The Similarity of FSH-Releasing Factor to Lamprey Gonadotropin-Releasing Hormone III (l-GnRH-III) (44519)

W. H. YU,* S. KARANTH,* S. A. SOWER,† A. F. PARLOW,‡ AND S. M. MCCANN*,[1]

**Pennington Biomedical Research Center (Louisiana State University), Baton Rouge, Louisiana 70808–4124; †Department of Biochemistry and Molecular Biology, University of New Hampshire, Biological Science Center, Durham, New Hampshire 03824; and ‡Harbor-UCLA Medical Center, Torrance, California 90509–2006*

Abstract. **To validate further the existence of a specific hypothalamic follicle stimulating hormone releasing factor (FSHRF), stalk-median eminence (SME) fragments from sheep and whole hypothalami from male rats were purified by gel filtration on Sephadex G-25, and the gonadotropin-releasing activity on hemipituitaries of rats incubated *in vitro* was determined by bioassay and compared with the radioimmunoassayable luteinizing hormone releasing hormone (LHRH) and lamprey gonadotropin releasing hormone (l-GnRH) activities in the fractions. The FSH-releasing fractions eluted in the same sequence of tubes from the Sephadex column found earlier by *in vivo* bioassay and were clearly separated from the immunoassayable and bioassayable LHRH. The radioimmunoassay (RIA) for l-GnRH recognized equally l-GnRH-I and -III but had negligible cross-reactivity with LHRH. Fractionation of rat hypothalamic extract by gel filtration on Sephadex G-25 revealed three peaks of l-GnRH determined by RIA, all of which eluted prior to the peak of LHRH. Only the second peak had FSH-releasing but not LH-releasing activity. To determine if this FSH-releasing activity was caused by the presence of l-GnRH in the fraction, the pituitaries were incubated with normal rabbit serum or the l-GnRH antiserum (1:1000), and the effect on the FSH- and LH-releasing activity of the FSH-releasing fraction and the LH-releasing activity of LHRH was determined. The antiserum had no effect on basal release of either FSH or LH but eliminated the FSH-releasing activity of the active fraction without altering the LH-releasing activity of LHRH. Since l-GnRH-I has little activity to release FSH or LH, and its activity is nonselective, whereas previous experiments have shown that l-GnRH-III highly selectively releases FSH with a potency equal to that of LHRH to release LH, the results support the hypothesis that the FSH-releasing activity observed in these experiments was caused by l-GnRH-III or a closely related peptide.**

[P.S.E.B.M. 2000, Vol 224:87–92]

Although the follicle stimulating hormone (FSH)-releasing activity of hypothalamic extracts was discovered in 1964 (1), separated from luteinizing hormone releasing hormone (LHRH) by gel filtration on Sephadex G-25 in 1965 (2), and further purified by carboxymethyl cellulose chromatography (3), the existence of FSH-releasing factor (FSHRF) has remained controversial (4–6). The existence of FSHRF and its separation from luteinizing hormone-releasing hormone (LHRH) were confirmed in 1966 (7). With the determination of structure of LHRH (8, 9), it was found to have intrinsic FSH-releasing activity (5, 10, 11). Furthermore, Schally *et al*. were unable to separate FSHRF from LHRH by gel filtration on Sephadex (12). Therefore, they concluded that a separate FSH-releasing factor did not exist and changed the name of LHRH to gonadotropin-releasing hormone (GnRH) (5).

Because of discrepancies between bioassay and radioimmunoassay (RIA) of gonadotropins, such as the presence

This work was supported by NIH Grants DK43900 (S.M.M.) and MH51853 (S.M.M.).

[1] To whom requests for reprints should be addressed at the Pennington Biomedical Research Center (Louisiana State University), 6400 Perkins Road, Baton Rouge, LA 70808–4124. E-mail: mccannsm@pbrc.edu

Received November 1, 1999. [P.S.E.B.M. 2000, Vol 224]
Accepted January 26, 2000.

