ACTIVATION OF VASOPRESSIN HORMONOGENS BY KIDNEY AMINOACYLARYLAMIDASE; STUDY *in vitro*

Karel HAUZER, Tomislav BARTH and Karel JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received June 9th, 1978

Under conditions *in vitro* the mammalian kidney aminoacylarylamidase splits off the individual glycine residues from the hormonogens of vasopressin and its analogs of the triglycyl-vasopressin type (triglycyl[8-lysine]vasopressin, triglycyl[8-ornithine]vasopressin and des-glycineamide triglycyl[8-lysine]vasopressin) generating consequently the hormone or its analogues. The time course of the reaction was followed by the biological method (pressoric assay) and by determing the concentrations of the liberated glycine. The generated hormone or its analogues are resistant to the action of the enzyme used.

Since the preparation of the first synthetic hormonogen Gly-Cys[1]-oxytocin by du Vigneaud[1] some years have elapsed. The programmed synthesis of the hormonogen series was performed several years later in both the oxytocin[2,3] and vasopressin[4-6] analogue series. From the aminoacylated analogues so far synthetized and acting as hormonogens only one, namely triglycyl[8-lysine]vasopressin[6], was successfully applied in medical practice. Pharmacokinetic studies were performed aiming at the determination of the rate at which triglycyl[8-lysine]vasopressin was generated under conditions *in vivo*[7-9]. The model studies performed with isolated enzymes (aminopeptidases) indicated the possible participation of some enzymes from special tissues in the liberation of the hormone proper[10]. In this paper we present the results of an *in vitro* study of the activation of some hormonogens by pig kidney aminoacylarylamidase[11].

EXPERIMENTAL

Materials

Triglycyl[8-lysine]vasopressin and desglycine amide triglycyl[8-lysine]vasopressin were prepared as indicated in the paper of Procházka and coworkers[12], triglycyl[8-ornithine]vasopressin according to the paper of Lebl and coworkers[13]. [8-Lysine]vasopressin was synthetized in the Department of Organic Synthesis and purified by free-flow electrophoresis[14]. The pig kidney aminoacylarylamidase was isolated according to the procedure of Pfleiderer and Celliers[11], using

L-leucine *p*-nitranilide as substrate, its specific activity was 80 mkat $\times kg^{-1}$ at 37 °C and pH 7.5. The remaining tryptic activity of the enzyme preparation (trypsin was used for the preparation of enzyme)[11] was determined by means of the chromogenic substrate N-benzoyl-D,L-arginine *p*-nitranilide[15]. Proteins were estimated according to Lowry and coworkers[16]. Thin layer sheets (Silufol^R) were purchased from the firm Kavalier, Votice.

Methods

Incubation conditions for enzyme and substrate: 40 μL of enzyme having the indicated activity were incubated with 50 μL of 10 mM substrate solution and 10 μL of 0·2M Na-phosphate buffer pH 7·5 for 30, 60 and 120 min at 37°C. The reaction was stopped by 5 min boiling.

Paper electrophoresis. After the termination of the reaction, the reaction mixture was separated by paper electrophoresis according to Durrum[17] in pyridine–acetic acid buffer, pH 5·7, at a potential drop of 20 V/cm.

Thin-layer chromatography. The reaction products of enzymic hydrolysis of triglycyl[8-lysine]-vasopressin and of the other hormonogens were separated by chromatography in a system of 98% EtOH–H_2O (7 : 3, v/v). The amount of glycine liberated from triglycyl[8-lysine]vasopressin was determined using the Automatic Amino Acid Analyzer (The Development Workshops ČSAV, type 6020).

Biological assay. The pressoric assay performed on pithed rats[18] was used for the estimation of the rate of conversion of triglycyl[8-lysine]vasopressin to [8-lysine]vasopressin.

RESULTS AND DISCUSSION

The enzyme preparation used for the activation study of vasopressin hormonogens was devoid of tryptic activity. It was fully confirmed by both methods applied, *i.e.* thin-layer chromatography and electrophoresis, that free glycine was liberated during the incubation of individual substrates with the enzyme. The liberation of the glycyl-glycine dipeptide was not observed under the given experimental conditions. In the case of [8-lysine]vasopressin only a slight decrease of the biologic potency was found when the hormone was incubated with the enzyme. This confirms the generally accepted view that tissue aminopeptidases are unable to split the cyclic nonapeptide unless the cyclic structure of the hormone is abolished, *e.g.* by the reduction of the disulfide bridge.

The rate at which the corresponding hormone was generated from the two hormonogens with pressoric activity, namely triglycyl[8-lysine]vasopressin and triglycyl[8-ornithine]vasopressin, was estimated on the basis of the shortly lasting change of blood pressure characteristic for the effect of the parent hormone. The hormogens used are without intrinsic activity in the pressoric assay and the slow change in the blood pressure is attributed to the effect of generated hormones[19]. A mixture of hormone and hormonogen injected *i.v.* results in a biphasic course

of blood pressure response with two maxima. The amount of hormone generated was calculated with regard to this finding (Table I).

