Molecular and Cellular Endocrinology, 85 (1992) 99–107

MOLCEL 02755

A second endogenous molecular form of mammalian hypothalamic luteinizing hormone-releasing hormone (LHRH), (hydroxyproline^9)LHRH, releases luteinizing hormone and follicle-stimulating hormone in vitro and in vivo

J.P. Gautron, P. Leblanc, M.T. Bluet-Pajot, E. Pattou, A. L'Héritier, F. Mounier, G. Ponce, V. Audinot, R. Rasolonjanahary and C. Kordon

Unité de Dynamique des Systèmes Neuroendocriniens (U159) de l'INSERM, Centre Paul Broca, 75014 Paris, France

(Received 20 November 1991; accepted 24 January 1992)

Key words: (Hyp^9)Luteinizing hormone-releasing hormone; Luteinizing hormone-releasing hormone; Mammalian; Pituitary; Luteinizing hormone; Follicle-stimulating hormone

Summary

In vitro and in vivo release of pituitary hormones were studied in the presence of (hydroxyproline^9)LHRH ((Hyp)LHRH), a newly characterized endogenous molecular form of LHRH. Results were compared to those obtained with LHRH itself. (Hyp)LHRH, as LHRH, stimulated both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release in a homothetic manner. The hydroxylated compound was, however, 24 times (in vitro) and 5 times (in vivo) less potent than LHRH. The lower activity of (Hyp)LHRH than of LHRH in the in vitro assay correlated well with a 28-fold lesser potency in a binding test using pituitary membrane preparations. The higher relative potency and the prolonged effect of (Hyp)LHRH in the in vivo test were related to a lesser susceptibility of the hydroxylated form to proteolytic degradation. Effects of LHRH and of (Hyp)LHRH were not additive, both peptides were equally able to desensitize gonadotrophs to a subsequent challenge by the other. Taken together, these observations suggest that both forms of LHRH act at the same receptor site. The lesser affinity of the hydroxylated compound is compensated to a certain extent by its higher resistance to enzymatic degradation. It is concluded that in spite of its lesser potency, (Hyp)LHRH may participate in the regulation of gonadotropins.

Introduction

We have recently reported isolation from the rat hypothalamus of an endogenous, slightly modified molecular form of mammalian LHRH (Gautron et al., 1991). After a 70,000-fold purification, amino acid analysis and enzymatic cleavages permitted to identify that molecule as (hydroxyproline^9)LHRH ((Hyp)LHRH), a peptide derived from a post-translational enzymatic modification of the LHRH precursor. (Hyp)LHRH was found in the brain of several mammalian species including man. It was particularly abun-

Correspondence to: J.P. Gautron, Unité de Dynamique des Systèmes Neuroendocriniens (U159), Centre Paul Broca, 2ter rue d'Alésia, 75014 Paris, France.

dant in the fetal brain, in which it represented about 70% of the total LHRH-like immunoreactivity.

In the present work, we have characterized the biological activity of (Hyp)LHRH by evaluating its capacity to displace LHRH bound to pituitary membranes and to release pituitary hormones, in particular luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in vitro and in vivo.

Materials and methods

Chemicals

Reagents were obtained from the following sources: LHRH, (Hyp)LHRH, (1–9)LHRH and (1–9,Hyp^9)LHRH from Neosystem Laboratoires (France); (D-Ala^6,des-Gly^{10},Pro^9-ethylamide)-LHRH from Peninsula; bovine serum albumin (fraction V), trypsin, trypsin inhibitor, deoxyribonuclease (DNase) and bacitracin from Sigma; Dulbeco's modified Eagle's medium (DMEM); fetal calf serum from Boehringer-Mannheim.

Animals

Male (250–300 g) or female (200–230 g) Wistar rats (Charles Rivers Breeding Laboratories, St. Aubin les Elbeuf, France) were maintained in a 12 h light, 12 h dark photoperiod (07.00/19.00 h, light/dark) in a controlled temperature (22°C) environment with free access to food and water.

