STUDIES ON THE STRUCTURE–ACTIVITY RELATIONSHIPS OF SYNTHETIC LHRH ANALOGS

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Received 1 July 1974

1. Introduction

A molecular model for the hypothalamic hormone LHRH, Pyr–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly-NH_2, was proposed by Chang et al. [1] and the significance of the Arg side chain at position 8 was emphasized because of a postulated ionic interaction between the guanidino group and 'a negative charge on the receptor site'. To obtain further information about this problem we studied the corresponding citrulline (Cit), homoarginine (Har) and lysine (Lys) [2,3] analogs.

2. Materials and methods

Cit^8-LHRH (I) was obtained by condensation of Pyr–His–Trp–Ser–Tyr–Gly [4] (IV, R_f^{A*} 0.45–0.55, R_f^C 0.15–0.25, R_f^F 0.52–0.62) and Leu–Cit–Pro–Gly–NH_2 (V, R_f^C 0.3–0.4, R_f^F 0.3–0.4) with dicyclohexylcarbodiimide in the presence of pentachlorophenol [5]. Both I and II were prepared in a stepwise manner to avoid the risk of racemisation. Pure I was obtained by precipitation from hot ethanol (R_f^C 0.33–0.43, R_f^F 0.38–0.48, α_D^{22} −27.8, (*c*=1 in dimethylformamide, amino acid analysis gave correct ratios).

Har^8–LHRH (II): Z-Leu–Lys (Boc)–Pro–Gly–NH_2 (VI, R_f^E 0.45–0.5) obtained in a stepwise manner was treated with trifluoroacetic acid to yield the partially protected tetrapeptide (VII, R_f^D 0.7–0.8) which was reacted with 1-guanyl-3.5-dimethyl-pyrazole. The ensuing Har-derivative (VIII, R_f^D 0.3–0.4) was purified by chromatography in a silica gel column using mixture E. After decarbobenzomylation the free tetrapeptide amide IX (R_f^C 0.2–0.3) was coupled with IV to yield II. The pure product was obtained by precipitation from water–ethanol (R_f^A 0.32–0.42, R_f^F 0.4–0.5, α_D^{22} −39.8, (c=1 in 1% acetic acid, amino acid analysis gave correct ratios).

Lys^8-LHRH (III): VI was decarbobenzoxylated (X, R_f^D 0.45–0.55) and coupled with IV to give a crude decapeptide which was purified by chromatography using the solvent mixture B. The pure product (XI, R_f^B 0.42–0.52) was deblocked by trifluoroacetic acid–anisol–water (7:2:1) mixture to afford III (R_f^A 0.32–0.42, R_f^F 0.4–0.5, α_D^{20} −49.3, c=1 in 5% acetic acid, amino acid analysis gave correct ratios).

3. Biological assay. Results

The ovulation inducing activities of LHRH analogs have been studied in diestrus rats employing the method of Yamasaki and Nakayama [6] and in androgen sterilized female rats by a procedure developed in our laboratory [7].

LH-releasing activities of the LHRH analogs were tested in ovariectomized, estrogen–progesterone blocked rats (OEP-rat), Ramirez and McCann [8]. The rats were used 3 months after ovariectomy, they were given 50 μg estradiolbenzoate and 25 mg progesterone in Ol.hel. 3 days prior to assay. The test materials

* Solvent systems for TLC and column chromatography: A: ethyl acetate–pyridine–formic acid–water (20:20:6:6); B: ethyl acetate–pyridine–formic acid–water (30:20:6:6); C: ethyl acetate–pyridine–acetic acid–water (40:20:6:11); D: ethyl acetate–pyridine–acetic acid–water (60:20:6:11); E: ethyl acetate–pyridine–acetic acid–water (240:20:6:11); F: ethyl acetate–n butanol–acetic acid–water (1:1:1:1).

Table 1
Ovulation-inducing activity of LHRH analogs in diestrus rats. (Synthetic LHRH and its analogs were administered by two subsequent s.c. injections)

	LHRH			Har^8-LHRH			Cit^8-LHRH		
Total dose μg/100 g body weight	No. of rats	Ovulating	%	No. of rats	Ovulating	%	No. of rats	Ovulating	%
0.2	6	3	50	6	0	0	4	0	0
0.8	5	5	100	7	3	43	7	1	14
3.2	4	4	100	7	5	71	6	3	50

Table 2
Ovulation-inducing activity of LHRH analogs in androgen sterilized rats. (Androgen sterilization was performed by injecting testosterone (100 μg) into 3-day old female rats. LHRH and its analogs were administered by two subsequent s.c. injections)

	LHRH			Har^8-LHRH			Cit^8-LHRH			Lys^8-LHRH		
total dose μg/100 g body weight	No. of rats	Ovulating	%	No. of rats	Ovulating	%	No. of rats	Ovulating	%	No. of rats	Ovulating	%
0.2	11	5	44	4	0	0	4	0	0			
0.4	8	8	100	8	0	0	4	0	0			
0.8	20	18	90	13	8	61	3	0	0	3	0	0
1.6				4	3	75	4	0	0			
3.2	5	5	100	4	4	100	4	2	50	4	2	50
6.4	5	5	100	6	6	100	5	3	66			
12.8										5	5	100

were injected into the tail vein. The effects of the analogs on plasma LH-activity following stimulation were followed by the ovarian ascorbic acid depletion assay (OAAD) [9].

