BIOLOGICAL EVIDENCE THAT SEPARATE HYPOTHALAMIC HORMONES RELEASE THE FOLLICLE STIMULATING AND LUTEINIZING HORMONES

by

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SUMMARY

A preparation from porcine hypothalami essentially free of the decapeptide releasing hormone, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, releases FSH and LH. It released, *in vitro*, a greater amount of FSH than the decapeptide. A certain dose stimulated an unequivocal release of FSH but virtually no LH. Because this activity is chemically separate from that of the decapeptide, and because the relative FSH/LH releasing ratio is greater than that of the decapeptide, it is concluded to be due to FSH-RH. Thus, the decapeptide is LH-RH, and FSH-RH is a separate hypothalamic releasing hormone.

Biological and chemical evidence of Johansson *et al.* (1) and Currie *et al.* (2) indicate that the decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, recently reported by Matsuo *et al* (3), is only LH-RH.

Schally *et al.* (4) proposed that this hypothalamic releasing hormone be designated LH-RH/FSH-RH since they concluded this single releasing hormone was the physiological regulator of both LH and FSH.

From our *in vivo* studies in rats and man, however, we believed that this hormone is only the physiological regulator of LH (5-7). The decapeptide stimulated release of LH was dramatic while the concomitant effect on FSH release was definite but relatively much less; only at high dosages was there a significant release of FSH in rats. In normal men and in women, except at ovulation, the LH releasing activity of the decapeptide was 10 to 20 times greater than its FSH releasing activity. Because of the relatively greater

*Hypothalamic Hormones XLV.

FSH releasing activity of the decapeptide at or near ovulation, it became apparent that the LH and FSH surge at ovulation could be explained solely by the LH-FSH releasing activity of the decapeptide, and, thus, it seemed likely that this was the hypothalamic hormone which regulates ovulation.

Of importance is the observation that for clinical patients dissociated serum levels of LH and FSH are not infrequently observed which would be difficult to explain by only the action of the decapeptide or, preliminarily, even by the effects of the gonadal hormones on the LH and FSH releasing activity of the synthetic decapeptide (8).

Our in vitro studies with the decapeptide and its analogs or derivatives indicate the very close relationship of the LH and FSH activities of these peptides (9). Using pituitaries from 20 day old female rats, the minimal amount of the decapeptide and the related peptide analogs necessary for stimulating LH release was found always to stimulate FSH release; as the levels of the peptides were increased, there was a greater release of both pituitary hormones. These results suggest a uniqueness in the chemical structural relationship of LH-RH and FSH-RH. The hormonal data described herein are compatible with the data of Johansson et al. (1) on biosyntheses and an apparant FSH-RH, and with the data of Currie et al. (2) on the chemical existence and partial purification of FSH-RH.

METHODS: Sprague-Dawley rats were obtained from the Charles River or Carworth Laboratories. The synthetic decapeptide was synthesized by the method of Chang et al. (10). Partially purified fractions of FSH-RH having low LH-RH activity were obtained from porcine hypothalami as described by Currie et al. (2). Continuation of chromatographic fractionation yielded a preparation essentially free of the decapeptide (the FSH-RH preparation) which was used in these biological studies and will be detailed in combination with the details of the first steps (2). In vitro studies were performed as described (2,9). After two one hour preincubations (P-1 and 2), the preparation tested was added at each incubation period (I-3 to I-6). The medium was changed hourly. Reagents for the radioimmunoassay of FSH were kindly supplied by Dr. A. Parlow of NIAMD, NIH. Dr. G. Niswender generously supplied the anti-ovine LH serum No. 15 for the rat LH assay and Dr. L. Reichert the ovine LH preparation for labelling and the rat LH reference preparation. The values for these assays are calculated in terms of ng of the following standards: LH-LER-1240-2(0.60 NIH-LH-S1 units/mg) and FSH 2.1xNIH-FSH-S1 units/mg).