0037-9727/00/2242-0087$15.00/0

in plasma of a bioactive LH that could not be detected by RIA, we fractionated ovine stalk-median eminence (SME) fragments on Sephadex G-25 and incubated the fractions with hemianterior pituitaries of male rats assaying the medium by the Steelman-Pohley bioassay and by RIA for FSH and by RIA for LH. Fractions possessing FSH-releasing activity distinct from those possessing LH-releasing activity were identified by bioassay in the same elution position as had been reported many years earlier (4); however, when FSH released from hemipituitaries *in vitro* was monitored by RIA, all FSH release from these fractions could be accounted for by their LHRH content (4). In those experiments, the extracts were incubated in a static system for 6 hr that maximized the FSH-releasing activity of LHRH. We concluded that a bioactive FSHRF was separable from LHRH and that this bioactive FSH was not recognized by the then current RIA (4). Since intravenous (iv) injection of low doses of LHRH in the rat induced only LH release, whereas FSH release was also stimulated by infusions of the peptide (10), we subsequently fractionated another lot of ovine SME fragments on Sephadex G-25 and tested fractions off the Sephadex column for FSHRF using our original assay for FSHRF, in which hypothalamic extracts were pulse-injected into ovariectomized, estrogen progesterone-blocked rats (1, 2). Using this assay, FSH-releasing activity was obtained in the same sequence of fractions that contained it in our previous experiments. These fractions were essentially free of LHRH activity by both bio- and immunoassay (13).

Since *in vivo* results could be related to not only an action on the pituitary directly, but also a possible action in the brain, in the present experiments, we reevaluated the activity of hypothalamic extracts to release FSH and LH in a modified hemipituitary incubation system in which the extracts were incubated for 3 hr rather than the 6 hr used previously. Here we report that gel filtration on Sephadex G-25 of either sheep or rat hypothalamic extracts resulted in the elution of fractions containing only FSH-releasing activity in the same sequence of tubes found in all our previous work. Recently, we discovered that lamprey l-GnRH-III has highly potent selective FSH-releasing activity, an activity that was unique among some 30 LHRH analogs tested. We believe that l-GnRH-III either is FSHRF or a closely related molecule (14).

In this communication, we report that the active fraction containing FSHRF is in a peak of immunoassayable l-GnRH, and that a specific antiserum against l-GnRH completely blocked the FSH-releasing activity of the active fraction providing further evidence that l-GnRH-III either is FSHRF or a closely related molecule.

Materials and Methods

Preparation of Hypothalamic Extracts. *Sheep hypothalamic tissues.* About 1600 sheep stalk median eminence fragments (SMEs) were collected at a slaughter house from freshly sacrificed animals and immediately frozen on dry ice. These SMEs were kept at −70°C and, subsequently, homogenized in acetone/0.01 *N* HCl (80:20 V/V) using a Polytron Homogenizer (Brinkmann Instruments, Inc., Newbury, NY) and centrifuged at 2,000*g* for 15 min. The supernatant was decanted, and the extraction was repeated on the residue twice. The three supernatants were pooled, and the lipids were removed by repetitive (four times) extraction with petroleum ether (40–60 B.P.). The aqueous phase was concentrated by rotary evaporation and lyophilized. The lyophilized extract obtained was dissolved in 0.2 *N* acetic acid (15 ml) and centrifuged at 25,000*g* for 30 min to remove small amounts of insoluble materials. The supernatant was applied to a Sephadex G-25 fine column (4.5 × 150 cm, exclusion volume = 1060 ml), and 200 fractions (20 ml each) were collected. These fractions were frozen at −70°C and aliquots (1 ml) from each fraction were lyophilized and dissolved in 1 ml of buffer just prior to use.

Rat hypothalamic tissues. About 1000 pieces of rat hypothalamic tissue were homogenized and extracted as described above. The final extract was dissolved in 5 ml of 0.2 *N* acetic acid and applied to a smaller Sephadex G-25 column (2.8 × 160 cm, exclusion volume = 440 ml), and 5-ml fractions were collected. Aliquots (1 ml) from each fraction were lyophilized and dissolved in 1 ml of buffer just before bioassay or RIA. Fractions were stored frozen at −70°C.

Bioassays for FSH and LH-Releasing Activity *In Vitro.* Adult male rats (200–250 g) of the Sprague-Dawley strain (Holtzmann, Madison, WI) were housed two per cage under controlled conditions of temperature (23°C–25°C) and lighting (on from 5:00 to 17:00 hr). The animals had free access to a pellet diet and tap water.

