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ON THE EXISTENCE AND SEPARATION OF THE FOLLICLE STIMULATING HORMONE RELEASING HORMONE FROM THE LUTEINIZING HORMONE RELEASING HORMONE

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SUMMARY

Porcine hypothalamic fragments were extracted by 2M AcOH at 4°C, and the extractives were subsequently processed in the presence of one protease inhibitor and one anti-oxidant. Gel filtration was performed on Bio-Gel P-2, and supplementary [^{3}H]-LHRH and [^{14}C]-<GluOH were used. The fractions of elution were assayed for both LH- and FSH-releasing activities and for radioactivities. An entity which unambiguously released FSH was separated from [^{3}H]-LHRH, and was differentiated from [^{14}C]-<GluOH and N-Ac-AspOH. This entity may be presumed to be FSHRH, and if so, it may have a molecular weight larger than that of the decapeptide, LHRH.

INTRODUCTION

Many investigators have studied the existence of the follicle stimulating hormone releasing hormone (FSHRH), which has been controversial.

Fawcett et al. (1) described chromatographic evidence for the existence of what they called "another species" of the luteinizing hormone releasing hormone (LHRH). They extracted batches of 50-250 rat hypothalami, and tested fractions with two bioassays. One measured LHRH in vitro, and the other was an RIA for LHRH. Two elution peaks were observed in fractionations. The major peak coincided with synthetic LHRH. Their data could possible be supportive of FSHRH as "another species" of LHRH.

Johansson et al. (2) conducted biosynthesis with ^{14}C-glutamic acid and glutamine in hypothalamic systems, followed by fractionation with Bio-Gel P-2, CMC, Sephadex G-25 and partition chromatography. Certain fractions released levels of 40,000-128,000 ng/ml of FSH; LHRH generally released about 18,000 ng/ml. Bowers et al. (3) summarized the biological evidence supporting the conclusion that separate hypothalamic hormones release FSH and LH. It was found that a fraction from porcine hypothalami,

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essentially free of LHRH, released both FSH and LH. This fraction released a greater amount of FSH, in vitro, than the synthetic LHRH. It was concluded that the decapeptide is LHRH, releasing primarily LH and secondarily FSH, and that FSHRH is a separate hypothalamic releasing hormone.

Igarashi et al. (4) published evidence supporting the existence of an FSHRH which was different from the decapeptide, LHRH. Yu et al. (5) described evidence for the existence of FSHRH. Their investigation was based on separate patterns of LH and FSH release from isolated pituitary tissues which were evoked by synthetic LHRH or by hypothalamic extracts of female rats, respectively, in a continuous perfusion system. Their data indicated that the hypothalamus of the female rat contains a substance other than LHRH, capable of releasing both LH and FSH. Such an unidentified component, in particular circumstances, caused a differential release of LH and FSH.

Folkers et al. (6) summarized and interpreted the evidence for and against the existence of FSHRH, and described a dissociated release of LH and FSH. Koch et al. (7) found that an antiserum to the synthetic LHRH suppressed in rats the surge of both LH and FSH and supported the view that this LHRH naturally regulates both LH and FSH unless the antiserum was not absolutely specific.

Schwartz (8) has stated that physiological data indicate that there must be some separate secretory control of FSH.

This report describes our new investigation on FSHRH.

MATERIALS AND METHODS

Lyophilized porcine hypothalamic fragments were used which had been stored for about 4-5 years at ca. 4°C. A batch of ca. 250 of these fragments (ca. 200 g) was put in dialysis bags which were then filled with 2M AcOH. These bags were then placed for dialysis in 800-ml jars which contained 2M AcOH. Another batch of ca. 250 of these fragments was placed directly into 800-ml jars containing 2M AcOH for extraction. These jars were maintained on a magnetic stirrer for 8 days at 4°C. The dialysate and the extract which was obtained without dialysis were combined and defatted with hexane:ethyl acetate (3:1). The organic layer was washed 3 times with 1M AcOH, and the combined aqueous layers were lyophilized to give a residue. This residue was largely dissolved by 2M AcOH:CH_3OH (1:1) which contained 0.001% phenylmethylsulfenylfluoride (a protease inhibitor) and 0.01% thiodiglycol (9). This solution was centrifuged at 10,000 RPM for 20 min at 4°C, and the supernatant was lyophilized to yield a residue. An aliquot (1.5 g) of this residue (ca. 40 g) was dissolved in ca. 5 ml of 2M AcOH, and 3 µg [^{3}H]-labeled LHRH (0.1 µci) (New England Nuclear) and 12 µg [^{14}C]-labeled < GluOH (0.025 µci) (New England Nuclear) were added. The residue was then applied to a Bio-Gel P-2 column (Bio-Rad Laboratories), using 2M AcOH containing 0.01% thiodiglycol and 0.0001% pentachlorophenol (9) as eluant. The elution was monitored at 254 nm and 206 nm with a 2089 Uvicord III (LKB). Ca. 25% of each fraction was bioassayed as described (10), and ca. 10% of the remaining 75% was used to measure the radioactivity in each fraction. A liquid scintillation counter (Beckman LS-150) using