RESULTS: As recorded in Table I, all levels of the synthetic decapeptide stimulated release of LH and FSH, and for both hormones, a dose-relationship

TABLE I. EFFECT OF SYNTHETIC LH-RH ON RELEASE OF LH AND FSH FROM PITUITARIES OF 20 DAY OLD FEMALE RATS

	nanograms LH-RH/ml added to I-3 thru I-6									
	0.1	0.3	0.9	10	25	25	100	500	500	
	ng FSH/ml medium									
P-1	1,900	2,500	2,250	1,000	1,600	1,000	2,100	1,500	1,000	1,700
P-2	1,600	3,150	2,500	3,600	2,000	2,500	2,350	2,500	2,500	2,300
I-3	1,960	8,100	6,750	13,250	10,000	15,250	23,250	18,750	15,200	15,500
I-4	1,940	8,500	17,500	24,250	22,000	26,750	20,650	35,000	28,000	24,000
I-5	1,800	8,000	15,000	25,300	25,700	28,500	28,900	25,600	17,350	13,250
I-6	1,850	4,500	17,000	29,500	25,000	27,000	27,000	24,000	16,150	14,350
	ng LH/ml medium									
P-1	68	80	109	45	212	20	59	40	38	68
P-2	45	85	70	54	158	23	60	50	40	20
I-3	77	225	250	415	245	395	840	775	945	540
I-4	66	253	593	1,063	830	863	1,583	1,215	1,563	1,225
I-5	52	295	705	1,650	1,750	925	1,275	1,425	1,193	990
I-6	50	265	418	1,550	1,783	618	1,315	1,463	1,008	850

TABLE II. IN VITRO EFFECT OF PARTIALLY PURIFIED FSH-RH AND SYNTHETIC LH-RH ON RELEASE OF FSH AND LH FROM PITUITARIES OF 20 DAY OLD FEMALE RATS

P-1	P-2	I-3	I-4	I-5	I-6	P-1	P-2	I-3	I-4	I-5	I-6
ng LH/ml medium						ng FSH/ml medium					
					FSH-RH*						
-	-	0.3	1	3	10	-	-	0.3	1	3	10
138	50	85	54	121	465	1,500	1,500	1,850	3,300	13,200	35,100
250	38	83	28	52	460	<1,000	1,900	2,050	2,600	12,600	65,500
30	27	49	58	92	>714	<1,000	<1,000	2,000	4,200	11,700	62,500
					ng LH-RH						
-	-	.03	0.1	0.3	0.1	-	-	.03	0.1	0.3	1.0
21	20	80	430	685	>714	1,400	1,150	3,950	14,250	17,900	27,900
50	53	255	607	580	682	<1,000	1,250	8,400	16,350	18,500	24,700
40	18	115	90	215	622	<1,000	1,000	2,150	5,250	14,550	15,850

*Doses recorded as equivalents of porcine hypothalamic fragments. Releasing hormones added to medium of I_3-I_6.

occurred between 0.1 and 0.9 ng. Essentially the same amounts of FSH and LH were released by 10, 25, 100 and 500 ng of the decapeptide. Noteworthy is that in only one instance (I-4 at 100 ng) was the FSH release greater than 30,000.

In the study recorded in Table II increasing doses of the FSH-RH preparation or the decapeptide were consecutively added to each incubate. Especially important were the results obtained when doses of the FSH-RH preparation equivalent to 3 hypothalamic fragments were added to the medium, when there was an unequivocal stimulation of FSH release and virtually no stimulation of LH release. Comparable amounts of FSH were stimulated by 0.1 ng of the decapeptide and, in contrast to the FSH-RH preparation, the stimulated release of LH was marked. The highest dose of the FSH-RH preparation

TABLE III. IN VITRO EFFECT OF PARTIALLY PURIFIED FSH-RH AND SYNTHETIC LH-RH ON THE FSH AND LH RELEASE FROM THE PITUITARY

20 Day Old Rats

		FSH-RH			LH-RH	
	#Dose	Female	Male	Dose	Female	Male
		ng FSH/ml medium ±SE		ng/ml	ng FSH/ml medium ±SE	
P-1	-	1,850±229*	<666±0	-	1,366±196	<754±88
P-2	-	2,833±667	<666±0	-	2,083±267	<666±0
I-3	1.5	4,533±840	1,799±572	0.3	19,533±509	4510±145
I-4	1.5	4,650±473	1,944±695	0.3	23,233±3084	4021±563
I-5	7.5	38,666±1167*	12,995±2744	100	30,460±974	9143±826
I-6	7.5	33,766±2879*	14,653±2225	100	22,500±3070	8513±1103
		ng LH/ml medium ±SE			ng LH/ml medium ±SE	
P-1	-	36±6	40±4	-	40±6	57±19
P-2	-	34±4	59±10	-	43±3	43±23
I-3	1.5	34±3	13±3	0.3	427±66	74±12
I-4	1.5	35±9	19±3	0.3	698±11	203±27
I-5	7.5	340±43	140±52	100	>710±0*	388±4*
I-6	7.5	795±146	237±62	100	>710±0	375±39
		Adult Rats**				
		ng FSH/ml medium ±SE			ng FSH/ml medium ±SE	
P-1	-	326±63	1,753±289	-	267±66	1698±482
P-2	-	287±49	2,410±473	-	283±60	1189±116
I-3	1.5	722±29	5,110±670	0.3	778±110	5077±202
I-4	1.5	<666±0	5,422±1031	0.3	721±55	3966±853
I-5	7.5	1,577±179	16,187±742*	100	2,022±269	8277±452
I-6	7.5	2,355±892	15,576±1131*	100	1,555±309	8143±458
		ng LH/ml medium ±SE			ng LH/ml medium ±SE	
P-1	-	85±15	142±48	-	103±34	105±44
P-2	-	91±15	183±36	-	58±22	65±17
I-3	1.5	33±0	78±26	0.3	111±17	176±50
I-4	1.5	33±1	109±62	0.3	153±15	191±43
I-5	7.5	124±13	407±162	100	351±58	505±107
I-6	7.5	217±56	828±372	100	327±47	516±109