Cell culture

Anterior pituitaries were obtained rapidly after decapitation of male rats. Cell dispersion was performed as previously described (Hopkins and Farquhar, 1973). Briefly, anterior pituitaries were dissected in small fragments (about 0.5 mm diameter) and incubated for 15 min with 0.5% trypsin in DMEM at 37°C. DNase (2 μg/ml) was then added for 1 min to the medium. After enzymatic digestion the medium was removed and anterior pituitaries were incubated for 5 min in DMEM with trypsin inhibitor (1 mg/ml). Medium was changed and pituitaries were incubated in Ca^{2+},Mg^{2+}-free medium containing 2 mM EDTA for 5 min followed by a 15 min incubation in the same medium containing 1 mM EDTA. Cells were then mechanically dispersed in Ca^{2+},Mg^{2+}-free medium containing 0.3% bovine serum albumin (fraction V). Cells were counted using a Coulter counter ZBI (Coultronics) and plated in multiwell plates (24 wells) (Nunc, Roskilde, Denmark). Cells were maintained in DMEM supplemented with 10% fetal calf serum pretreated with charcoal (1%), dextran (0.1%) and with antibiotics (penicillin, 1%; streptomycin, 5 mg/ml) for 3–4 days under a humidified atmosphere of 7% CO_2 to 93% air at 37°C. The cells were then washed, pre-incubated for 30 min and incubated for 1 h in serum-free medium at 37°C under the same humidified atmosphere. LH and FSH releases were determined after 1 h incubation in the presence or absence of various doses of LHRH and (Hyp)LHRH. Bacitracin (2×10^{-5} M) was added to the incubation medium to avoid peptide degradation. At the end of the incubation the medium was removed and stored at −20°C until radioimmunoassays (RIAs).

Binding study on the pituitary membranes

Preparation of the membrane fraction. Anterior pituitaries, obtained rapidly after decapitation of male rats, were washed, weighed and homogenized in cold (4°C) 0.25 M sucrose by means of a glass 'Thomas' homogenizer fitted with a Teflon pestle (rotation at 3000 rpm, three up-and-down strokes). The homogenate was centrifuged at 1000 rpm ($650 \times g$ per min) for 3 min in a Sorvall RP-3 refrigerated centrifuge. The supernatant was collected then centrifuged at 42,000 rpm ($50{,}000 \times g$ per min) for 40 min in a L50 Spinco centrifuge. The resulting pellet was resuspended in 5 mM Tris pH 7.4, containing 0.1% bovine serum albumin (BSA) and centrifuged again at 42,000 rpm for 40 min. Finally the pellet was resuspended (110 μl per mg of pituitary tissue) in cold Krebs-Ringer medium containing 120 mM NaCl, 3 mM KCl, 0.67 mM $CaCl_2$, 1.2 mM KH_2PO_4, 5.9 mM glucose, 25 mM Hepes pH 7.4.

Ligand iodination. The superactive LHRH agonist, (D-Ala^6,des-Gly^{10},Pro^9-ethylamide)-LHRH (LHRHa) was used as ligand after its labelling with 125-iodine (IMS-30, Amersham France) according to the chloramine-T method (Nett et al., 1973). The resulting specific activity ranged from 1.1 to 1.5 Ci/mmol and binding to pituitary membranes was 40%.

Binding assay. 25 μl (227 μg of pituitary tissue) were added to 200 μl of Krebs-Ringer medium containing the iodinated LHRHa at a final concentration of 3.3×10^{-9} M and 25 μl from solutions with different concentrations of various unlabelled LHRHa, LHRH and (Hyp)-LHRH diluted in 5 mM Tris containing 2% BSA, pH 7.4. The final assay volume was 250 μl. After 22 min incubation at 25°C, the reaction was stopped with addition of 2 ml of cold 50 mM Na/Na_2 phosphate buffer, pH 7.0 containing 0.9% NaCl (weight/volume) (PBS) and immediately filtered under vacuum through glass fibre filters (Whatman GF/B) presoaked during 3 h before filtration in 1.2% polyethylamine (50% aqueous solution, Sigma) in water at 0.5°C as previously described (Bruns et al., 1980). Assay tubes were washed twice with 2 ml PBS and filters were counted. Each experimental point was run in triplicates.

In vivo experiments

Experiments were performed on freely moving female rats castrated for 4 weeks. Two to 3 days before the experiments, an indwelling cannula was inserted into the right atrium under ether anesthesia as previously described (Bluet-Pajot et al., 1986). After surgery, the cannula was filled with heparinized saline (250 IU/ml), and the animals were allowed to recover in individual cages. On the day of the experiment, a polyethylene tubing was filled with heparinized saline (25 IU/ml) and connected to the end of the cannula 2 h before blood collection. Serial blood samples (0.3 ml) were withdrawn and collected on ice 15 min before and just before, as well as at regular intervals during 6 h after intravenous injection of LHRH or of (Hyp)LHRH (injection started at 10.00 h). After centrifugation, red blood cells from samples collected before injection were resuspended in saline and reinjected in order to minimize hemodynamic changes. Plasma was stored at -20°C until RIAs. Treatments were randomized among three experiments.

