Stimulatory and Inhibitory Analogs of Luteinizing Hormone Releasing Hormone†

David H. Coy,* Esther J. Coy, Andrew V. Schally, Jesus A. Vilchez-Martinez,‡ Luciano Debeljuk,§ Weldon H. Carter, and Akira Arimura

ABSTRACT: [Des-Gly[10]]-LH-RH ethylamide, a nonapeptide closely related to LH-RH, has about two and one-half times the luteinizing hormone releasing activity and a comparable FSH releasing activity to the natural hormone when assayed *in vivo*. In addition to being enhanced, the LH-RH–FSH-RH activity was prolonged. In an effort to produce competitive inhibitors of LH-RH, the [des-His[2],des-Gly[10]]- and [des-Trp[3], des-Gly[10]]-LH-RH ethylamides were prepared. The peptides, as well as [des-Gly[10]]-LH-RH ethylamide, were synthesized by the solid-phase method *via* protected peptides which were cleaved from the solid support by treatment with ethylamine. Protecting groups were then eliminated by reaction with hydrogen fluoride, to give peptides which were purified by column chromatography. Both compounds exhibited virtually no LH or FSH releasing activity *in vivo*. However, the [des-His[2]] peptide was a potent inhibitor of LH-RH *in vivo* whereas the [des-Trp[3]] analog showed little antagonist activity.

The search for antagonists of LH-RH[1] upon which a new contraceptive method might be based has so far resulted in compounds which, at best, are only inhibitory in artificial *in vitro* systems. Among these is [des-His[2]]-LH-RH (Vale *et al.*, 1972) which weakly inhibits the effect of LH-RH on cultures of dispersed rat anterior pituitary cells but which does not produce detectable inhibition *in vivo* (Schally *et al.*, 1973). The synthesis (Fujino *et al.*, 1972, 1973) of the first analog of LH-RH, [des-Gly[10]]-LH-RH ethylamide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH-CH_2-CH_3), more active than the parent hormone, suggested to us that more effective antagonists might be found among analogs based on the ethylamide structure. Presumably the increased activity of this peptide is attributable to increased affinity for receptor sites or a decrease in the rate of enzyme inactivation and clearly these features would ideally be possessed by an efficient antagonist.

In order to investigate more fully the properties of [des-Gly[10]]-LH-RH ethylamide, the nonapeptide was first synthesized by a version of the Merrified solid-phase method (Marshal and Merrified, 1965) described, in part, previously (Coy *et al.*, 1973a,b). Its properties relative to LH-RH were then examined. Since [des-His[2]]-LH-RH is an *in vitro* inhibitor and also has low inherent LH releasing potency (Monahan *et al.*, 1972), it was decided to synthesize [des-His[2],des-Gly[10]]-LH-RH ethylamide in the expectation that a more vigorous antagonist would result. In addition, since [des-Trp[3]]-LH-RH also possesses very low intrinsic activity (Yanaihara *et al.*, 1973), the corresponding octapeptide ethylamide was also synthesized and similarly tested.

Materials and Methods

L isomers of amino acid derivatives used as starting materials were purchased from Bachem, Inc., Marina del Rey, Calif. Microchemical analyses were performed in duplicate by PCR, Inc., Gainesville, Fla. Amino acid analyses were carried out on samples which were hydrolyzed (18 hr) in 6 M HCl containing 4% thioglycolic acid (Matsubara and Sasaki, 1969). Peptide–resins were prepared in a Beckman Model 990 automatic peptide synthesizer. The following thin layer chromatography (tlc) solvent systems were used: R_F[1], 1-butanol–acetic acid–water (4:1:5, upper phase); R_F[2], 1-butanol–ethyl acetate–acetic acid–water (1:1:1:1); R_F[3], ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Sample sizes of *ca.* 20 μg were applied to Brinkman precoated layers of cellulose or silica and spots were visualized using Ehrlich reagent (for peptides containing tryptophan), Pauly reagent (for peptides containing histidine), and iodine vapor.