In the case of triglycyl[8-lysine]vasopressin we compared the rate at which [8-lysine]vasopressin was generated with the amount of liberated glycine (Table I). The rate constants were not determined, owing to the complexity of the reaction (three-substrate consequent reaction with glycine as a product of all three reactions). Nevertheless, we were able to show that after 30 min of reaction, final conversion to [8-lysine]vasopressin occurred in one out of 25 molecules of substrate attacked (*e.g.* tri-, di-, and monoglycyl[8-lysine]vasopressins) and that, after 60 and 120 min of incubation, the total conversion increased to one out of 14 and 12 molecules attacked, respectively.

Des-glycineamide triglycyl[8-lysine]vasopressin, an analogue, described only recently had none of the biological activities typical of vasopressin with the exception of an effect on the stabilization of the memory process[12]. We followed the generation of the parent hormone only on the basis of the chromatographically determined glycine. As we expected the rate of glycine liberation was consistent with the rate observed in the case of the above-mentioned hormonogens.

Our results show that the enzyme studied under conditions *in vitro* can generate the corresponding hormone or analogue from hormonogens. Owing to the fact that aminopeptidases are known to be present in many tissues we may expect the generating process to occur in different organs. After the application of triglycyl[8-lysine]-

TABLE I

The Generation of [8-Lysine]vasopressin and [8-Ornithine]vasopressin by the Kidney Aminoacylarylamidase

Time of incubation min	The amount of [8-lysine] vasopressin generated from triglycyl[8-lysine] vasopressin %	The amount of liberated glycine %	The amount of [8-ornithine] vasopressin generated from triglycyl[8-ornithine] vasopressin %
0	0	0	0
30	0·4	10	0·3
60	1·0	14·7	0·4
120	1·6	18·7	0·9

vasopressin, the amount of [8-lysine]vasopressin generated was determined by the biological assay both in urine and blood plasma of experimental animals[7-9].

In one of the recent papers the specificity of enzyme generating the hormone under physiological conditions was discussed[20]. It was shown that the persistence of disubstituted hormonogens is less than that of monosubstituted analogues. This could indicate that the hormonogens with the longer substituent are preferentially hydrolyzed in the dipeptidase mode, and such a reaction is more rapid than when a single amino acid residue is split off from the monoaminoacylated hormone. In our reflections on the mode of enzymic generation of hormone from hormonogens we shall have to take into account the participation of dipeptidases or even endopeptidases in cases when hormonogens with a longer substituting chain are concerned. The question of the possible participation of different hormone-generating systems could be solved by using suitably labelled hormonogens.

REFERENCES

1. Vigneaud du V., Fitt P. S., Bodanszky M., O'Conell M.: Proc. Soc. Exp. Biol. Med. *104*, 653 (1960).
2. Jošt K., Rudinger J., Šorm F.: This Journal *28*, 2021 (1963).
3. Beránková-Ksandrová Z., Bisset G. W., Jošt K., Krejčí I., Pliška V., Rudinger J., Rychlík I., Šorm F.: This Journal *26*, 615 (1966).
4. Zaoral M., Pliška V., Řežábek K., Šorm F.: This Journal *28*, 747 (1963).
5. Zaoral M., Šorm F.: This Journal *30*, 2812 (1965).
6. Kasafírek E., Rábek V., Rudinger J., Šorm F.: This Journal *31*, 4581 (1966).
7. Kynčl J., Rudinger J.: J. Endocrinol. *48*, 157 (1970).
8. Pliška V., Chard T., Rudinger J., Forsling M. L.: Acta Endocrinol. (Copenhagen) *81*, 474 (1976).
9. Pliška V., Chard T., Rudinger J., Forsling M. L.: Experientia *29*, 767 (1973).
10. Beránková-Ksandrová Z., Rychlík I., Šorm F. in the book: *Oxytocin, Vasopressin and Their Structural Analogues* (J. Rudinger, Ed.), p. 181. Pergamon Press, Oxford 1964.
11. Pfleiderer G., Celliers P. G.: Biochem. Z. *339*, 186 (1963).
12. Procházka Z., Krejčí I., Kupková B., Slaninová J., Bojanovská V., Prusík Z., Vosekalna I. A., Maloň P., Barth T., Frič I., Bláha K., Jošt K.: This Journal *43*, 1285 (1978).
13. Lebl M., Barth T., Jošt K.: This Journal *40*, 1167 (1978).
14. Vaněčková J., Barth T., Prusík Z., Jošt K., Rychlík I.: This Journal *40*, 3230 (1975).
15. Erlanger B. L., Kokowsky N., Cohen W.: Arch. Biochem. Biophys. *95*, 271 (1961).

16. Lowry O. H., Roseborough N. J., Farr A. L., Randall R. J.: J. Biol. Chem. *193*, 265 (1951).
17. Durrum E. L.: J. Amer. Chem. Soc. *72*, 2943 (1950).
18. Krejčí I., Kupková B., Vávra I.: Brit. J. Pharmacol. Chemother. *30*, 497 (1967).
19. Kluh I., Sedláková E., Barth T., Cort J. H.: Mol. Pharmacol. *9*, 414 (1973).
20. Kynčl J., Řežábek K., Kasalírek E., Pliška V., Rudinger J.: Eur. J. Pharmacol. *28*, 294 (1974).

Translated by L. Servitová.