#Dose recorded as equivalents of porcine hypothalamic fragments. Each value is the mean result of 3 incubates. *Female vs male - p-value <.01
**200 gm rats.

which was added to the medium stimulated a much greater amount of FSH-release than was ever observed for the synthetic decapeptide. For example, the FSH-RH preparations frequently stimulated FSH release to >128,000 ng/ml (2). In this assay system, seldom have the stimulated FSH levels of the decapeptide, even at high dosages, ever been greater than 30,000 and only on rare occasions 35,000 or 40,000. The FSH-RH preparation in larger dosages also released a large amount of LH in this assay system.

Results recorded in Table III show that the activities of the FSH-RH preparation and the decapeptide can be more easily distinguished, in vitro, when pituitaries were obtained from 20 day old female rather than male rats. In both assay systems, there was a relatively greater release of FSH than LH release when the FSH-RH preparation was added; however, these relative

TABLE IV. COMBINED EFFECT OF SYNTHETIC LH-RH AND PARTIALLY PURIFIED FSH-RH ON FSH AND LH RELEASE FROM PITUITARIES OF 20 DAY OLD RATS, IN VITRO

	Dose LH-RH	Dose* FSH-RH	Female		Male	
			FSH	LH	FSH	LH
	ng/ml		ng/ml medium			
P-1	-	-	4,300	112	<666	36
P-2	-	-	2,800	66	733	11
I-3	0.3	7.5	54,000	533	18,315	302
I-4	0.3	7.5	55,600	>714	21,335	460
I-5	100	7.5	25,974	1688	15,984	532
I-6	100	7.5	37,100	1500	17,798	583

*Dose recorded as equivalents of porcine hypothalamic fragments.

effects of the FSH-RH preparation, at the doses added, were only significant when female pituitaries were used.

In the adult rats somewhat the reverse results were obtained since pituitaries of male rather than female rats were better for separating the activities of the two releasing hormones. Doses of 1.5 h.f.e. of the FSH-RH preparation and 0.3 ng of the synthetic decapeptide both slightly stimulated release of FSH; however, at these doses only the decapeptide stimulated release of LH. When the larger amounts were added to the medium, there was a significantly greater amount of FSH released by the FSH-preparation than by decapeptide. The LH-response of the FSH-RH preparation in the adult female, but not the adult male, was slightly less than that from the decapeptide. In the dosages added to the medium, the difference in the relative degree of FSH and LH activities of the FSH-RH preparation and LH-RH could not be distinguished when the adult female pituitaries were used. Also, the stimulated release of FSH and LH was much less from the female than male pituitary.

Another point of interest is the combined effect of the FSH-RH preparation and the decapeptide. When added to the medium together, the effect appears to be both additive and/or antagonist depending on the ratios of the dosages (Table IV). FSH release upon addition of 0.3 ng of the decapeptide plus the dosage of 7.5 h.f.e. of the FSH-RH preparation was greater than that from either of the hormones alone. As recorded in Table III, when the hormones were individually added to the medium, these dosages of the FSH-RH preparation and the decapeptide for the FSH values were about 36,000 and 21,000 ng, respectively, from the pituitaries of 20 day old female rats. The additive effects of the hormones on FSH release are apparent; in combination, the FSH-values were about 55,000 in comparison to the total of 57,000 when calculated from the individual values. The LH-values were not additive

and may indicate that the release at this dosage-ratio may be antagonistic for LH. In contrast, when the dosage of the decapeptide was increased to 300 fold (100 ng) without changing the dosage of the FSH preparation, there was inhibition of the amount of FSH released, but an increase in the amount of LH released. When the pituitaries were obtained from 20 day old male rats, the combined effects of the releasing hormones on release of both FSH and LH were additive but not seemingly antagonistic.