After acclimatization for 7 or more days in the vivarium, animals were sacrificed by decapitation. Following removal of the posterior lobe, the anterior pituitary (AP) was bisected longitudinally, and each AP was incubated in a tube containing 1 ml of Krebs-Ringer bicarbonate buffer (KRB) in an atmosphere of 95% O_2/5% CO_2 in a Dubnoff shaker (50 cycles/min) for a period of 60 min. After this preincubation, APs were incubated for 3 hr in fresh KRB buffer alone or KRB containing dissolved fractions of hypothalamic extract of sheep (50 μl) or rat (150 μl). The medium was then aspirated and stored frozen at −20°C until radioimmunoassay of FSH and LH.

Immunoneutralization of FSHRF with l-GnRH Antisera *In Vitro.* Fraction #116 of rat hypothalamic extract had selective FSH-releasing activity, suggesting that this fraction contained FSHRF. This fraction (1 ml)(20% of #116) was lyophilized and dissolved in 1 ml of KRB prior to incubation. Anterior pituitaries (APs) of male rats were obtained and incubated as described above. Following a 1-hr preincubation, APs were incubated for 3 hr in fresh KRB buffer or KRB containing normal rabbit serum (NRS)(1:1000), l-GnRH antiserum (1:1000), fraction #116 (150 μl) or fraction #116 together with l-GnRH antiserum. The medium was obtained and stored frozen until radioimmunoassay.

Radioimmunoassays. FSH and LH were measured by kits supplied by the National Institute of Arthritis Digestive Diabetes and Kidney Disease, and hormone values were expressed as NIH-rFSH-RP-2 and NIH-rLH-RP-3 standards. The inter- and intra-assay coefficients of variation for FSH assays were 7.2% and 5.0%, respectively, and 6.4% and 4.5% for LH, respectively.

LHRH was measured by RIA as previously described (15) using synthetic LHRH (Peninsula Laboratories, Belmont, CA) as standard and a highly specific LHRH antiserum kindly donated by Dr. A. Barnea (University of Texas Southwestern Medical Center, Dallas, TX). The minimal detectable LHRH concentration was 0.2 pg/100 μl/tube.

Lamprey GnRH was measured as previously described (16) using synthetic l-GnRH-III (American Peptide Company, Sunnyvale, CA) as standard and an antiserum raised against l-GnRH. This antiserum has 100% cross-reactivity with l-GnRH-I or l-GnRH-III and only 0.02% cross-reactivity with mammalian LHRH and no detectable cross-reactivity with chicken GnRH-II or salmon GnRH (< 0.001%). The minimal detectable l-GnRH was 9.8 pg/100 μl/tube.

Statistics. The significance of differences among multiple groups was determined by the analysis of variance (ANOVA) with subsequent Newman-Keuls multiple comparisons. Student's *t* test was used to determine the significance of differences between two groups.

Results

Purification of FSHRF by Gel Filtration on Sephadex G-25. In our initial experiments, we used SME obtained from lambs, and the column fractions were assayed for LHRH by RIA. This demonstrated a peak concentration of LHRH in Tube 110. The activity first appeared in Tube 95 and was minimal until the upward slope began at Tube 102 with a rapid rise to the peak concentration of LHRH. The values declined rapidly beginning with Tube 114 to return to 0 at Tube 125 (Fig. 1). By bioassay, there was no significant stimulation of LH release with any of the tubes tested up to Tube 104, just before the onset of the rapid upswing of immunoassayable LHRH concentration (Fig. 2). In fact, there was an inhibitory zone that extended from Tube 98 to Tube 103. Significant inhibition was obtained with Tubes 100, 102, and 103.

The results with FSH were quite different in that Fractions 98 and 100 individually gave significant stimulatory activity. If the data for Fractions 98–100 were pooled, the overall activity of the three fractions was significant ($P < 0.01$). Activity declined to almost basal values until Fraction 104 that gave a significant stimulation of FSH (Fig. 2). As a control, LHRH was tested at 2 and 20 ng/ml concentrations, and it gave significant stimulation of both FSH and LH (data not shown).

