Synthetic Polypeptide Antagonists of the Hypothalamic Luteinizing Hormone Releasing Factor

Abstract. *Two analogs of the hypothalamic luteinizing hormone releasing factor modified at the histidine-2 position were tested for biological activity (secretion of luteinizing hormone) in cultures of dispersed rat anterior pituitary cells. The analog in which glycine was substituted for histidine at position 2, [Gly²]LRF, behaves as a partial agonist releasing less than 50 percent of the luteinizing hormone secreted at maximum concentrations of the releasing factor, while the analog in which histidine at position 2 is deleted has no significant agonist activity at any of the doses tested. When added to the cultured cells at molar ratios 10^3 to 10^4 times that of the luteinizing hormone releasing factor, both analogs decrease the amount of luteinizing hormone secreted in response to the releasing factor.*

The primary structure of porcine (*1*) and ovine (*2*) luteinizing hormone releasing factor (LRF) has been demonstrated to be pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (*3*). As part of a program aimed at investigating the structure-function relations in the hypothalamic peptide with the ultimate goal of devising antagonists to LRF, we have synthesized, purified, and tested several LRF analogs. We now describe the biological activities in vitro of two LRF analogs, [Gly²]LRF and des-His²-LRF; furthermore, data are presented to show that both suppress the LRF-stimulated secretion of luteinizing hormone (LH) by cultured dispersed anterior pituitary cells.

The peptides were examined for biological activities by observing their effect on the rate of LH secretion by primary cultures of dispersed rat anterior pituitary cells. This method of assay for releasing factors has been described and validated (*4*). Cell cultures were used in secretion experiments 4 to 6 days after their establishment in vitro. After the medium was changed several times and LRF or LRF analogs were added to the desired concentrations to two or three dishes of cells, the test period was continued for 3 hours; in experiments designed to study the effect of an analog on the rate of LH secretion mediated by LRF, the analog was added immediately before the LRF. The medium was removed at the end of the test period, diluted with 1 percent bovine serum albumin in saline, and frozen for future assay of its LH concentration.

Concentration of LH in the culture fluids was measured by a double-antibody radioimmunoassay in which rat LH and rabbit antibody to ovine LH were used (*5*). Using the method of Monahan *et al.* (*6*), we prepared LRF, [Gly²]LRF, and des-His²-LRF, by solid-phase synthesis on a benzhydrylamine resin (*7*).

The effects of these analogs and of LRF on LH secretion rate by the pituitary cells in culture are shown in Table 1. The variations in LH secretion observed between experiments reflect differences in the number of cells per dish as was discussed in (*4*); variations in LH secretion within experiments are related to treatment effects. While having some agonist activity (Table 1, experiments A–D), [Gly²]LRF exhibits a dose response curve with a lower slope than that of LRF and shows less intrinsic activity (*8*), releasing at the highest doses tested ($10^{-4}M$) less than 50 percent of the amount of LH released by the highest concentrations of LRF. At the doses tested ($10^{-6}M$ to $10^{-4}M$) des-His²-LRF has no statistically significant agonist activity (Table 1, experiments D and E). In four experiments [Gly²]LRF shows a negative interaction with LRF: at high concentrations ($3 \times 10^{-5}M$ to $10^{-4}M$), [Gly²]LRF is not additive with low concentrations ($10^{-9}M$) of LRF (experiment B) and decreases the response to high concentrations ($10^{-8}M$ to $10^{-7}M$ of LRF.

The secretion rate of LH, as stimulated by LRF, is reduced 50 percent or more in the presence of 1 to 4×10^3 times the molar ratios of des-His²-LRF over LRF (Table 1, experiments D and E). Results of experiments C and D (Table 1) demonstrate that the inhibition by either antagonist of one concentration of LRF can be overcome by higher levels of LRF, thereby indicating that each analog acts as a competitive inhibitor of LRF.