tert-Butyloxycarbonyl (Boc)-protected amino acids (3.0 mmol) were coupled in turn to a 2% cross-linked, polystyrene–divinylbenzene–proline resin (1.0 mmol of proline) in the presence of dicyclohexylcarbodiimide (3.0 mmol) by a procedure which has been described previously (Coy *et al.*, 1973a, b). Boc protecting groups were removed at each stage by treatment with 1 M HCl in glacial acetic acid. Reactive side chains of amino acids were protected as follows: histidine, dinitrophenyl (Dnp), or tosyl; serine and tyrosine, benzyl; arginine, tosyl. Dinitrophenyl or tosyl groups were removed from histidine during cleavage of the corresponding peptide from the resin and the remaining protecting groups were removed by treatment of the purified protected peptide intermediates with hydrogen fluoride in the presence of anisole (Sakakibara *et al.*, 1967).

Hydrogen fluoride cleaved peptides were first eluted on a column (2.7 × 91 cm) of Sephadex G-25 in 0.2 M acetic acid and always emerged as major peaks. All peptides were finally applied on a column (1.4 × 94 cm) of carboxymethylcellulose (CM-cellulose) equilibrated with 0.002 M ammonium acetate buffer at pH 4.6. A pH and concentration gradient was immediately started by introducing 0.1 M ammonium acetate (pH 7.0) through a 250-ml mixing flask containing starting

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‡ Fellow of the University of Zulia, Maracaibo, Venezuela.

§ Present address: Centro de Investigaciones sobre Reproduccion, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

[1] Abbreviations used are: LH, luteinizing hormone; RH, releasing hormone; FSH, follicle stimulating hormone; Boc, *tert*-butyloxycarbonyl; Dnp, dinitrophenyl.

TABLE I: LH-RH Activity of [Des-Gly[10]]-LH-RH Ethylamide in Ovariectomized, Estrogen–Progesterone-Treated Rats.

Sample	Dose (ng/rat)	Mean LH (ng/ml ± SE) 20 min	40 min	60 min
Saline		6.1 ± 0.2	6.1 ± 0.3	6.1 ± 0.2
Natural LH-RH	0.5	10.4 ± 1.1	7.7 ± 0.9	8.1 ± 0.5
	2.5	29.2 ± 4.4	17.2 ± 3.0	11.8 ± 1.4
[Des-Gly[10]]-LH-RH ethylamide (Coy)	0.25	10.1 ± 1.0	8.2 ± 1.0	7.9 ± 0.8
	1.25	28.2 ± 6.3	17.4 ± 3.7	14.8 ± 3.2
Des-Gly[10]]-LH-RH ethylamide (Fujino)[a]	0.25	9.6 ± 1.3	8.1 ± 0.8	6.8 ± 0.8
	1.25	28.3 ± 2.8	18.6 ± 4.6	14.6 ± 4.3

	Potency Rel to LH-RH with 95% Limits (%)
20 min	
Ethylamide (Coy)	190 (73–476)
Ethylamide (Fujino)	186 (106–322)
40 min	
Ethylamide (Coy)	248 (94–780)
Ethylamide (Fujino)	230 (88–676)
60 min	
Ethylamide (Coy)	308 (50–2294)
Ethylamide (Fujino)	248 (49–2320)

[a] Fujino *et al.*, 1972, 1973.

TABLE II: FSH-RH and LH-RH Activity of [Des-Gly[10]]-LH-RH Ethylamide When Infused into Immature Male Rats.

Sample	Dose (ng/rat)	LH (ng/ml)	FSH (ng/ml)
Saline		0.23 ± 0.08	324 ± 54
LH-RH	50	16.9 ± 1.0	1970 ± 230
	150	28.7 ± 2.6	3770 ± 434
[Des-Gly[10]]-LH-RH ethylamide (Coy)	25	16.3 ± 3.0	2950 ± 535
	75	35.5 ± 3.0	5897 ± 714
[Des-Gly[10]]-LH-RH ethylamide (Fujino)[a]	25	20.3 ± 0.5	3030 ± 351
	75	29.1 ± 2.6	4810 ± 480

	FSH Potency with 95% Limits (%)
Ethylamide (Coy)	410 (213–1749)
Ethylamide (Fujino)	381 (205–1313)