There were problems obtaining FSH-releasing factor activity from sheep hypothalami, probably related to seasonal variations in the abundance of FSHRF in lamb brains. Indeed, collecting SMEs in late spring and summer, we obtained specific FSH-releasing activity in the same elution position as shown here on all six trips to the slaughterhouse. On two occasions in late fall and winter, selective FSH release was not obtained after gel filtration in Sephadex G-25. Therefore, we decided to evaluate rat hypothalami. We fractionated rat hypothalami on Sephadex three times and found the FSH-releasing activity in every case from the

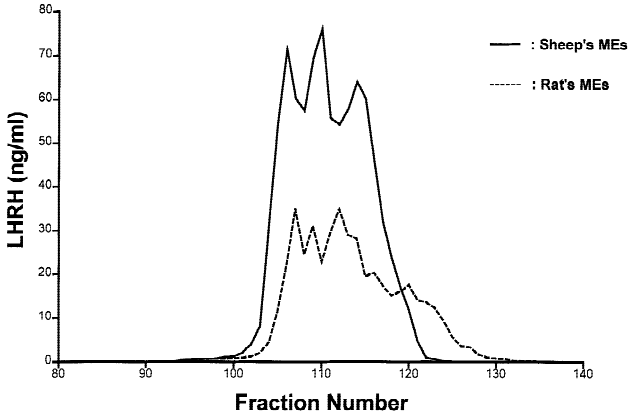

Figure 1. RIA of LHRH in column fractions of Sephadex-purified sheep and rat hypothalami.

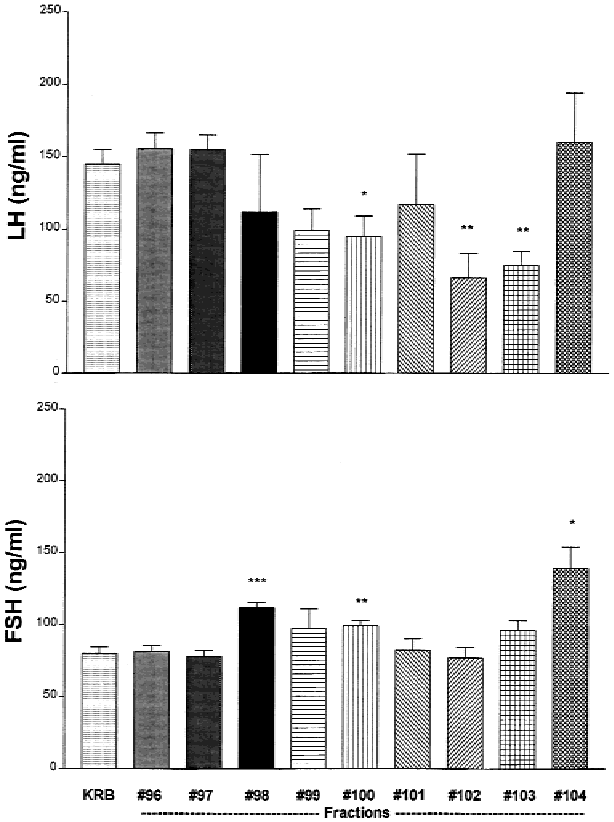

Figure 2. FSH- and LH-releasing activity of fractions purified on Sephadex G-25 from sheep SME extracts. In this and subsequent figures, results are mean plus one standard error of the mean $*P < 0.05$; $**P < 0.01$; and $***P < 0.001$ vs control KRB incubates ($n = 7$ in each group).

Sephadex column in the same position as the activity from sheep SMEs. Because of the decreased weight of tissue fractionated, we ran our initial experiment on a smaller column of Sephadex G-25. The flow rate was adjusted to be comparable in terms of elution position of GnRHs to that obtained with sheep hypothalami. Indeed, LHRH eluted from the column in a very similar pattern to that obtained with sheep hypothalami, with the first detectable activity at Tube 96 and a rapid upward slope beginning at Tube 103, reaching peak concentrations between Tubes 107–112, followed by a decline to undetectable levels reached at Tube 132 (Fig. 1). Interestingly, the quantity of LHRH recovered from 1000 rat hypothalami was equal to 21% of that recovered from the same number of sheep SME fragments. Since the average weight of sheep SMEs was 150 mg and that of rat SMEs was 1.5 mg, the concentration of LHRH in rat SMEs was ≈ 9 times greater than that in the sheep. Since the sheep SMEs were dissected at the slaughterhouse, and rat SMEs were similarly dissected freehand, undoubtedly these fragments contained considerable tissue overlying the ME, but the weights probably reflect the relative size of the SME in the two species.