Both [Gly²]LRF and des-His²-LRF can antagonize the action of LRF; the antagonistic activity of [Gly²]LRF is obscured, however, by the fact that it is also a partial agonist; therefore, only at levels of LRF which stimulate a greater LH release than that due to the partial agonist, will its suppression of LRF-mediated LH secretion be manifested. The antagonism of LRF by des-His²-LRF is more easily visualized

Table 1. Secretion of LH by rat anterior pituitary cell cultures: Interaction of LRF and [Gly²]LRF or des-His²-LRF.

[Gly²]-LRF (M)	LRF (M)	LH secreted per dish (ng ± S.E.M.)
Experiment A		
		334 ± 33
	10^{-9}	1037 ± 26
	10^{-8}	1842 ± 99
8×10^{-5}		820 ± 65
8×10^{-5}	10^{-8}	773 ± 8.5
Experiment B		
		364 ± 0
	10^{-9}	986 ± 85
	10^{-8}	2440 ± 26
3×10^{-5}		901 ± 119
3×10^{-5}	10^{-9}	1046 ± 128
3×10^{-5}	10^{-8}	1428 ± 153
Experiment C		
		< 85
	10^{-9}	90 ± 2.9
	10^{-8}	237 ± 36
	10^{-7}	317 ± 21
10^{-4}		< 85
10^{-5}	10^{-8}	189 ± 5.1
2.5×10^{-5}	10^{-8}	178 ± 8.5
10^{-4}	10^{-8}	124 ± 5.1
10^{-5}	10^{-7}	328 ± 18
2.5×10^{-5}	10^{-7}	330 ± 30
10^{-4}	10^{-7}	228 ± 51

[Gly²]LRF (M)	des-His²-LRF (M)	LRF (M)	LH secreted per dish (ng ± S.E.M.)
Experiment D			
			76 ± 15
		10^{-9}	176 ± 19
		5×10^{-9}	384 ± 24
		2.5×10^{-8}	711 ± 92
10^{-5}			235 ± 45
3×10^{-5}			249 ± 1
9×10^{-5}			315 ± 16
10^{-5}		2.5×10^{-8}	641 ± 38
3×10^{-5}		2.5×10^{-8}	548 ± 13
9×10^{-5}		2.5×10^{-8}	385 ± 19
	3×10^{-6}		97 ± 8
	3×10^{-5}		113 ± 1
	3×10^{-5}	2.5×10^{-8}	298 ± 2
Experiment E			
			325 ± 31
		10^{-9}	901 ± 70
		5×10^{-9}	1598 ± 17
		2.5×10^{-8}	1700 ± 34
	10^{-6}		274 ± 36
	10^{-5}		270 ± 66
	10^{-4}		297 ± 49
	10^{-6}	5×10^{-9}	1530 ± 85
	10^{-5}	5×10^{-9}	707 ± 102
	10^{-4}	5×10^{-9}	384 ± 3
	10^{-6}	2.5×10^{-8}	1819 ± 102
	10^{-5}	2.5×10^{-8}	1751 ± 51
	10^{-4}	2.5×10^{-8}	1028 ± 161

because of its lack of intrinsic activity.

The affinity of the two analogs for the receptor are less than that of LRF, as is evidenced by the high ($\geq 10^3$) molar ratios (antagonist/LRF) required for the inhibition of LRF. Because the affinity of des-His^2-LRF for the LRF receptor is the same or higher than that of [Gly^2]LRF, we can propose that either the presence of the imidazole ring or an amino acid in the L configuration in LRF is important but not obligatory for the binding of LRF to its receptor.

The competitive antagonism to LRF by the two analogs, and the lack of LH releasing activity of des-His^2-LRF at a concentration tenfold higher than that required to suppress the response to LRF indicate a dissociation of the binding and secretory processes. This assumption is supported by our observation (*9*) that des-His^2-LRF competes with [^{3}H-Pro^9]LRF for specific binding to anterior pituitary LRF receptors.