DISCUSSION: The in vitro biological activity of the partially purified preparation from porcine hypothalami used in these studies which was essentially free of the decapeptide indicates LH-RH and FSH-RH are separate hypothalamic releasing hormones, but our results demonstrate the difficulties in distinguishing their biological activities. Both the synthetic decapeptide or LH-RH and the FSH-RH preparation released LH and FSH, and the relative degrees of LH or FSH releasing activities differentiate them. At certain dosages, however, the FSH-RH preparation stimulated an unequivocal release of FSH with virtually no release of LH. In this same assay system, the decapeptide or even several of the decapeptide analogs or derivatives have never stimulated FSH release without concomitantly stimulating LH release. These results only emphasize the subtlety of the biological relationship of these two hormones, and probably the similarities in their chemical structural relationship.

In vitro, the maximal LH releasing activity of LH-RH and the FSH-RH preparation were the same (unpublished); however, the maximal FSH releasing activity of this and other FSH-RH preparations in comparison with LH-RH was much greater (1). Although these are important points for characterization and demonstration of their separate activities, in vitro, they may have only indirect physiological meaning.

Previous reports have amply described the marked, in vivo, LH but the relatively small FSH releasing activity of LH-RH in rats and man. What is somewhat surprising is that FSH-RH has a remarkable degree of LH releasing activity. It is possible that the serum levels of LH and FSH observed under physiological conditions may be a result of the concomitant secretion of both FSH-RH and LH-RH rather than either alone. If so, evaluation of the relative secretion rates of these two hypothalamic hormones and the interplay of the feedback effects of gonadal hormones will be rather complex. As indicated by the data, the possibility that LH-RH and FSH-RH are natural inhibitors of each other may be considered. Our preliminary results suggest that FSH-RH under some circumstances may inhibit the LH activity of LH-RH while LH-RH may inhibit the FSH activity of FSH-RH. If they do inhibit each

other's effects when secreted in physiological amounts, it only further emphasizes the necessity of studying their concomitant effects. The high LH releasing activity of FSH-RH could indicate that only FSH-RH is secreted during the first and last half of the menstrual cycle while only LH-RH is secreted at ovulation. Of course, to be considered is that FSH-RH is secreted in the first half of the cycle and LH-RH at ovulation and in the last half of the cycle or as previously suggested both are always secreted concomitantly in varying ratios.

From our results, new evidence has been presented which shows that FSH and LH are regulated by separate hypothalamic releasing hormones. Even from this preliminary study on the activities of synthetic LH-RH and the FSH-RH preparation, fundamental questions arise about the way in which LH-RH and FSH-RH may be secreted and interact to regulate LH and FSH release.

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REFERENCES

1. Johansson, K.N.G., Currie, B.L., Folkers, K. and Bowers, C.Y., Biochem. Biophys. Res. Commun., 49, (1972).
2. Currie, B.L., Johansson, K.N.G., Folkers, K. and Bowers, C.Y., Biochem. Biophys. Res. Commun., 49, (1972).
3. Matsuo, H., Baba, Y., Nair, R.M.G., Arimura, A. and Schally, A.V., Biochem. Biophys. Res. Commun., 43, 1334 (1971).
4. Schally, A.V., Arimura, A., Kastin, A.J., Matsuo, H., Baba, Y., Redding, T.W., Nair, R.M.G., Debeljuk, L. and White, W.F., Science, 173, 1036 (1971).
5. Bowers, C.Y., Sievertsson, H., Chang, J.K. and Folkers, K., Fed. Proc., 31, 9 (1972) (abstract).
6. Bowers, C.Y., Malacara, J., Gomez, F., Hernandez, S., Chang, J. and Folkers, K., J. Clin. Invest., 51, 13 (1972).
7. Malacara, J., Bowers, C.Y., Hernandez, S., Gomez, F. and Folkers, K., Serono Foundation Conference, Acapulco, Mexico. Excerpta Medica (1972) (in press).
8. Bowers, C.Y., Malacara, J., Gomez, F., Hernandez, S., Friesen, H., Chang, J. and Folkers, K., 5th International Congress of Pharmacology, San Francisco (1972) (abstract).
9. Bowers, C.Y., Chang, J.K. and Folkers, K., Serono Foundation Conference, Acapulco, Mexico. Excerpta Medica (1972) (in press).
10. Chang, J.K., Sievertsson, H., Currie, B.L., Bogentoft, C., Folkers, K. and Bowers, C.Y., J. Med. Chem., 15, 623 (1972).