Degradation studies

LHRH and (Hyp)LHRH (5–60 μM) were incubated at 37°C for 20 min in 100 μl of 50 mM Tris, pH 7.3 containing 15 mM dithiothreitrol (DTT) and an aliquot (18 μg protein) of male rat hypothalamic homogenates centrifuged at $100{,}000 \times g$ for 60 min. Incubations were stopped by heating at 90°C for 10 min. Samples were diluted in 1 ml of 14% acetonitrile (ACN) in H_2O containing 0.1% (v/v) trifluoroacetic acid (TFA), centrifuged and injected under high performance liquid chromatography (HPLC) conditions on an ODS Spherisorb column (50×4.1 mm) (Interchim, France) eluted for 15 min with a linear gradient from 14 to 19% ACN in H_2O containing 0.1% TFA at a flow rate of 1 ml/min. Elution profiles were monitored at 210 nm. LHRH, (Hyp)LHRH and their degradation products were identified by coelution with synthetic LHRH-like peptides previously chromatographed for the calibration of the column.

Hormonal determination

Levels of LH, FSH, prolactin (PRL), growth hormone (GH) and thyroid stimulating hormone (TSH) were determined by validated RIAs according to methods previously described (Midgley, 1967; Niswender et al., 1968, 1969; Kieffer et al., 1974; Bluet-Pajot et al., 1978). Briefly, rat (r) LH RP (reference preparation) -1, LH CSU120 antiserum (from Dr. Niswender, Colorado State University, Boulder, CO, USA); rFSH RP-1, rFSH-S11 antiserum (from Dr. Parlow, Pituitary Hormone and Antisera Center, California, USA); rPRL RP-3, rPRL S9 antiserum; rGH RP-2, rGH S5 antiserum and rTSH RP-2, rTSH S5 antiserum were used for the assays.

All hormones were iodinated by the chloramine-T method. Separation of bound from free radiolabelled hormones was achieved in all cases except GH by the second antibody method. For GH, a charcoal adsorption method was used. The sensitivity was 5 ng/ml for LH, 5 ng/ml for FSH, 1.5 ng/ml for PRL, 1 ng/ml for TSH. Inter- and intraassay variabilities were below 15%.

Statistics

Data are represented as the mean ($\pm$SEM).

For in vivo experiments, the area under the curve (AUC) was calculated as the integration of hormone levels in conventional units and time intervals in minutes without deduction of baseline values. Results were analyzed by Dunnett's *t*-test.

In vitro data were submitted to a variance analysis with parametric tests allowing calculation of parallelism and potency ratios. Binding data were subjected to a non-linear least-square fitting analysis using one- or two-site models as previously described (Martres et al., 1984).

Results

Binding experiments

Specific binding of the superactive radiolabelled LHRH agonist (LHRHa) to a pituitary membrane preparation from normal male rats was displaced in a parallel manner by increasing concentrations of cold LHRHa or LHRH with respective IC_{50} of $3.5 \pm 0.6 \times 10^{-9}$ M and $6.7 \pm 0.6 \times 10$–8 M (Fig. 1). Under the same conditions, (Hyp)LHRH also competed with radiolabelled LHRHa binding with a 28-fold lesser potency than that of LHRH itself (IC_{50}: $1.9 \pm 0.5 \times 10^{-6}$ M).

In vitro gonadotropin release

Addition of LHRH to dispersed pituitary cells sampled from normal male rats and maintained in primary culture induced a dose-dependent release of FSH and of LH (Fig. 2). Maximal medium levels of both hormones reached almost 30-fold basal values after 60 min stimulation; concentrations of LHRH which elicited half-maximal stimulation (EC_{50}) of both hormones were comparable and slightly lower than the nanomolar range.

Addition of (Hyp)LHRH stimulated LH release with a very similar amplitude; larger doses,

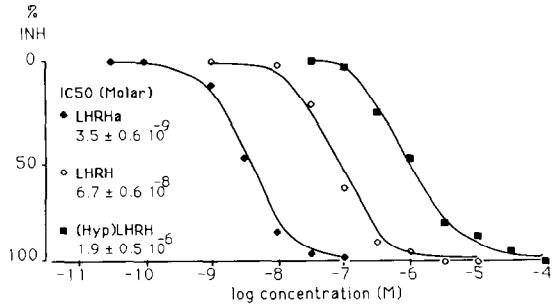

Fig. 1. Competitive inhibition curves of [^{125}I]LHRHa specific binding by cold LHRHa, LHRH and (Hyp)LHRH. Experiments were performed at 25°C on pituitary membrane fractions from normal male rats in the presence of a fixed concentration (3.9×10^{-9} M) of [^{125}I]LHRHa. The data are the means (±SEM) from three independent experiments where each point was determined in triplicate. The data are expressed as the percentage of inhibition (INH) of [^{125}I]-LHRHa specific binding. IC_{50} for each peptide are indicated.