With the discovery of the potent FSH-releasing activity of l-GnRH-III (14), we decided to see whether the elution position of FSHRF corresponded with that of immunoassayable l-GnRH in the fractions. Consequently, we fractionated another 1000 rat hypothalami on Sephadex G-25 (Fig. 3). The FSH-releasing activity emerged in a single tube 116 (Fig. 4) that was devoid of significant LH-releasing activity (Fig. 5). In the case of FSH and LH, there was no significant activity on either LH or FSH release of higher numbered fractions from 117 to 123 (Figs. 4 and 5), the latter being at the beginning of the upsweep of immunoassayable LHRH concentrations (Fig. 3).

The relevant fractions were assayed not only by immunoassay for LHRH but also by immunoassay for l-GnRH. To our surprise, the results revealed not one but three detectable peaks of l-GnRH (Fig. 3). The first of these peaks was at Tube 108 that was removed from biological activity on either FSH or LH in a number of previous experiments.

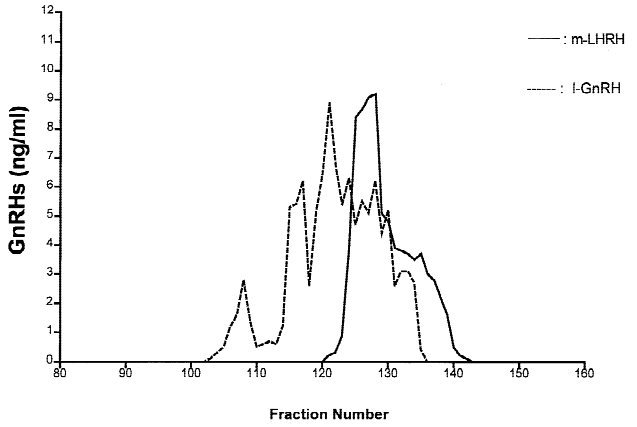

Figure 3. RIA of m-LHRH and l-GnRH in column fractions of rat hypothalami purified by gel filtration on Sephadex G-25.

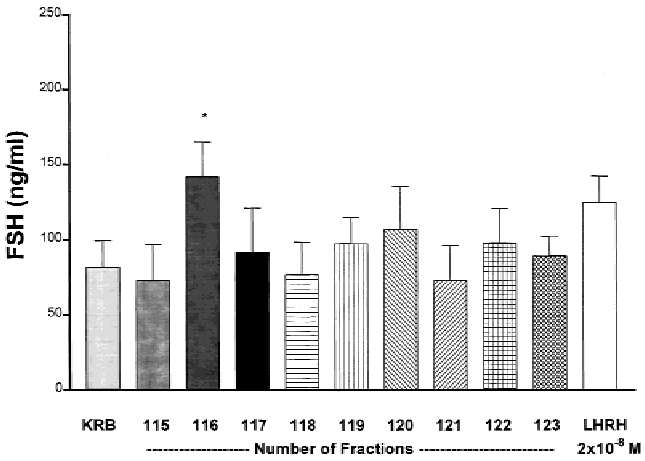

Figure 4. FSH-releasing activity of Sephadex purified rat hypothalamic fractions. The lack of significant activity of LHRH (2×10^{-8} *M*) is also shown. Only Tube 116 had significant FSH-releasing activity ($n = 7$ in KRB controls and $n = 6$ in all other groups).

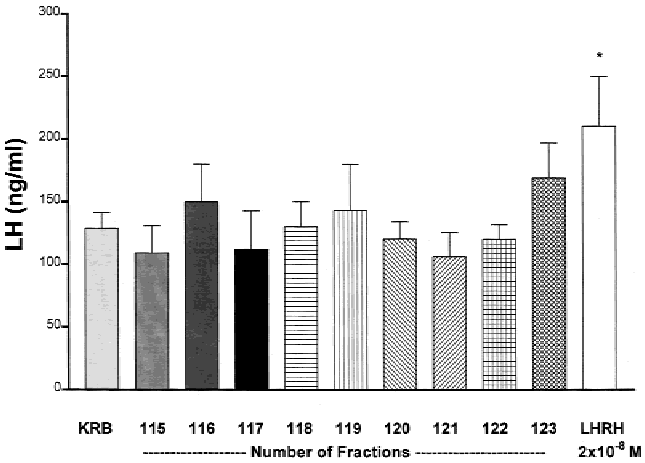

Figure 5. Lack of LH-releasing activity of the same fractions from rat hypothalami purified by gel filtration on Sephadex G-25. The significant activity of LHRH is shown ($n = 7$ in KRB controls and $n = 6$ in all other groups).