	LH-RH Potency with 95% Limits (%)
Ethylamide (Coy)	249 (143–500)
Ethylamide (Fujino)	244 (138–492)

[a] Fujino *et al.*, 1972, 1973.

buffer. Peptides in the effluents were located by measurement of optical density at 280 nm.

pGlu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-NH-CH_2-CH_3 (*I*). Amino acids, including Boc-histidine (Dnp), were joined to the proline–resin (2.78 g, 1.00 mmol). The resulting peptide–resin (3.90 g) was placed in a round-bottomed flask and ethylamine (*ca.* 15 ml) was condensed on top of it. The mixture was stirred at 0° (3 hr) and excess amine was then allowed to evaporate at room temperature. The resin was extracted with methanol (50 ml) and dimethylformamide (three 5-ml portions) and the methanol was removed *in vacuo*. Ethyl acetate (200 ml) when added to the deep yellow dimethylformamide solution produced a copious precipitate of the protected peptide I which was filtered, washed with ethyl acetate, and dried to yield 1.26 g of cream-colored powder (84%). A sample reprecipitated from ethanol–ethyl acetate gave one spot by tlc: R_F[1] (silica), 0.43, $[\alpha]_D^{26}$ −20° (*c* 1.24, dimethylformamide). Amino acid analysis gave: Trp, 0.96; His, 1.09; $EtNH_2$, 1.01; Arg, 0.96; Ser, 0.93; Glu, 0.91; Pro, 1.01; Gly, 1.00; Leu, 1.05; Tyr, 0.90. *Anal.* Calcd for $C_{76}H_{94}N_{16}O_{14}S \cdot H_2O$: C, 59.36; H, 6.29; N, 14.57. Found: C, 59.00; H, 6.04; N, 14.45.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH-CH_2-CH_3 (*II*). The protected peptide I (150 mg), anisole (3.5 ml), and hydrogen fluoride (20 ml) were stirred at 0° for 45 min. After rapid removal of hydrogen fluoride *in vacuo*, the sticky peptide was extracted into 0.1 M acetic acid (50 ml) and the solution washed with ethyl acetate (four 10-ml portions). Lyophilized peptide was subjected to chromatography on Sephadex G-25 followed by CM-cellulose, whereupon a single peak emerged between 875 and 1050 ml. White powder (62 mg, 52%) was obtained by lyophilization to constant weight from water. Tlc revealed one component: R_F[1] (cellulose), 0.58; R_F[2] (silica), 0.48; R_F[3] (silica), 0.56; $[\alpha]_D^{26}$ −55° (*c* 0.97, 0.1 M acetic acid), lit. $[\alpha]_D^{24}$ −56.2° (Fujino *et al.*, 1972). Amino acid analysis gave: Trp, 1.01; His, 1.08; $EtNH_2$, 0.98; Arg, 0.90; Ser, 0.91; Glu, 1.00; Pro, 0.95; Gly, 1.00; Leu, 1.02; Tyr, 0.91.

pGlu-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg-Pro-NH-CH_2-CH_3 (*III*). When the protected peptide III was synthesized in an analogous fashion to peptide I, a white powder was obtained in 39% yield upon reprecipitation from boiling ethanol–ethyl acetate. This material exhibited one spot by tlc: R_F[1] (silica), 0.61; $[\alpha]_D^{27}$ −20° (*c* 0.98, dimethylformamide). Amino acid analysis gave: Trp, 0.93; $EtNH_2$, 0.99; Arg, 1.05; Ser, 0.76, Glu, 0.96: Pro, 1.09; Gly, 1.03; Leu, 0.95; Tyr, 0.93. *Anal.* Calcd for $C_{76}H_{87}N_{13}O_{13}S \cdot 2H_2O$: C, 60.63; H, 6.61; N, 13.13. Found: C, 60.59; H, 6.40; N, 13.35.

