or norarginine were hydrolyzed more slowly. Similarly as in our case, no hydrolysis of the ester of protected 3-guanido--2-aminopropionic acid was observed.

The situation in the series of lysine homologues does not correspond to our findings. The methylester of protected ornithine is hydrolyzed by trypsin, whereas the analogue of vasopressin with L-ornithine in position 8 is resistant to the action of trypsin. Vasopressin analogues containing shorter homologues of ornithine in position 8 were also proved to be more stable. We failed to find any report on the treatment of esters or amides of shorter homologues of ornithine by trypsin.

The position of the guanido group or the amino group in the side chain not only influenced the rate of tryptic hydrolysis, but also determined whether the compound

TABLE I

Tryptic Inactivation of Vasopressin Analogues; Rate Constants of the First Order

Compound	k_1 min^{-1}	Compound	k_1 min^{-1}
I[a]	0·229	*XI*[a]	0·321
II, *III*	0·0	*VII*	0·046
IV, *V*		*VIII*	0·054
VI		*IX*	0·022
		X	0·0

[a] See the Experimental, incubation conditions.

could serve as a substrate for the enzyme. This fact may influence the further synthesis of analogues of lysine or arginine vasopressin. The basic amino acid could be replaced by its lower homologues, which might result in a peptide resistant to the action of trypsin or trypsin-like enzymes.

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REFERENCES

1. du Vigneaud V., Lawler H. C., Popenoe E. A.: J. Amer. Chem. Soc. *75*, 4880 (1953).
2. Acher R., Chauvet J.: Biochim. Biophys. Acta *12*, 487 (1953).
3. Acher R., Chauvet J.: Biochim. Biophys. Acta *14*, 421 (1959).
4. Barth T., Pliška V., Rychlík I.: This Journal *32*, 1058 (1967).
5. Baines N. J., Baird J. B., Elmore D. T.: Biochem. J. *90*, 470 (1964).
6. Roberts D. V., Elmore D. T.: Biochem. J. *141*, 545 (1974).
7. Baird J. B., Curragh E. F., Elmore D. T.: Biochem. J. *96*, 733 (1965).
8. Curragh E. F., Elmore D. T.: Biochem. J. *93*, 163 (1964).
9. Elmore D., Roberts D., Smyth J.: Biochem. J. *102*, 728 (1967).
10. Klesov A. L.: Bioorg. Chim. *3*, 1100 (1977).
11. Vaněčková J., Barth T., Prusík Z., Jošt K., Rychlík I.: This Journal *40*, 3230 (1975).
12. Berde B., Boissonnas R. A., Huguenin R. L., Stürmer E.: Experientia *20*, 42 (1964).
13. Jošt K., Procházka Z., Cort J. H., Barth T., Škopková J., Prusík Z., Šorm F.: This Journal *39*, 2835 (1974).
14. Zaoral M., Brtník F., Barth T., Machová A.: This Journal *41*, 2088 (1976).
15. Zaoral M., Kolc J., Šorm F.: This Journal *35*, 1716 (1970).
16. Zaoral M., Krchňák V.: Unpublished results.
17. Zaoral M., Brtník F.: This Journal *40*, 905 (1975).
18. Huguenin R. L., Boissonnas R. A.: Helv. Chim. Acta *49*, 695 (1966).
19. Zaoral M., Brtník F., Flegel M., Barth T., Machová A.: This Journal *44*, 1179 (1979).
20. Prusík Z., Sedláková E., Barth T.: Hoppe Seyler's Z. Physiol. Chem. *353*, 12 (1972).
21. Krejčí I., Kupková B., Vávra I.: Brit. J. Pharmacol. Chemother. *30*, 497 (1967).
22. Lindeberg E. G. G.: Int. J. Peptide Protein Res. *7*, 395 (1975).

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