a Scintillation toluene cocktail (Beckman #187990), was used. This purification step by P-2 was later repeated. One column (II) was run with radioactive LHRH and < GluOH, and the other (III) without the radioactive entities. The two columns gave identical UV-patterns at 280 nm. The third column was also calibrated with radioactive LHRH and < GluOH, and, the radioactive LHRH and < GluOH had the same V_R as in the previous columns of identical size (2.5 x 85 cm). UV was measured manually at 280 nm with the Beckman ACTA CIII; and 1 ml was used to measure radioactivity. 1.5 mg of each fraction was taken for bioassay.

RESULTS AND DISCUSSION

The fractions from the P-2 column were found to contain LH-and FSH-releasing entities in two different areas of fractions.

Exemplary data from column III are in Table I, and because of the complexity, data on FSH and LH in ng/ml are recorded in Figure I as the differential between incubation (I_3) and control (P_2) to reveal better the separation of the entities. The data in Figure I include biological activities and radioactivities.

These data reveal a different distribution of entities which released LH and FSH in comparison to the elution behavior of [^{3}H]-LHRH and [^{14}C]-< GluOH. The release of LH from fractions 15-23 is apparently partially or totally due to the presence of < GluOH and possibly also to some N-Ac-AspOH. There was also release of FSH from fractions 15-23. The release of FSH in the first fractions of this peak can be due to N-Ac-AspOH. The release of FSH in fractions 18-22 can be due to < GluOH. These LH- and FSH-releasing activities of N-Ac-AspOH and < GluOH were previously recognized, and these compounds may be differentiated (11).

The release of LH and FSH, however, from the earlier fractions 6 and 7 from the P-2 column is represented by one distinct peak of biological activity which is well-separated from the peak (fractions 9-15) containing [^{3}H]-LHRH. One could consider that the biological activities of fractions 6 and 7 might be due to LH and FSH. However, porcine LH and FSH do not cross-react in our rat RIA. The other two columns, I and III, gave similar profiles of the release of LH and FSH.

All these data may be considered as a three-fold confirmation of the chromatographic separation of an entity which releases FSH from LHRH as indicated by the supplementary [^{3}H]-LHRH. This entity may be presumed to be FSHRH. In any case, this entity, which may be FSHRH, is less strongly retarded on Bio-Gel P-2 than the synthetic decapeptide, LHRH, and which may indicate that FSHRH may be larger in molecular weight than LHRH.

Table I. Data on Release of LH and FSH.

Fraction No.	Elution ml.	LH		FSH	
		P_2	I_3	P_2	I_3
5	165-173	13	274	1182	5,450.
6	174-182	89	1159	3983	> 42,667.
7	183-191	285	632	5819	17,762.
8	192-199	102	210	4288	6,314.
9	200-210	43	101	1984	4,983.
10	211-230	78	261	3085	8,120.
11	231-249	53	77	2928	3,994.
12	250-269	79	117	4339	7,442.
13	270-289	83	253	3719	11,922.
14	290-308	91	311	3679	9,676.
15	309-328	23	190	2093	14,922.
16	329-348	80	1187	2097	41,943.
17	349-368	83	1238	3045	> 42,667.
18	369-387	161	1148	4715	> 42,667.
19	388-406	80	1345	2861	> 42,667.
20	407-426	80	1389	2307	> 42,667.
21	427-446	99	1549	2219	> 42,667.
22	447-466	101	1385	4393	> 42,667.
23	467-484	86	1286	2260	> 42,667.
24	485-502	93	231	3269	4,866.
25	503-519	162	295	2823	7,078.
26	520-537	184	535	4680	18,407.
27	538-555	99	236	3317	7,356.