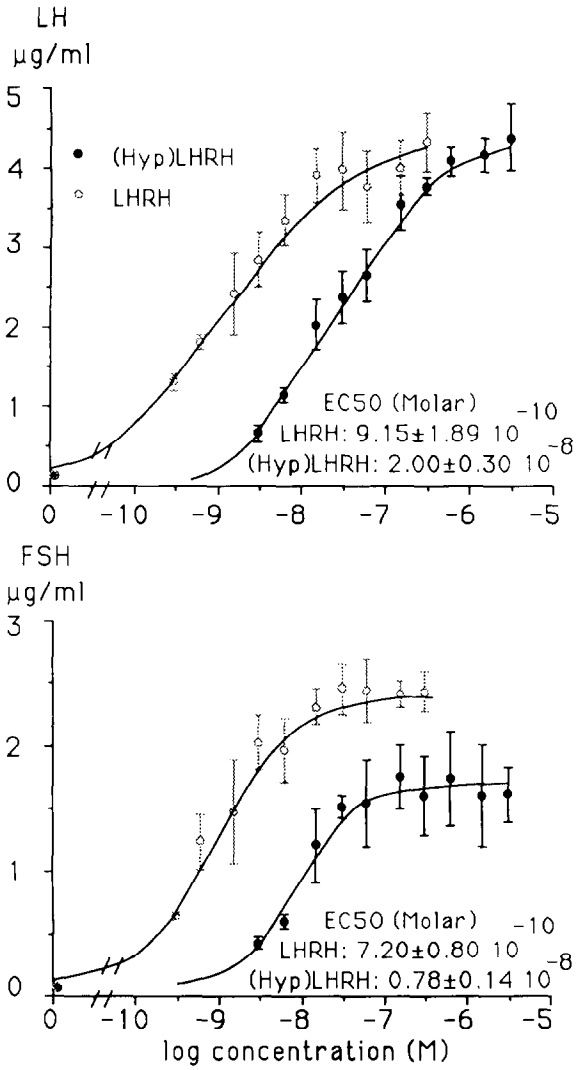

Fig. 2. Dose-dependent effect of LHRH and of (Hyp)LHRH on the in vitro gonadotropin release. LH and FSH secretion was determined in the culture medium after 1 h incubation at 37°C in the presence of 2×10^{-5} M bacitracin and various concentrations of LHRH or of (Hyp)LHRH. Culture dishes contained 7×10^5 pituitary cells from normal male rats. EC_{50} for each peptide are indicated and they are calculated with the means from four independent experiments which gave the same results. Each point and vertical bars represent the mean (±SEM) of eight determinations for one experiment.

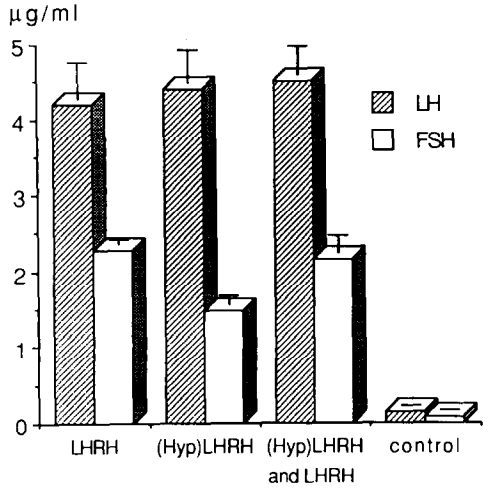

Fig. 3. Non-additivity between LHRH and (Hyp)LHRH effect on the in vitro gonadotropin release. LH and FSH release was determined in the culture medium after 1 h incubation at 37°C in the presence of 2×10^{-5} M bacitracin with either 3×10^{-7} M LHRH or 3×10^{-6} M (Hyp)LHRH or mixed peptides. Culture dishes contained 7×10^{5} pituitary cells from normal male rats. Results are mean (±SEM) of eight determinations.

however, were necessary to produce the effect. Potency ratios calculated by a 4-point linear regression analysis for two independent experiments indicated that (Hyp)LHRH was 24 times less potent than LHRH with confidence limits for $P \geq 0.95$ of 10.23 and 58.14. The hydroxylated peptide was also able to stimulate FSH, although the maximal stimulation obtained for that hormone was slightly lower than when LHRH itself was used.

Parallely, neither LHRH nor (Hyp)LHRH had any effect on PRL, GH or TSH release (data not shown).