Thus the histidyl residue in LRF is somehow required not only for the recognition of LRF by its receptor but for the intrinsic activity of the molecule. Although important, the imidazole ring (or the presence of an amino acid in the L configuration) is not obligatory for LRF intrinsic activity since substitution of glycine for histidine in LRF yields a molecule with almost 50 percent of the LH releasing activity of LRF. However, the peptide linkage in the 2-position seems to be a requisite for the intrinsic activity of the LRF decapeptide, since des-His^2-LRF has little or no LH releasing ability. In the absence of data on the conformation of either LRF or the structural analogs discussed here, we cannot confidently ascertain whether the pharmacological properties of the LRF analogs are a result of alteration of functional groups or are secondary to changes in the conformation of the molecule.

Several proposed hypotheses could explain the observed dissociation of ligand-receptor interactions and subsequent biological responses (*10, 11*). According to the model presented by Changeux and Podleski (*10*) our results could mean that the LRF receptor site and elements mediating the secretory process can exist in two forms in equilibrium: a secretion-triggering state and a resting state, with LRF having preferential affinity for the "secretory" state. The antagonism of des-His^2-LRF would be a consequence of interaction with and stabilization of the LRF receptor in the "resting" inactive configuration. The partial agonist-antagonist [Gly^2]LRF might have affinity for both states, leading at maximum levels to a distribution of the two states determined by its relative binding affinity for the two forms. Of course, other hypotheses which could explain our results based on an induced fit model [see Koshland and Neet (*11*)] are also plausible.

We have previously described a hypothalamic releasing factor analog that probably competes (with a releasing factor) for binding to a biological receptor site: the dipeptide pGlu-His-OMe apparently functions as a competitive inhibitor of the plasma enzyme that inactivates pGlu-His-Pro-NH_2 (TRF) (*12*). Competitive antagonists of the action of other peptide hormones, vasopressin (*13*), angiotensin (*14*), and glucagon (*15*) have been reported. Also, another des-histidine peptide, des-His^1-glucagon, is a competitive antagonist of glucagon (*15*).

These two LRF analogs are the first peptides reported to be competitive antagonists of the biological activity of LRF. The physiological and potential clinical significance of these LRF antagonists is not yet known.

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3. The abbreviations and notations for amino acid residues are as follows: pGlu, pyroglutamic acid; His, histidine; Trp, tryptophan; Ser, serine; Tyr, tyrosine; Gly, glycine; Leu, leucine; Arg, arginine; Pro, proline; the numbering begins at the residue furthest from the NH_2 group. [Gly^2] means that glycine has been substituted at position 2; des-His^2 means that His has been deleted at position 2.
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Effects on Humans of Δ^9-Tetrahydrocannabinol Administered by Smoking

Abstract. *Twelve chronic marijuana users received Δ^9-tetrahydrocannabinol by smoking. The magnitude of their pulse increment was highly correlated with their subjective experiences. Three of the 12 subjects subsequently received Δ^9-tetrahydrocannabinol labeled with carbon-14; the time course of its concentration in plasma was highly correlated with the pulse increment. Subjective symptoms, however, appeared later and dissipated more slowly.*

Numerous studies have been carried out to assess the effects of marijuana (*1*). In many of these studies, natural marijuana or its putative active component, Δ^9-tetrahydrocannabinol (Δ^9-THC), was administered by smoking. Correlations of the concentration of Δ^9THC in plasma with psychological and physiologic effects after administration by smoking were not, however, made.

We report here on a comparison between a 10-mg dose of synthetic Δ^9-THC and placebo marijuana material, both administered to 12 subjects by smoking. The subjective description of effects was qualitatively similar but quantitatively different for the two states. The magnitude of the syndrome as described subjectively by individuals receiving active Δ^9THC correlated very highly with their respective pulse increments.

In order to assess the time course of these variables, we administered to three of the subjects the same dose of