GONADOTROPHIN RELEASE FROM HUMAN FOETAL PITUITARY CULTURES INDUCED BY FRAGMENTS OF THE LUTEINIZING HORMONE-RELEASING HORMONE

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1. Introduction

Following the isolation from hypothalamic tissue of a decapeptide (LH-RH) with luteinizing hormone (LH) and follicle stimulating hormone (FSH)-releasing activity [1], several groups have described the synthesis of fragments and analogues of the molecule [2, 3]. Although the biological properties of these materials have been extensively investigated in the experimental animal, studies in the human have been mainly limited to the *in vivo* effects of the decapeptide itself. Because results of work on rodent tissues may not be generally applicable to the human subject, we have employed here organ cultures of human foetal pituitaries [4, 5] as means of correlating structure with biological activity for peptides derived from LH-RH. The results have been used to construct a tentative model of the receptor site for LH-RH on the human pituitary cell.

2. Materials and methods

The following peptides were tested: the LH-RH decapeptide (*p*Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly-NH_2) designated $C_{(1-10)}$ [1]; *p*Glu–Gln–Ala-NH_2 [6]; *p*Glu–Tyr–Arg–Trp-NH_2 [7]; fragments of the LH-RH decapeptide, designated $C_{(1-9)}$, $C_{(1-8)}$, $C_{(1-6)}$, $C_{(3-10)}$, $C_{(3-9)}$, $C_{(7-10)}$; a tetrapeptide consisting of the first and last pairs of residues of the decapeptide (*p*Glu–His–Pro–Gly-NH_2). All materials were dissolved in distilled water before addition to the culture medium (1 μl water/ml medium). The medium was then sterilized by passage through a Millipore(R) filter (0.2 μm pore size).

Organ cultures of human foetal pituitaries were maintained in TC199 medium as described previously [4, 5]. Media were changed every 24 hr (with 2 × 15 min wash between changes) and stored at −20°C until assayed. The hormone content of media from two successive 24 hr incubations was measured by double antibody radioimmunoassays for FSH and LH [4]. The concentration of gonadotrophin in medium removed at the end of the second period was expressed as a percentage of that in the medium removed from the same culture at the end of the first incubation period.

2.1. *Effect of peptides on hormone release*

Paired pituitary explant cultures were set up; cultures from one hemipituitary acting as controls for those prepared from the contralateral half. During the second period, the medium in experimental cultures was supplemented with the peptide under test. Control cultures contained medium alone through the experiment.

2.2. *Effect of fragments of LH-RH induced gonadotrophin release*

2.2.1. Studies on the N-terminus $C_{(1-6)}$

Thirty-two non-paired cultures were prepared from 8 pituitaries. In 11 cultures, the medium present in the second period was supplemented with LH-RH (10 ng/ml). In a further 11 cultures LH-RH (10 ng/ml) and the $C_{(1-6)}$ fragment (6.4 μg/ml) were present during the second period. 10 control cultures contained medium alone throughout.

Table 1
Effect of synthetic peptides on gonadotrophin release by paired organ cultures of human foetal pituitaries.

Peptide	Concentration	No. of pairs	LH (%)		FSH (%)	
			Control	Test	Control	Test
*p*Glu–Gln–Ala-NH_2 [6]	5.7 μg/ml	4	92 ± 10	92 ± 6	41 ± 42	53 ± 60
*p*Glu–Tyr–Arg–Trp-NH_2 [7]	4.2 μg/ml	5	77 ± 35	80 ± 34	54 ± 26	65 ± 18
*p*Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly-NH_2 [1]	10 ng/ml	10	69 ± 15	275 ± 189†	56 ± 35	720 ± 640

Hormone release measured during 2 successive 24 hr periods; hormone content of medium removed at end of second period expressed as a percentage of that removed from same culture at the end of the first period.

† P(v controls) < 0.002 (Student's paired t-test). Mean ± S.D.

Studies on the C-terminus were performed in a similar manner.

2.3. *Statistical analysis*

The data were analysed by Student's paired t-test or by non-parametric methods [8].

Table 2
Effect of fragments of LH-RH ($C_{(1-10)}$) on gonadotrophin release by paired organ cultures of human foetal pituitaries (see legend to table 1).

Peptide	Concentration (μg/ml)	No. of Pairs	LH (%)		FSH (%)	
			Controls	Test	Controls	Test
$C_{(1-9)}$	5.0	4	92 ± 27	210 ± 91†	58 ± 21	249 ± 114*
	0.5	8	87 ± 51	322 ± 126†	22 ± 9	810 ± 587†
	0.1	4	39 ± 18	560 ± 130††	19 ± 5	189 ± 35††
$C_{(1-8)}$	5.0	6	65 ± 18	256 ± 268*	71 ± 14	123 ± 40**
	0.5	6	87 ± 16	101 ± 21	26 ± 4	54 ± 19*
$C_{(1-6)}$	6.4	5	89 ± 19	94 ± 45	56 ± 19	52 ± 24
$C_{(3-10)}$	3.0	7	99 ± 16	122 ± 30	71 ± 63	87 ± 48
$C_{(7-10)}$	5.7	5	82 ± 25	76 ± 33	34 ± 37	54 ± 34
$C_{(3-9)}$	4.8	5	90 ± 29	84 ± 13	103 ± 113	79 ± 34

P(v controls); * < 0.025; ** < 0.01; † < 0.005; †† < 0.001 (Student's paired t-test). Mean ± S.D.