Joint addition of both peptides to the incubation medium in order to test their possible additivity resulted in LH stimulation to an identical extent than when each peptide was added alone (Fig. 3). As already shown in Fig. 2, maximal stimulation of FSH was slightly lower after incubation with (Hyp)LHRH than with LHRH; coincubation with both peptides resulted in the same amplitude of stimulation as that induced by LHRH alone.

When tested for its capacity to desensitize the FSH or the LH response to repeated activation of LHRH receptors, (Hyp)LHRH showed identical properties to those of the traditional decapeptide. After a 4 h pre-incubation with either peptide, a subsequent 1 h challenge with the other indicated an identical loss of responsiveness of the pituitary cells (Fig. 4).

In vivo effects of LHRH and (Hyp)LHRH on plasma gonadotropin levels

Intravenous infusion of LHRH or (Hyp)LHRH to cannulated, free moving 4-weeks castrated female rats induced an elevation of plasma LH levels in a dose-dependent manner (Fig. 5). In both cases, maximal plasma concentrations were observed between 5 and 15 min after injection. For the highest doses used, plasma levels were still maximally stimulated as long as 30 min after injection. Five-fold higher doses of (Hyp)LHRH than of LHRH itself were necessary to produce a

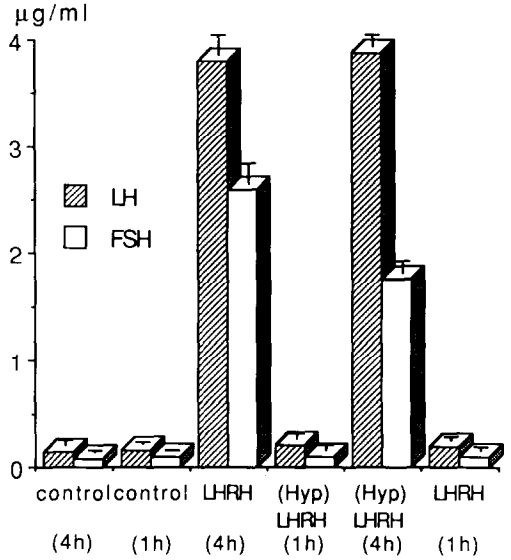

Fig. 4. In vitro desensitization of gonadotrophs by LHRH and (Hyp)LHRH. Pituitary cells (7×10^{5}/dish) from normal male rats were pre-incubated for 4 h in the absence of peptide (control) or in the presence of 3×10^{-7} M LHRH or 3×10^{-6} M (Hyp)LHRH. After that preincubation period, dishes were washed (2 times) with fresh medium, then cells, pre-incubated in the absence of peptide or in the presence of LHRH or (Hyp)LHRH, were incubated for 1 h without peptide (control) or with 3×10^{-6} M (Hyp)LHRH or with 3×10^{-7} M LHRH respectively. All the procedures were performed in the presence of 2×10^{-5} M bacitracin. Results are the mean (±SEM) of eight determinations.

comparable amplitude of stimulation, as shown in Fig. 6. Depending upon the dose of LHRH injected, LH levels returned to basal levels between 60 and over 240 min after the peptide was infused. In the case of (Hyp)LHRH, the time needed for restoration of basal levels was slightly longer than for LHRH, in particular for the intermediate and the largest doses applied. In those cases, the LH response even tended to show a biphasic profile, with a second peak within 90 and 120 min of injection (Fig. 5). That second peak was significant when compared to the LH response produced by LHRH during the same test period when calculated according to the AUC method (Fig. 6).

The situation was not as clear for plasma FSH levels because of high dispersion, in spite of a great number of determinations. Nevertheless, the general tendency was an elevation of plasma FSH levels in an apparent dose-dependent manner after injection of LHRH or (Hyp)LHRH (data not shown). In contrast to the LH response, no significant difference was observed between the FSH response to LHRH and (Hyp)LHRH.