pGlu-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH-CH_2-CH_3 (*IV*). The octapeptide (III) (150 mg) was allowed to react with liquid hydrogen fluoride under conditions described for the synthesis of peptide II. The crude material was purified on Sephadex G-25 followed by CM-cellulose where it emerged in the region 690–710 ml. Compound IV (44 mg, 46%) was recovered by lyophilization. Tlc exhibited one spot: R_F[1] (cellulose), 0.75; R_F[1] (silica), 0.55; R_F[3] (silica), 0.69; $[\alpha]_D^{26}$ −38° (*c* 0.95, 0.1 M acetic acid). Amino acid analysis gave: Trp, 0.86; $EtNH_2$, 1.01; Arg, 1.03; Ser, 0.79; Glu, 1.00; Pro, 1.04; Gly, 1.00; Leu, 1.03; Tyr, 0.93.

pGlu-His-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-NH-CH_2-CH_3 (*V*). The protected peptide V was synthesized in the usual manner, histidine being incorporated as its *im*-tosyl derivative. A white powder was obtained in 37% yield after reprecipitation of the crude ethylaminolysis product from boiling ethanol–ethyl acetate. This gave one spot by tlc: R_F[1] (silica), 0.41; $[\alpha]_D^{26}$ −32° (*c* 1.33, dimethylformamide). Amino acid analysis gave: His, 0.90; $EtNH_2$, 0.99; Arg, 1.10; Ser, 0.80; Glu, 1.02; Pro, 1.05; Gly, 1.04; Leu, 1.00; Tyr, 0.84.

TABLE III: LH-RH Activity of [Des-His^2,Des-Gly^{10}]-LH-RH and [Des-Trp^3,Des-Gly^{10}]-LH-RH Ethylamide in Ovariectomized, Estrogen–Progesterone-Treated Rats.

Sample	Dose (ng/rat)	Mean LH (ng/ml ± SE)	Potency with 95% Limits (%)
Saline		4.0 ± 0.2	
Natural LH-RH	0.5	6.4 ± 0.5	
	2.5	16.9 ± 2.9	
[Des-His^2,Des-Gly^{10}]-LH-RH ethylamide	50,000	8.7 ± 1.3	0.001 (0.0004–0.0003)
	250,000	15.5 ± 2.1	
[Des-Trp^3,Des-Gly^{10}]-LH-RH ethylamide	50,000	3.5 ± 0.3	
	250,000	10.9 ± 1.7	0.0004 (0.00015–0.0009)

TABLE IV: Inhibitory Effect of [Des-His^2,Des-Gly^{10}]-LH-RH Ethylamide (Peptide IV) on the Response to LH-RH in Ovariectomized, Estrogen–Progesterone-Treated Rats.

I.C. Infusion	I.V. Injection	Serum LH (ng/ml) + SE: Before Injection	Serum LH (ng/ml) + SE: After Injection	Change in LH (ng/ml)
Saline	Saline	4.3 ± 0.3	4.0 ± 0.5	−0.3 ± 0.88
Saline	LH-RH (2.5 ng/rat)	4.1 ± 0.7	35.6 ± 1.1	31.9 ± 1.3[a]
Peptide IV (100 μg/rat)	Saline	6.4 ± 0.5	8.6 ± 0.4	1.6 ± 0.1
Peptide IV (100 μg/rat)	LH-RH (2.5 ng/rat)	9.1 ± 0.5	20.8 ± 1.3	11.9 ± 0.7
Peptide IV (10 μg/rat)	LH-RH (2.5 ng/rat)	4.4 ± 0.4	19.2 ± 2.3	14.4 ± 1.9
Peptide IV (1.0 μg/rat)	LH-RH (2.5 ng/rat)	4.4 ± 0.3	21.5 ± 0.7	17.0 ± 0.5

[a] Significantly different ($p < 0.01$) from all other ΔLH values (Duncan's new multiple range test).

Anal. Calcd for $C_{65}H_{84}N_{14}O_{13}S \cdot 3H_2O$: C, 57.59; H, 6.69; N, 14.47. Found: C, 57.60; H, 6.31; N, 14.53.

pGlu-His-Ser-Tyr-Gly-Leu-Arg-Pro-NH-CH$_2$-CH$_3$ (VI). The octapeptide V (150 mg) was allowed to react with hydrogen fluoride under the usual conditions. Peptide VI was eluted off CM-cellulose between 860 and 920 ml and weighed 41 mg when lyophilized. Tlc exhibited one spot: R_F^1 (cellulose), 0.54; R_F^2 (silica), 0.35; R_F^3 (silica), 0.50; $[\alpha]_D^{26}$ −62° (*c* 0.52, 0.1 M acetic acid). Amino acid analysis gave: His, 1.01; $EtNH_2$, 0.97; Arg, 1.00; Ser, 0.74; Glu, 1.00; Pro, 1.00; Gly, 1.00; Leu, 1.09; Tyr, 0.97.

