ON THE TRYPTOPHAN RESIDUE IN PORCINE LH AND FSH-RELEASING HORMONE*

Yoshihiko Baba, Akira Arimura and Andrew V. Schally

VA Hospital and Tulane University School of Medicine, New Orleans, La.

Received August 17, 1971

Summary. The effect of 2-hydroxy-5-nitrobenzyl bromide on the biological activity of a preparation of pure porcine LH and FSH-releasing hormone (LH-RH/FSH-RH) was reinvestigated. Since this treatment as well as performic acid and incubation with anhydrous trifluoroacetic acid, caused a complete inactivation of LH-RH/FSH-RH, tryptophan residue is thought to be essential for the biological activity of this polypeptide.

The structure of hypothalamic polypeptide of porcine origin endowed with luteinizing hormone-releasing hormone and follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) activity was recently elucidated in our laboratory (1, 2). It was shown to be a decapeptide, containing a residue of tryptophan, with the following amino acid sequence: (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. At an earlier stage of our structural analyses, we investigated the effect of various chemical and enzymatic treatments on the biological activity of this polypeptide (3, 4). Because the amounts of pure LH-RH/FSH-RH were limited, we utilized in this work a partially purified material (3, 4). The results obtained were generally in good agreement with our proposed structure. However the data on tryptophan residue were inconsistent. In this communication, we summarized our recent inactivation experiments concerning tryptophan, which suggest the importance of this residue for the biological activity of LH-RH/FSH-RH.

MATERIALS AND METHODS

LH-RH/FSH-RH used in the present investigation consisted of essentially pure samples of the material made as described previously (3-5) or by a new isolation method based mainly on countercurrent distribution (6).

* Supported by grants from VA, USPHS grant AM-07467 and Population Council, New York, New York to AVS.

The reaction with 2-hydroxy-5-nitrobenzyl bromide (7) was performed as follows: To the solution of 10 µg of pure LH-RH/FSH-RH in 100 µl of 0.1 N Na citrate buffer pH 3.0, 50 µg of 2-hydroxy-5-nitrobenzylbromide in 250 µl acetone were added. The mixture was kept at room temperature for 16 hours and then evaporated to dryness in vacuo. An aliquot consisting of one one-hundredth of this preparation was employed for bioassays, and the remainder was used for the estimation of tryptophan residue. Performic acid oxidation was carried out as described by Hirs (8). Incubation with anhydrous trifluoroacetic acid was performed at room temperature for 16 hours. Tryptophan was estimated using an automatic Spinco-Beckman model 120 C amino analyzer provided with microcuvettes and 1 mV range card after hydrolysis in constant boiling hydrochloric acid containing 5% thioglycollic acid (9). As a model of the peptide containing tryptophan, (pyro)Glu-His-Trp NH_2 (Abbott Laboratories) and Met-Glu-His-Phe-Arg-Trp-Gly (Ciba) were used. Chymotryptic digestion and dansylation experiments were carried out as reported previously (1, 2).

LH-RH activity was determined in vivo by the stimulation of release of LH in ovariectomized rats pretreated with estrogen and progesterone as described previously (3). FSH was determined in duplicate by the double antibody radioimmunoassay method using NIAMD-rat-FSH-RIA kit. The values were expressed in terms of NIAMD-rat-FSH-RP-1 (10, 11).

RESULTS AND DISCUSSIONS

In our previous experiment, 2-hydroxy-5-nitrobenzyl bromide was allowed to react with a purified preparation of LH-RH/FSH-RH in 0.001 N HCl-acetone solution, without monitoring pH, because the reaction scale was too small to permit it. Model experiments using (pyro)Glu-His-Trp NH_2 and Met-Glu-His-Phe-Arg-Trp-Gly established that about 50 percent of tryptophan residue was modified under these conditions. However, these experiments had no detectable effects, neither on the LH-RH, nor on the FSH-RH activity. As our structural investigation of LH-RH/FSH-RH, continued, some evidence for the presence

of tryptophan residue became apparent. Althouth no cystine residue was found, even in the impure material, both LH-RH and FSH-RH activities were abolished by performic acid (Table I). Moreover, anhydrous trifluoroacetic acid also caused a similar inactivation suggesting the oxidative decomposition of tryptophan residue in acidic medium. Finally tryptophan was clearly established during the composition and structural analyses of the pure material. These findings prompted us to reinvestigate the effect of 2-hydroxy-5-nitrobenzylbromide. Pure material was treated by this reagent in citrate buffer instead of 0.001 N hydrochloric acid. The tryptophan residue was modified by 93% under these conditions, and both LH-RH and FSH-RH activities were abolished (Table I).