This was followed by another peak of l-GnRH emerging in Tubes 115–117. As indicated above, bioassay of these three tubes revealed no LH-releasing activity (Fig. 5). Significant FSH-releasing activity was detected only in Tube 116 (Fig. 4). There was no FSH or LH releasing activity in the location of the third peak at Tube 121. Proceeding higher from this tube, l-GnRH immunoreactivity declined to a plateau at Tube 123, that was maintained until Tube 130 that was located on the rapid down slope of the curve of radioimmunoassayable LHRH. Since the crossreactivity of this antiserum with LHRH was only 0.02%, it appears that none of these results can be accounted for by m-LHRH; however, since the antiserum recognized l-GnRH-I and III equally, the activity detected by RIA could have been due to either peptide or a mixture of the two.

l-GnRH-II was discovered but has not been characterized (17). It is possible that it might also crossreact with the l-GnRH antiserum employed. The area under the curve of

l-GnRH was 112.35 ng fraction/ml, whereas that for LHRH was 79.55 ng fraction/ml. Therefore, there was 1.41 times as much immunoreactive l-GnRH as LHRH in rat SMEs.

Immunoneutralization of FSHRF with Antisera Against l-GnRH. If l-GnRH-III is FSHRF, the activity should be abolished by incubating it in the presence of antisera against l-GnRH. Indeed, incubation with the antibody used for RIA at a dilution of 1–1000 had no effect on FSH release alone, but completely inactivated the FSH-releasing activity in Tube 116 (Fig. 6). The antiserum had no effect on basal LH release or the LH-releasing activity of LHRH (Fig. 7).

Discussion

The results of this study clearly indicate that FSHRF is active *in vitro* on incubated anterior pituitaries. Therefore, it directly stimulates the release of FSH from the pituitary gland. It can be purified from either rat or sheep hypothalami. We do not know the reason why extracts obtained from sheep were not always active. Examination of the times when we collected sheep hypothalami suggests that there was no activity demonstrable when we collected in late fall and winter, but activity was demonstrable in spring and summer (Yu WH, *et al*., unpublished data). Since most of the lambs were born in early spring, this suggests that this change may be due to a developmental change in the content of FSHRF present in sheep hypothalami. Further work is necessary to determine if this is the cause of the problem. In the meantime, we have been able to recover FSHRF from three consecutive batches of rat hypothalami collected from adult male rats.

We have recently shown that l-GnRH-III is a potent FSH-releasing factor with activity to release FSH in the same concentration range as that of LHRH to release LH (14). In the present experiments, we showed that the FSH-releasing fraction also contained immunoassayable l-GnRH; however, this was detected in a peak of three tubes, 115–117, of which only one had detectable biological activity.

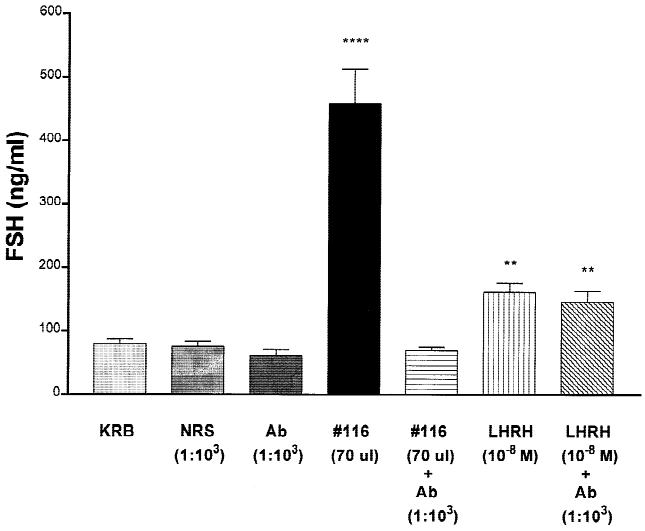

Figure 6. The effect of antisera directed against l-GnRH (1:1000 dilution) on the FSH-releasing activity of Fraction #116 purified by gel filtration on Sephadex G-25 of rat hypothalami. This is the same fraction illustrated in Figure 4. The lack of effect of this antiserum on the FSH-releasing activity of LHRH is also illustrated (n = 7 in each group).