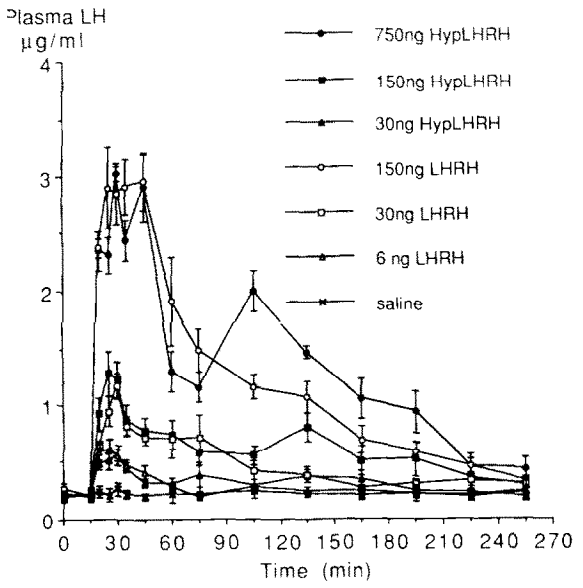

Fig. 5. Kinetics of plasma LH levels after intravenous injection of various doses of LHRH or of (Hyp)LHRH. Each point and vertical bars represent the mean (±SEM) of 12 determinations randomized among three experiments performed on freely moving 4-weeks castrated female rats bearing a cannula into the right atrium in which 750, 150 and 30 ng for (Hyp)LHRH, 150, 30 and 3 ng for LHRH and vehicle (saline) were injected (0.1 ml/injection). Injection occurred 15 min after the first blood collect (0.3 ml).

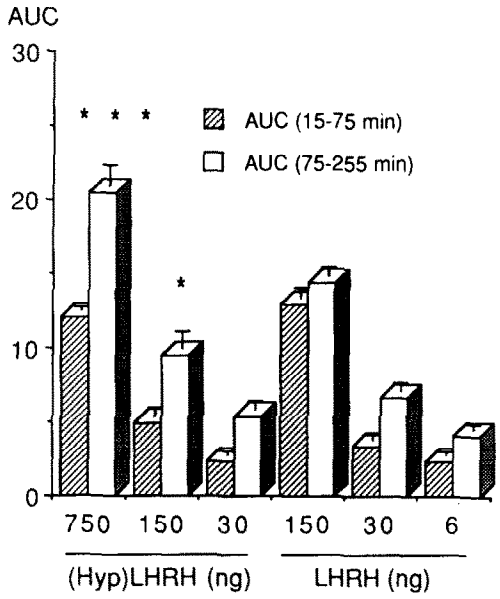

Fig. 6. Areas under the curves (AUC) from kinetics of plasma LH levels after intravenous injection of LHRH or of (Hyp)LHRH. AUC from 15–75 min and 75–255 min test periods were calculated as the integration of LH levels in conventional units and time of testing in minutes without deduction of the baseline values. Results are the mean (±SEM) of 12 determinations. *** $P < 0.001$ vs. 150 ng LHRH effect; * $0.05 < P > 0.01$ vs. 30 ng LHRH effect.

Degradation

During incubation with DTT in the presence of hypothalamic homogenates, the maximal velocity of (Hyp)LHRH hydrolysis (11 pmol/min) was 9-fold lower than that of LHRH (100 pmol/min) (Fig. 7). LHRH only generated the (1–9) N-terminal fragment resulting of cleavage of the Pro^9-Gly-$NH_2{}^{10}$ bond by the post-proline cleaving enzyme.

Discussion

(Hyp)LHRH, a naturally occurring derivative of the LHRH precursor recovered from the hypothalamus of several species of mammals, but also present in amphibians (Gautron et al., 1991), thus seems to exhibit biological properties similar

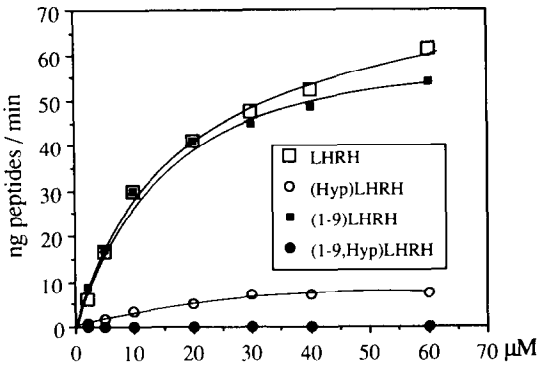

Fig. 7. Kinetics of LHRH and (Hyp)LHRH degradation and (1–9)LHRH production during incubation with DTT in the presence of hypothalamic homogenate. Incubations were performed at 37°C during 20 min and stopped by heating at 90°C for 10 min. LHRH, (Hyp)LHRH and their degradation products were separated on a reverse phase column pre-calibrated with synthetic LHRH-like peptides.