As shown in table 1, 50%, 43% and 50% ovulation was caused by 0.2 μg LHRH, 0.8 μg Har^8-LHRH, and 3.2 μg of Cit^8-LHRH respectively.

In androgen sterilized female rats (table 2), 0.2 μg LHRH, 0.8 μg Har^8-LHRH, 3.2 μg Cit^8-LHRH, and 3.2 μg Lys^8-LHRH yielded ovulation in 44, 61, 50 and 50% of the animals respectively. The biological activity of Lys^8-LHRH is known in the literature, it was only tested as a reference preparation.

In our experiments LH-releasing activity of LHRH in the OEP-rats, as measured by bioassay, was generally seen at dose levels of 75–150 ng/rat i.v.

The degrees of OAAD caused by 75–150 ng of LHRH can be achieved with 145.9–540 ng of Har^8-LHRH (extrapolated values) and 314.9–504.6 ng of Cit^8-LHRH respectively (fig. 1).

4. Discussion

The postulated ionic interaction between the basic side chain of Arg^8 in the LHRH molecule and a 'negative charge' on the receptor site was supported by the findings that lysine and ornithine derivatives retained 10–30% activity of the parent hormone while the neutral norvaline or leucine [10] analogs were practically inactive. The relatively high potency of Gln^8-LHRH reported by Yanaihara et al. [10], however, is not in full accord with this supposition.

The hormonal evaluation of the neutral Cit^8-LHRH

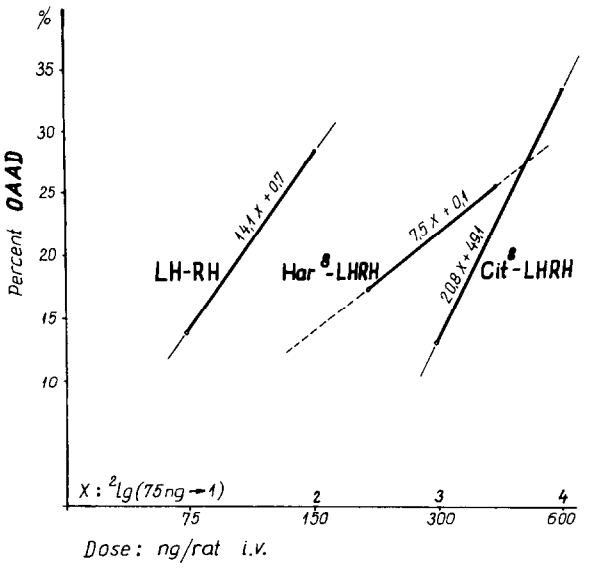

Fig. 1. LH-Releasing activities of LHRH and of its analogs; bioassayable LH in OEP-plasma, 15 min after i.v. injection, as measured by OAAD. OEP: ovariectomized, steroid-pretreated rats.

has revealed that it shows 6–33% activity of the parent hormone depending on the assay used. So it can be presumed that the supposed 'negative charge' on the receptor site is that of a carboxyl group which can combine with the guanidino function of Arg^8 through some bidentate structure formed by both ionic bonds and hydrogen bridges. Considering the data obtained for Cit^8-LHRH (and for Gln^8 derivative as well [10]) it is reasonable to assume that H-bridge forming capacity of the side chain function may substitute the ionic bond in such bidentate structures, nevertheless the H-bridge bond or the electrostatic bond affords by itself a weaker interaction between the carboxyl group and the side chain function than together.

Har^8-LHRH retained 25–50% of the biological potency of the native hormone indicating that the distance between the side chain function and the peptide backbone has a significant influence on the activity.

References

[1] Chang, J-K., Williams, R. H., Humphries, A. J., Johansson, N-G. and Folkers, K. (1972) Biochem. Biophys. Res. Commun. 47, 727.

[2] Chang, J-K., Sievertsson, H., Currie, B. L., Bogentoft, C. and Folkers, K. (1972) J. Med. Chem. 15, 623.

[3] Fujino, M., Kobayahi, S., Obayashi, M., Fukuda, T., Shinagawa, S., Yamazaki, I., Nakayama, R., White, W. F. and Rippel, R. H. (1972) Biochem. Biophys. Res. Commun. 49, 698.

[4] Bajusz, S., Turán, A., Fauszt, I. (1972) in: Chemistry and Biology of Peptides (Meienhofer, J., ed.), p. 325–329. Ann Arbor Sci. Publ. Inc., Ann Arbor, Michigan.

[5] Bajusz, S., Medzihradszky, K. (1967) in: Peptides, Proc. 8th European Peptides Symposium (Beyerman, H. C., Van de Linde, A. and Maasen Van Den Brink, W., eds.), p. 209 North-Holland, Amsterdam.

[6] Yamazaki, I. and Nakayama, R. (1973) Folia Endocrinologica Japonica 49, 189.

[7] Borvendég, J., Hermann, I. and Bajusz, S. (1972) J. Endocr. 55, 207.

[8] Ramirez, V. D. and McCann, S. M. (1963) Endocrinology 73, 193.

[9] Parlow, A. F. (1960) Ciba Found. Coll. endocrin. 13, 300.

[10] Yanaihara, N., Yanaihara, C., Hashimoto, T./Kenmochi, Y., Kaneko, T., Oka, H., Saito, S. (1972) Biochem. Biophys. Res. Commun. 49, 1280.