I. The level tested was always 1500 μg.

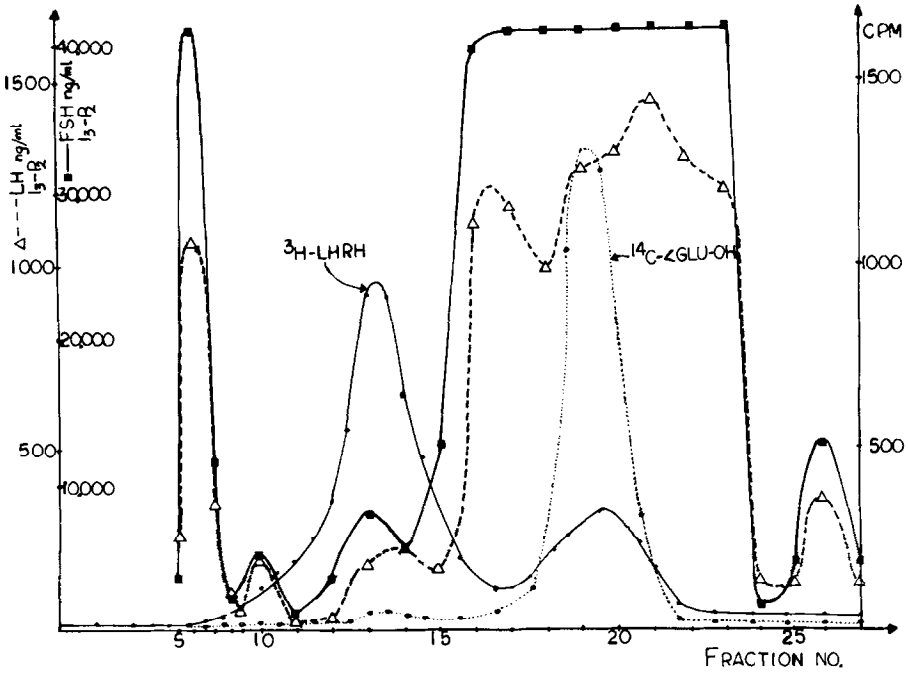

Figure I. Data on Release of FSH and LH

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REFERENCES

1. Fawcett, C.P., Beezley, A.E., and Wheaton, J.E., Endocrinology, 96, 1311, (1973).
2. Johansson, K.N.G., Currie, B.L., Folkers, K., and Bowers, C.Y., BBRC, 50, 8, (1973).
3. Bowers, C.Y., Currie, B.L., Johansson, K.N.G., and Folkers, K., BBRC, 50, 14, (1973).
4. Igarashi, M., Taya, K., and Ishikawa, J., in N.H.T. Micken (ed.) Psychoneuroendocrinology, Workshop Conf. Int. Soc. Psychoneuroendocrinology, Pub. Karger, 1978, Basel, 1974.
5. Yu, J.Y.L., Namiki, H., and Gorbman, A., Life Science, 22, 269, (1978).
6. Folkers, K., Fuchs, S., Humphries, J., Wan, Y.P., and Bowers, C.Y., in Novel Aspects of Reproductive Physiology (C.H. Spillman and J.W. Wilks, eds.,) p. 319; New York: S.P. Medical and Scientific Books, 1978.
7. Koch, Y., Chobsieng, V., Zor, V., Fridkin, M., and Linduer, H.R., BBRC, 55, 623, (1973).
8. Schwartz, N.B., in Novel Aspects of Reproductive Physiology, pp. 336-338; New York: S.P. Medical and Scientific Books, 1978.
9. Rubenstein, M., Stein, S., Gerber, D., and Udenfriend, S., Proc. Nat'l Acad. Sci. USA, 74, 3052, (1977).
10. Lam, Y.K., Knudsen, R., Folkers, K., Frick, W., Daves, G.D., Jr., Barofsky, D.F., and Bowers, C.Y., BBRC, 81, 680, (1978).
11. Fuchs, S., Lam, Y.K., Knudsen, R., Folkers, K., and Bowers, C.Y., BBRC, in press.