Bioassays. LH-RH activity was first determined *in vivo* by the stimulation of LH release at two dose levels in ovariectomized rats (four per group) pretreated with estrogen and progesterone (Ramirez and McCann, 1963; Schally *et al.*, 1971) followed by radioimmunoassay (Niswender *et al.*, 1968) for LH. Serum LH levels were obtained after administration of controls with saline and 0.5- and 2.5-ng doses of LH-RH isolated from natural sources. Two preparations of the highly active [des-Gly^{10}]-LH-RH ethylamide peptide made by ourselves and by Dr. M. Fujino were tested and the LH content of serum samples was measured at 20, 40, and 60 min after injection.

The FSH releasing activity and the LH releasing activity of the peptide were then determined by infusion into 25-day-old male rats (four per group) for 4 hr (Arimura *et al.*, 1972).

[Des-His^2,des-Gly^{10}]-LH-RH ethylamide and [des-Trp^3,des-Gly^{10}]-LH-RH ethylamide were investigated for antagonism to LH-RH by infusion of the particular peptide into the carotid artery of ovariectomized rats (estrogen–progesterone pretreated, four per group) for 2 hr followed by intravenous injection of a standard 2.5-ng dose of LH-RH (Vilchez-Martinez *et al.*, 1973). Increases in serum LH levels were compared with those found after infusion with saline followed by injection of saline, infusion of saline followed by injection of LH-RH, and infusion of potential antagonist followed by injection of saline.

Results

Biological Activities. Table I shows the effects of [des-Gly^{10}]-LH-RH ethylamide, prepared by both solid-phase and classical methods (Fujino *et al.*, 1972, 1973), on serum LH levels which were measured at intervals. Values, expressed relative to LH-RH itself, clearly demonstrate the high LH-releasing properties of the nonapeptide and, furthermore, suggest that these effects are more prolonged than those of the natural hormone. The relative LH releasing potency determined by infusion (Table II) of the two samples of the peptide, prepared by the two different routes, was almost identical. Its FSH releasing potency was also very high.

Inherent LH-RH activities of the two octapeptides IV and VI are shown in Table III and were extremely low, 0.001 and 0.0004%, respectively, of the potency of LH-RH.

Infusion of 100- and 10-μg amounts of octapeptide IV into ovariectomized rats dramatically reduced LH released by the 2.5 dose of LH-RH (Table IV).

Discussion

[Des-Gly^{10}]-LH-RH Ethylamide. The nonapeptide was synthesized conveniently and in good yield by the solid-phase method *via* a protected peptide intermediate which was readily cleaved from the resin by treatment with ethylamine alone at

0°. The free hydrogen fluoride deprotected peptide possessed identical physical and chemical characteristics with material prepared by fragment condensation (Fujino *et al.*, 1973). Samples of both materials gave, within experimental limits, the same levels of biological activity in all the tests which were performed.

The releasing activity calculated from the quick injection (Table I) and infusion (Table III) assays was in the range of 200–250% when compared with LH-RH. This is in agreement with previous estimates (Fujino *et al.*, 1973). The higher FSH-RH activity (*ca.* 400%) is within the experimental error of the assay and does not represent a real dissociation of LH-RH and FSH-RH activity.

The results of the time studies on LH released by LH-RH and the nonapeptide indicate that the activity of the latter is maintained at a higher level over the period of time examined. This is presumably due to a decrease in the rate of physiological destruction of the peptide coupled with increased affinity for pituitary receptor sites. The data emphasize the potential that the analog has for clinical and veterinary use.