to those of LHRH itself. Under our experimental conditions, the IC_{50} of homologous (D-Ala^6,des-Gly^{10},Pro^9-ethylamide)LHRH binding displacement is lower by one order of magnitude than the affinity of the analog usually reported in other studies. This is due to the fact that our experiments were performed in higher saline concentrations, a condition more compatible with joint evaluation of binding and secretion but known to yield lower K_d values (Loumaye et al., 1983; Leblanc et al., 1990). The affinity of (Hyp)LHRH for the LHRH receptor, as evaluated from its capacity to displace binding of the superactive agonist, (D-Ala^6,des-Gly^{10},Pro^9-ethylamide)LHRH, from pituitary membrane preparations, is 28-fold lower than that of LHRH. As previously suggested from their behavior under HPLC and from their crossreactivity with different anti-LHRH antibodies (Gautron et al., 1991), the conformation of (Hyp)LHRH and of LHRH is certainly not identical. This is probably due to the participation of the Pro^9 residue in the stabilization of the LHRH molecule (Deslauriers et al., 1973, 1975; Momany et al., 1976; Karten et al., 1986). That structural difference may explain the low efficiency of (Hyp)LHRH at the pituitary LHRH receptor level, an interpretation which agrees with the very restrictive selectivity of the mammalian pituitary LHRH receptor towards LHRH-like molecules (Clayton et al., 1980; Milton et al., 1983; Thau et al., 1985; Hattori et al., 1986; Karten et al., 1986; Millar et al., 1989).

When tested for its ability to release gonadotropins from dispersed pituitary cells in primary culture, the potency ratio of (Hyp)LHRH with respect to LHRH is practically identical to that inferred from binding tests (a 24-fold as compared to a 28-fold shift in activity). Under those conditions, release of FSH and of LH by (Hyp)LHRH and by LHRH are quite comparable. A similar result was obtained with pituitary cells sampled from spayed females pretreated or not with estrogen (data not shown). Surprisingly, the maximal amplitude of FSH stimulation appears slightly, but significantly and consistently lower with (Hyp)LHRH than with LHRH. This could theoretically be due to receptor heterogeneity; a corresponding bimodal binding displacement might have remained undetected due to minor representation of FSH cells in our culture. In contrast, this cannot be accounted for by the assumption than (Hyp)LHRH could behave as a partial LHRH agonist on FSH secreting cells, since LHRH is able to produce maximal stimulation even in the presence of maximal concentrations of (Hyp)LHRH. At any rate, however, the hydroxylated peptide cannot be assumed to correspond to the FSH releasing activity reported by some authors in hypothalamic extracts (McCann et al., 1983).

The higher potency ratio of (Hyp)LHRH over LHRH in vivo as compared to that observed in binding as in vitro experiments is not due to a lesser capacity of the hydroxylated peptide to desensitize the gonadotropin response, since preincubation of pituitary cells with either form of LHRH elicited a similar desensitization of the FSH and the LH responses to a subsequent challenge with the other. It cannot either be accounted for by a putative heterogeneity of the pituitary LHRH receptor (which would have a more important impact on the biological test than on binding), since effects of LHRH and (Hyp)LHRH were not additive. The most likely explanation for discrepant potency ratios of both peptides in different tests is thus that of a differential degradability and/or distribution in tissues. Highest LH-releasing activities in mam-

malian (Nestor et al., 1984; Karten et al., 1986) and in submammalian species (Peter et al., 1985) are generally observed for hydrophobic LHRH analogs; this is due to greater distribution or retention in tissues as compared to that of hydrophilic LHRH analogs (Karten et al., 1986). This, however, does not concern (Hyp)LHRH, a peptide which exhibits under HPLC conditions more hydrophilic properties than those of LHRH and most of its analogs (Gautron et al., 1991). In contrast, we were able to show that degradation of (Hyp)LHRH is slower than that of LHRH. The hydroxyl group on the Pro^9 residue of the peptide protects it against hydrolysis by the post-proline cleaving enzyme, a major agent of TRH and LHRH catabolism (Knisatschek and Bauer, 1979).

That interpretation is further substantiated by the kinetics of the in vivo response to intravenous administration of (Hyp)LHRH. Under those conditions, the hydroxylated form of LHRH is only 5 times less potent than the native form, a 5-fold gain over its binding potency ratio. A slightly prolonged action of moderate to high doses of (Hyp)LHRH, as well as their tendency to induce a second peak of LH plasma levels 90 min after their administration, is consistent with the hypothesis of a longer lasting pharmacokinetics of (Hyp)LHRH. Under those conditions as well as in vitro, the potency ratio of (Hyp)LHRH to LHRH itself did not seem to depend upon prior exposure to steroids; a similar situation was observed when test animals were treated with estrogen prior to administering the peptides (data not shown).

