ON THE CHEMICAL EXISTENCE AND PARTIAL PURIFICATION OF THE HYPOTHALAMIC FOLLICLE STIMULATING HORMONE RELEASING HORMONE

by

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SUMMARY

Others have stated that the hypothalamic hormone, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is the "gonadotropin-releasing hormone" which regulates both the luteinizing and follicle stimulating hormones (LH and FSH). Such dual regulation is contradictory to a long considered "unitary" interrelationship for hormones of the hypothalamus and pituitary. Four fractions at stage SP and thirteen at stage P-2 released, in vitro, FSH at levels as high as 40,000- >128,000 ng/ml. The release of FSH by synthetic LRH averaged about 16,700 (range 6150-28000) at 500 ng/ml. These data are basic and have priority, because they reveal the unknown FSHRH by differentiation of its activity from that of LHRH; this was not feasible before the availability of LHRH.

Over years of research on the endocrinology of the hypothalamic hormones, it was common thinking on the basis of extensive studies by many investigators that one hormone of the hypothalamus releases one corresponding hormone of the anterior lobe of the pituitary gland. This thinking was essentially a "unitary" interrelationship of the hormones of the hypothalamus and the pituitary. There have been two notable and possible exceptions to this "unitary" interrelationship. One exception was based on the chemically established decapeptide of the hypothalamus, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. Although the hypothalamic hormone now known to have the structure of this decapeptide was initially studied for its release of the luteinizing hormone of the pituitary, it was, in due time, unambiguously proposed to be the natural regulator of two pituitary hormones, namely, the luteinizing hormone (LH) and the follicle stimulating hormone (FSH). A second possible exception to the "unitary" interrelationship followed the finding by Bowers et al. (1) that the chemically established hypothalamic hormone, pGlu-His-Pro-NH_2, released both thyrotropin (TSH) and prolactin (PRL) in man at the low dosage of 10 μg;

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this release of prolactin has been observed by Jacobs et al. (2); (unpublished data, ref. 20, 21, 22 of (3)). Such a finding naturally raised the question of whether pGlu-His-Pro-NH_2 is a natural regulator of both TSH and PRL; a positive answer to this question would be only a partial exception to the "unitary" interrelationship, because the primary hypothalamic hormone which regulates release of PRL is well established to be the prolactin inhibiting factor (PIF). Also, there is known evidence for the dual regulation of the two pituitary hormones, GH and PRL, by both releasing and inhibiting hypothalamic hormones. To emphasize the complexity of determining whether TRH is also PRH, are the data of Valverde-R. et al. (3) which show that an apparent PRH was found in porcine and rat hypothalamic tissue which could be chromatographically separated from pGlu-His-Pro-NH_2 (TRH).

We now describe data which do not support statements that the decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is the natural hypothalamic regulator of both LH and FSH. However, there is no question that administration of this decapeptide to animals and man does release both LH and FSH under certain experimental conditions. Many of the other peptide hormones are known to elicit more than one distinct biological activity, such as, by oxytocin and vasopressin; bradykinin and gastrin are well known to have a spectrum of biological activities.

In 1970, White (4) reported from his studies to isolate LHRH that his final product showed activity in releasing both LH and FSH, at the level of 10-25 ng, and there was no separation of the two activities.

In 1971, Schally et al. and White jointly published (5) the isolation and properties of the FSH- and LH-releasing hormone. Since their isolated product was obtained in an apparently homogeneous state and, in doses of a few nanograms, stimulated the release of LH and FSH, in vivo, and in vitro, they stated "this polypeptide appears to represent the hypothalamic hormone which controls the secretion of both LH and FSH from the pituitary."

Matsuo et al. (6) reported the structure of the porcine LH- and FSH-releasing hormone which is the decapeptide described above.

Schally et al, and White jointly summarized (7) their crucial chemical and biological data on both the isolated and synthetic decapeptides which led them to introduce the name "gonadotropin-releasing hormone" for one polypeptide which regulates the secretion of LH and FSH.

METHODS

Preparation of Hormone Fractions From Porcine Hypothalami

Batches of 5,000 porcine hypothalamic fragments were lyophilized and homogenized with methanolic acetic acid. The homogenate was filtered, and

the filter cake was resuspended in methanolic acetic acid several times with thorough mixing and refiltering. The combined filtrates were evaporated to dryness. The resultant residue was defatted and then lyophilized (stage SP in Table I). The defatted extract was dissolved in 1 M acetic acid and submitted

TABLE I. ASSAY DATA FOR FSH-RH ACTIVITY, IN VITRO

No.	Stage	Pre-Incubation 1 hr.	Pre-Incubation 2 hrs.	Incubation 3 hrs.	Incubation 4 hrs.	Incubation 5 hrs.	Incubation 6 hrs.
					ng FSH/ml		
				ca. 0.5 h.f.e.		ca. 2.5 h.f.e.	
1	SP	<1,000	<1,000	6,500	35,750	69,000	77,000
2	SP	1,000	1,500	13,750	18,900	46,000	>128,000
3	SP	1,450	3,600	31,500	51,500	52,500	39,000
4	SP	2,850	3,600	20,600	51,500	54,750	26,250
1	P2	3,600	3,250	33,750	58,000	>128,000	100,000
2	P2	1,000	<1,000	7,500	12,000	42,750	42,500
3	P2	2,400	2,350	5,750	6,250	66,000	48,250
4	P2	2,400	3,600	38,500	104,500	>128,000	40,250
5	P2	4,000	3,550	58,250	>128,000	>128,000	53,500
6	P2	4,050	4,250	14,500	>128,000	>128,000	49,000
7	P2	2,800	2,500	36,750	92,500	>128,000	>128,000
8	P2	<1,000	1,600	40,500	51,750	38,500	24,000
9	P2	2,050	4,000	49,500	39,000	22,100	21,250
10	P2	6,000	8,000	25,250	22,750	49,000	39,000
11	P2	1,100	3,000	61,000	76,500	76,500	35,250
12	P2	2,850	3,250	40,500	73,500	83,000	40,000
13	P2	1,100	1,000	5,600	7,100	43,500	43,750