Similarly, when we investigated the effects of performic acid and incubation with trifluoroacetic acid using pure LH-RH/FSH-RH, chymotryptic digests of both inactivated materials showed by dansylation only glycine as a major new N-terminus. Since the chymotryptic digest of native LH-RH/FSH-RH showed both serine and glycine as N-terminal residues of cleavage fragments (1), it is concluded that tryptophan residue adjacent to the serine residue was modified by these treatments.

Our results suggest that the tryptophan residue in LH-RH/FSH-RH is essential both for LH-RH and FSH-RH activity. As Fawcett pointed out (12), the estimates of the nature of unknown substances on the basis of modification of biological activity, using impure materials, must be interpreted with great caution. In the case of LH-RH/FSH-RH, discrepancies were found often in the results of the inactivation experiments obtained in different laboratories (12, 13). Bogentoft et al. (13) reported recently that 2-hydroxy-5-nitrobenzylbromide inactivated LH-RH in extracts from pig and cattle hypothalami in agreement with our present results. They estimated the molecular size of LH-RH/FSH-RH as a decapeptide on the basis of their inactivation experiments (13) and our report (5) on the amino acid ratios of porcine LH-RH/FSH-RH after acid hydrolysis without thioglycollic acid. Such

Table I

LH-RH AND FSH-RH ACTIVITIES AFTER VARIOUS TREATMENTS

Treatment	Mean Serum LH Level (ng/ml) * Sample	Control LH-RH		Mean Serum FSH Level (ng/ml)** Sample	Control LH-RH	
2-hydroxy-5-nitro-benzyl Bromide	7.7 ± 0.3 (2.5 ng LH-RH)	blank	7.4 ± 1.0	0.91 ± 0.6 (50 ng LH-RH)	blank	0.91 ± 0.1
		0.5 ng	17.6 ± 0.7		10 ng	1.29 ± 0.6
		2.5 ng	67.2 ± 8.7		50 ng	1.40 ± 0.7
Performic Acid Oxidation	11.3 ± 1.98 (50 ng LH-RH)	blank	10.8 ± 1.95	inactivation reported already (4)		
		10 ng	44.3 ± 7.00			
		50 ng	74.1 ± 13.6			
Trifluoroacetic Acid Incubation	5.7 ± 1.2 (50 ng LH-RH)	blank	8.4 ± 1.7	not tested		
		50 ng	74.0 ± 11.0			

(Note) Pure LH-RH/FSH-RH used for the treatment with 2-hydroxy-5-nitrobenzyl bromide (AVS 77-33 #215-269) was over ten times more active than the material used previously (AVS 77-3 #320-339).

* In terms of NIH-LH-S-14.

** FSH-RH activity was determined *in vivo* by the stimulation of release of FSH in immature male rats.

an estimate is really not possible without quantitative analyses for tryptophan. These were not made since their materials were still impure.

REFERENCES

1. Matsuo, H., Baba, Y., Nair, R.M.G., Arimura, A. and Schally, A.V.: Biochem. Biophys. Res. Commun. 43: 1334, 1971.
2. Baba, Y., Matsuo, H. and Schally, A.V.: Biochem. Biophys. Res. Commun. 44: 459, 1971.
3. Schally, A.V., Baba, Y., Arimura, A., and Redding, T.W.: Biochem. Biophys. Res. Commun. 42: 50, 1971.
4. Schally, A.V., Baba, Y., and Redding, T.W.: Neuroendocrinology 8: 70, 1971.
5. Schally, A.V., Arimura, A., Baba, Y., Nair, R.M.G., Matsuo, H., Redding, T.W., Debeljuk, L., and White, W.F.: Biochem. Biophys. Res. Commun. 43: 393, 1971.
6. Schally, A.V., Nair, R.M.G., Arimura, A., and Redding, T.W.: J. Biol. Chem., submitted.
7. Horton, H.R. and Koshland, D.E., Jr.: Methods in Enzymology XI p. 556, Academic Press, 1967.
8. Hirs, C.H.W.: J. Biol. Chem. 219: 611, 1956.
9. Matsubara, H., and Sasaki, R.: Biochem. Biophys. Res. Commun. 35: 175, 1969.
10. Daane, T.A. and Parlow, A.F.: Endocrinology 88: 653, 1971.
11. Parlow, A.F., Daane, T.A., and Schally, A.V.: Program of the 51st Meeting of The Endocrine Society, pg. 83, June, 1969.
12. Fawcett, C.P., in "Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry" p. 242 (J. Meites, ed.), The Williams & Wilkins Co., 1970.
13. Bogentoft, C., Currie, B.L., Sievertsson, H., Chang, J-K., Folkers, K., and Bowers, C.Y.: Biochem. Biophys. Res. Commun. 44: 403, 1971.