In conclusion, the naturally occurring hydroxylated form of LHRH exhibits actions quite comparable to those of LHRH itself. (Hyp)LHRH may therefore be named mammalian GnRH-II or mammalian LHRH-II. Given its affinity, it may not play a major physiologic role in gonadotropic control under steady-state conditions of FSH and LH secretion. Its higher resistance to enzymatic degradation could, however, enable it to be functionally relevant under discrete situations in which a longer half life of the hypothalamic peptide may prove selective. This possibility is also supported by the observation that elevated proportions of (Hyp)LHRH over LHRH are present during ontogenic development as well as in seasonal mammals. Such situations involve clearly different constraints of gonadotropic control, and their constant association with much higher concentrations of the hydroxylated peptide suggests that processing of the LHRH precursor is selectively regulated. Alternately, the less degradable form of the peptide might also be involved in remote control of hypothalamic neurons over peripheral LHRH receptor expressing tissues.

References

Bluet-Pajot, M.T., Schaub, C. and Nassiet, J. (1978) Neuroendocrinology 26, 141–149.

Bluet-Pajot, M.T., Durand, D., Drouva, S., Mounier, F., Pressac, M. and Kordon, C. (1986) Neuroendocrinology 44, 70–75.

Bruns, R.F., Maxfield, F.R., Willingham, M.C. and Pastan, I. (1980) J. Biol. Chem. 255, 1239–1241.

Clayton, R.N. and Catt, K.J. (1980) Endocrinology 106, 1154–1159.

Deslauriers, R., Walter, R. and Smith, I.C. (1973) Biochem. Biophys. Res. Commun. 53, 244–250.

Deslauriers, R., Levy, G.C., McGregor, W.H., Sarantakis, K. and Smith, I.C. (1975) Biochemistry 14, 4335–4343.

Gautron, J.P., Pattou, E., Bauer, K. and Kordon, C. (1991) Neurochem. Int. 18, 221–235.

Hattori, A., Ishii, S. and Wada, M. (1986) Gen. Comp. Endocrinol. 64, 446–455.

Hopkins, C.R. and Farquhar, M.G. (1973) J. Cell Biol. 59, 276–303.

Karten, M.J. and Rivier, J.E. (1986) Endocr. Rev. 7, 44–66.

Kieffer, J.D., Weintraub, B.D., Brigelman, W., Leeman, S. and Maloof, F. (1974) Acta Endocrinol. 76, 495–505.

Knisatschek, H. and Bauer, K. (1979) J. Biol. Chem. 254, 10936–10943.

Leblanc, P., Haour, F., L'Héritier, A., Mounier, F. and Kordon, C. (1990) J. Neuroendocrinol. 2, 509–515.

Loumaye, E. and Catt, K.J. (1983) J. Biol. Chem. 255, 12002–12009.

Martres, M.P., Sokoloff, P., Delandre, M., Schwartz, J.C., Protais, P. and Costentin, J. (1984) Naunyn-Schmied. Arch. Pharmacol. 325, 102–108.

McCann, S.M., Mizunuma, H., Samson, W.K. and Lumpkin, M.D. (1983) Psychoneuroendocrinol. 8, 299–308.

Midgley, A.R. (1967) J. Clin. Endocrinol. Metab. 27, 295–300.

Millar, R.P., Flanagan, C.A., Milton, R.C.deL. and King, J.A. (1989) J. Biol. Chem. 264, 21007–21013.

Milton, R.C.deL., King, J.A., Badminton, M.N., Tobler, C.J., Lindsey, G.C., Fridkin, M. and Millar, R.P. (1983) Biochem. Biophys. Res. Commun. 111, 1082–1088.

Momany, F.A. (1976) J. Am. Chem. Soc. 98, 2990–2995.

Nestor, Jr., J.-J., Ho, T.L., Tahilramani, R., McRae, G.I. and Vickery, B.H. (1984) in LHRH and its Analogues (Labrie,

F., Belanger, A. and Dupont, A., eds.), pp. 24–35, Elsevier, New York.

Nett, T.M., Akbar, A.M., Niswender, G.D., Hedlund, M.T. and White, W.F. (1973) J. Clin. Endocrinol. Metab. 36, 880–885.

Niswender, G.D., Midgley, A.R., Monroe, J.E. and Reichert, L.E. (1968) Proc. Soc. Exp. Biol. Med. 128, 807–811.

Niswender, G.D., Chen, C.L., Midgley, A.R., Ellis, J. and Meites, J. (1969) Proc. Soc. Exp. Biol. Med. 130, 793–796.

Peter, R.E., Nahorniak, C.S., Sokolowska, M., Chang, J.P., Rivier, J.E., Vale, W.W., King, J.A. and Millar, R.P. (1985) Gen. Comp. Endocrinol. 58, 231–242.

Thau, R.B., Limonta, P., Schmidt, F. and Sundaram, K. (1985) J. Steroid Biochem. 23, 811–817.