to gel filtration on Bio-Gel P-2 (exclusion limit 1800 Daltons). The active regions from two batches were combined and passed through a Bio-Gel P-2 column again (Stage P-2 in Table I). Subsequent steps include partition Sephadex G-25 and CMC columns for chromatography. Samples for hormonal assay were taken after the defatting step and after pooling appropriate fractions from each gel filtration column. Aliquots for assay were lyophilized and dissolved in 0.05M phosphate buffer, pH 7.1. Aliquots of 10 and 50 µl were added to the incubation medium at I_3, I_4 and I_5, I_6, respectively.

In Vitro Assay Procedure

For the in vitro assays, pituitaries were obtained from female rats of the Sprague-Dawley strain which were 20 days old. Two pituitaries for each assay were incubated at 37° C in 1 ml of lactated Ringer's solution (Travenol Laboratories) in 10-ml Teflon beakers in a Dubnoff shaker. After a pre-incubation period of 1 hour (PI_1), the medium was removed for a control assay and fresh medium was added to the system. After a second hour of pre-incubation (PI_2), the medium was removed for assay and replaced. During the incubation

(I), the medium was removed and assayed for release of pituitary hormones four times at one-hour periods, I_3, I_4, I_5, I_6. The samples to be assayed were added after the two-hour pre-incubation period; the total experimental time was six hours. LH was determined by the radioimmunoassay method of Niswender et al.(8), and FSH was determined according to Daane and Parlow (9). The release of LH and FSH were evident by comparison of the pre-incubation and incubation values. The reagents for assay of FSH were generously distributed by Dr. A. Parlow of NIAMD, NIH; Dr. G. Niswender generously supplied the anti-ovine LH serum No. 15. Dr. L. E. Reichert, Jr. supplied the ovine LH preparation for labelling and the reference standard of LH from the rat. The values for these assays were calculated in terms of ng of the following reference standards: LH-LER-1240-2 (0.60 NIH-LH-S1 units/mg) and FSH 2.1 x NIH-FSH-S1 units/mg.

Results and Discussion

The data from the hormonal assays for the activity of FSH on a total of seventeen fractions prepared from porcine hypothalami are in Table I. Four of these fractions represent the hormonal activities after a defatting (stage SP). Thirteen of the fractions represent activities of preparations obtained from passing concentrates over Bio-Gel P-2 columns (stage P-2). In our experience, the procedures used for the preparation of both of these typical concentrates are reproducible.

Synthetic pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LRH) at a level of 0.9 ng in the assay released an average of 18,075 ng/ml of FSH; at a level of 500 ng of LRH, the release of FSH averaged only 16,710 ng/ml. Additional data on the release of FSH by synthetic LRH in this assay are recorded in the accompanying paper by Bowers et al. (10); in these data, only one out of 28 released levels of FSH was above 30,000 ng/ml. (i.e., 35,000).

The four fractions at stage SP (Table I) showed consistent control levels of FSH ranging from <1,000-3,600 ng/ml over the two one-hour periods. Eight of the sixteen assay values for two dose levels of the four samples at three and four-hour periods and at five and six-hour periods ranged from 46,000 to >128,000 ng/ml.

For thirteen fractions at stage P-2, the pre-incubation or control values for the one and two hour periods ranged from <1,000 to 8,000 ng/ml. These control values are in reasonable agreement with those for the four assays of the fractions at stage SP. Thirty-one of fifty-two assay values for the thirteen fractions showed a release of 40,000 to >128,000 ng/ml of FSH. Nine of these fifty-two values were greater than the upper level of the assay or >128,000 ng/ml. Sample 2 in Table I was observed to show a linear log dose-response curve.

It is apparent that there is a hormone present in the hypothalamic fractions at two stages of an isolation process which releases much more FSH-activity than can be attributed to the presence of the decapeptide or LRH. Consequently, these chemical fractionations and assay data constitute evidence for the existence of a hypothalamic FSH-RH which is different from the decapeptide, LRH. The data of Johansson et al. (11) on the biosynthesis of an apparent FSHRH, and the biological evidence that LHRH and FSHRH are separate hormones are compatible with the data described herein.

Geiger et al. (12) reported "two well separated preparations with LH-RH activity." Presently, it is not feasible to determine the relationship between their observations and our results.

Other investigators have detected and measured the activities of hypothalamic releasing hormones at early stages of fractionation. For example, Meites et al. (13) and Pasteels (14) independently observed a prolactin inhibiting factor; characterization of the activity of PIF was confirmed by McCann et al.(15); Chen et al. (16) and Kamberi et al. (17,18). Valverde-R. et al. (3) also utilized data on preliminary fractionation of extracts of porcine and rat hypothalamic tissue to project a prolactin releasing factor which appears different from TRH.

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