Table 3
Effect of peptides related to LH-RH ($C_{(1-10)}$) on gonadotrophin release by paired organ cultures of human foetal pituitaries (see legend to table 1).

Peptide	Concentration (μg/ml)	No. of pairs	LH (%)		FSH (%)	
			Controls	Test	Controls	Test
*p*Glu–His–Pro–Gly-NH_2 [1, 2, 9, 10]	6.6	6	85 ± 20	183 ± 62*	96 ± 60	330 ± 304*
$C_{(1-6)} + C_{(7-10)}$	5.0	4	76 ± 18	422 ± 137*	72 ± 21	343 ± 135*

P (v controls) < 0.025 (Student's paired *t*-test). Mean ± S.D.

3. Results

Table 1 shows the effects of the tripeptide of Igarashi et al. [6], the tetrapeptide of Chang et al. [7] and LH-RH, on gonadotrophin release. At concentrations 500 times greater than those at which the decapeptide was active, neither of the smaller molecules produced detectable hormone release. Removal of the N-terminal amino acids resulted in a total loss of potency (table 2). Modification of the C-terminal produced a more gradual change in properties; the $C_{(1-9)}$ and $C_{(1-8)}$ fragments retaining some of the releasing activity of the complete decapeptide.

Because the amino acid residues at the N and C terminals appeared to be essential for biological activity, the tetrapeptide representing the first and last pair of residues (*p*Glu–His–Pro–Gly-NH_2) was tested. When present in relatively high concentrations, this peptide stimulated gonadotrophin release (table 3). Although the peptides representing part of the N-terminal sequence $C_{(1-6)}$ and part of the C-terminal sequence $C_{(7-10)}$ of LH-RH were inactive (table 2), a mixture of these materials stimulated FSH and LH release (table 3).

It is possible that inactive fragments of LH-RH may block hormone release by competing with the intact molecule for binding sites on gonadotrophs. However, the presence of the $C_{(1-6)}$ fragment did not inhibit LH-RH-stimulated FSH release (table 4), on the contrary it enhanced release of LH, to a small but significant degree (table 4). The $C_{(7-10)}$ fragment did not potentiate or diminish the effects of the decapeptide. In all of these experiments, the ratio of LH to FSH released by active materials was fairly constant and was always greater than unity.

4. Discussion

Our results suggest that the reported gonadotrophin releasing activity of the tripeptide of Igarashi et al. [6] or the tetrapeptide of Chang et al. [7] may not be physiologically significant in man or is perhaps specific for rodent tissue alone. Loss of biological activity in our system, which was associated with deletion of the N-terminal residue, is in agreement with the results of Yanaihara et al. [9]; the rather lesser impor-

Table 4
Effect of the $C_{(1-6)}$ peptide (6.4 μg/ml) on LH release induced by LH-RH (10 ng/ml) (see legend to table 1).

Controls (%)	LH-RH (%)	LH-RH + $C_{(1-6)}$ (%)
78	176	657
70	158	478
70	200	438
52	666	177
48	562	240
71	450	178
57	171	267
66	163	250
82	169	174
100	153	203
	157	490 †

† *P* (v LH-RH) < 0.002 (Mann–Whitney *U*-test).

tance of the C-terminus confirms the conclusions of Chang et al. [10, 11] and Fujino et al. [3], which were based on experiments in the rat.

Because the N and C termini appear to be essential for full biological activity, it is possible that they represent specific sites of interaction with complementary regions on pituitary gonadotrophic cells. The releasing activity of the tetrapeptide consisting of these residues $C_{(1,2,9,10)}$ provides additional evidence for the exisence of C and N terminal receptor 'sites' for LH-RH on the cell surface. In this connection, it was significant that mixtures of the C and N terminal sequences $C_{(1-6)} + C_{(7-10)}$ developed releasing activity that was absent in solutions of the individual peptides. Possibly, at high concentrations of the peptides, a sufficient number of the N and C terminal 'sites' on the receptor may be simultaneously occupied by both fragments so as to produce a significant stimulation of hormone release. Although the conformation of LH-RH is unknown, molecular models of the decapeptide and the $C_{(1,2,9,10)}$ tetrapeptide can be easily aligned to produce very similar conformations of the relevant amino acid residues. It is of interest that the $C_{(1,2,9,10)}$ peptide which has also been reported as showing thyrotrophin releasing activity [12], contains the amino acid sequence of thyrotrophin releasing hormone (TRH) (*p*Glu–His–Pro-NH_2).

Neither of the fragments tested had any effect on the releasing action of LH-RH. Indeed, the $C_{(1-6)}$ fragment actually enhanced LH-RH-induced LH release, possibly because the peptide may have competitively inhibited tissue proteases capable of degrading LH-RH. A similar effect has been noted with certain analogues of TRH which can retard the destruction of TRH by plasma enzymes [13].

Because mixtures of the $C_{(1-6)}$ and $C_{(7-10)}$ peptides stimulated hormone release, it may be assumed that the fragments are capable of binding to the plasma cell membranes of gonadotrophs. Yet neither fragment inhibited the action of the decapeptide, presumably because they are easily displaced from the receptor by LH-RH. This suggests that co-operative effects may occur between the two halves of the molecule resulting in a firmer binding to the receptor than is possible with the fragments in isolation.

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