[Des-His^{2}des-Gly^{10}]-LH-RH Ethylamide and [Des-Trp^3,des-Gly^{10}]-LH-RH Ethylamide. The LH-RH activities of both these octapeptides were satisfactorily small, being, in fact, lower than the corresponding analogs based on the normal LH-RH structure. In view of the high doses which have so far been required for producing detectable inhibition, intrinsic activity should necessarily be as low as possible.

The [des-His^2] peptide was evaluated for antagonistic properties in rats *in vivo* at three dose levels. The results in Table IV are encouraging since they represent the first reduction in LH levels induced by LH-RH by peptides tested in this sensitive system. Thus, for instance, [des-His^2]-LH-RH itself was not effective in similar experiments (Schally *et al.*, 1973).

The [des-Trp^3] analog was not an inhibitor at doses as high as 100 μg. With respect to structure–function relationships in the LH-RH molecule, this result would argue that the tryptophan residue is responsible for both biological activity and receptor site binding, whereas the histidine residue, although important for biological potency, can be dispensed of with some retention of binding capability.

Analogs of the natural hormone which are more active than the natural material appear to offer excellent starting structures on which to base compounds for use as inhibitors. Very recently it has been reported (Monahan *et al.*, 1973) that [D-Ala^6]-LH-RH is superactive and we envisage that effective competitive inhibitors might also be based on this structure, particularly in conjunction with the modified C terminus.

References

Arimura, A., Debeljuk, L., and Schally, A. V. (1972), *Endocrinology 91*, 529.

Coy, D. H., Coy, E. J., and Schally, A. V. (1973a), *J. Med. Chem. 16*, 83.

Coy, D. H., Coy, E. J., and Schally, A. V. (1973b), *J. Med. Chem. 16*, 827.

Fujino, M., Kobayashi, S., Obayashi, M., Shinagawa, S., Fukuda, T., Kitada, C., Nakayama, R., Yamakazi, I., White, W. F., and Rippel, R. H. (1972), *Biochem. Biophys. Res. Commun. 49*, 863.

Fujino, M., Shinagawa, S., Yamakazi, I., Kobayashi, S., Obayashi, M., Fukuda, T., Nakayama, R., White, W. F., and Rippel, R. H. (1973), *Arch. Biochem. Biophys. 154*, 488.

Marshal, G. R., and Merrifield, R. B. (1965), *Biochemistry 4*, 2395.

Matsubara, H., and Sasaki, R. (1969), *Biochem. Biophys. Res. Commun. 35*, 175.

Monahan, M. W., Rivier, J., Vale, W., Guillemin, R., and Burgus, R. (1972), *Biochem. Biophys. Res. Commun. 47*, 551.

Monahan, M. W., Vale, W., Rivier, J., Grant, G., and Guillemin, R. (1973), *Program Endocrine Soc.*, 194.

Niswender, G. D., Midgeley, A. R., Monroe, S. E., and Reichert, L. E. (1968), *Proc. Soc. Exp. Biol. Med. 128*, 807.

Ramirez, V. D., and McCann, S. M. (1963), *Endocrinology 73*, 197.

Sakakibara, S., Shimonishi, Y., Kishida, Y., Okada, M., and Sugihara, H. (1967), *Bull. Chem. Soc. Jap. 40*, 2164.

Schally, A. V., Kastin, A. J., Arimura, A., Coy, D. H., Coy, E. J., Debeljuk, L., and Redding, T. W. (1973), *J. Reprod. Fert.* (in press).

Schally, A. V., Nair, R. M. G., Redding, T. W., and Arimura, A. (1971), *J. Biol. Chem. 246*, 7230.

Vale, W., Grant, G., Rivier, J., Monahan, M., Amoss, M., Blackwell, R., Burgus, R., and Guillemin, R. (1972), *Science 176*, 933.

Vilchez-Martinez, J. A., Schally, A. V., Debeljuk, L., Coy, D. H., and Coy, E. J. (1973), *Neuroendocrinology* (in press).

Yanaihara, N., Hashimoto, T., Yanaihara, C., Tsuji, K., Kenmochi, Y., Ashizawa, F., Kaneko, T., Oka, H., Arimura, A., and Schally, A. V. (1973), *Biochem. Biophys. Res. Commun. 52*, 64.