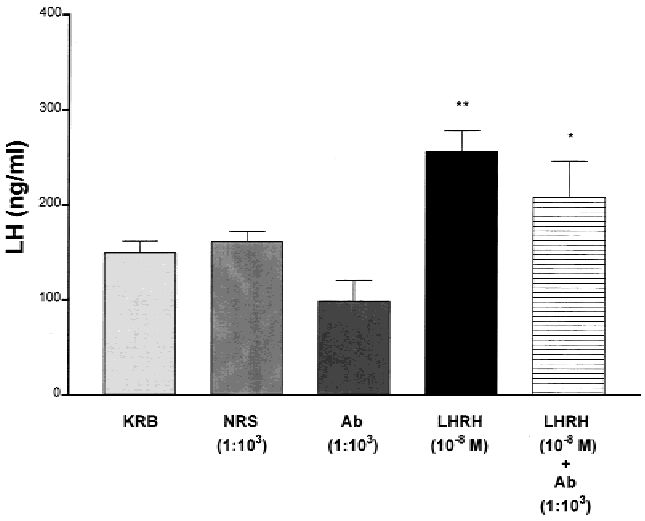

Figure 7. Lack of effect of antiserum directed against l-GnRH (1:1000) on basal LH-release and the LH-releasing activity of LHRH (2×10^{-8} M (n = 7 in each group).

Furthermore, there was immunoassayable l-GnRH in an earlier fraction 108, presumably containing a larger molecular-weight form of l-GnRH since Sephadex separates peptides primarily according to molecular size with larger molecules emerging in earlier tubes. This immunoassayable activity might also be caused by l-GnRH-I or the uncharacterized l-GnRH-II. We did not bioassay these tubes for FSH-releasing activity in this experiment, but comparable tubes were devoid of biological activity in our previous experiments with rat and sheep hypothalami. Therefore, we postulate that this is a biologically inactive form of l-GnRH that was detected by the radioimmunoassay. In addition, there was another peak at Tube 121, and a shoulder of this peak persisted to Tube 130, the last tube assayed. This peak was also inactive biologically and contained little or no immunoassayable LHRH. Since our antibody recognized l-GnRH-I and III equally, and l-GnRH-I has only weak activity to stimulate both FSH and LH (minimal effective dose of 10^{-5}–10^{-6} M *in vitro* versus 10^{-9} M for l-GnRH-III), we postulate that this later peak may also represent either l-GnRH-I or II. The shoulder, which overlaps the LHRH peak, may also be this compound. It is hardly possible that it could be LHRH since the crossreactivity with LHRH in the assay for l-GnRH was only 0.02%.

Further evidence that FSHRF may be l-GnRH-III was provided by the immunoneutralization studies indicating that the antisera used for immunoassay of l-GnRH, although having no effect on basal release of FSH, completely obliterated the FSH-releasing activity of the active fraction of FSHRF. This antiserum did not affect basal LH release and had no effect on the LH-releasing activity of LHRH. Fi-

nally, using the same antiserum employed in these experiments, we found by immunocytochemistry a l-GnRH neuronal system in the rat brain, with cell bodies in the preoptic area in regions that are devoid of LHRH neurons and axons that project to the ME (18). Lesions and stimulations of the region just caudal to the l-GnRH neuronal perikarya containing the axons of these neurons have previously been shown to be associated with selective effects on FSH release (19, 20).

In conclusion, the present results have established the activity of FSHRF directly on the pituitary gland, shown that it can be reproducibly separated from LHRH by gel filtration on Sephadex G-25, that the active fraction contained immunoassayable l-GnRH, and that an antiserum against l-GnRH inhibited the FSH-releasing activity of purified FSHRF. Therefore, further evidence has been provided that FSHRF is either l-GnRH-III or a closely related peptide.

1. Igarashi M, McCann SM. A hypothalamic follicle stimulating hormone releasing factor. Endocrinology **74:**446–452, 1964.
2. Dhariwal APS, Nallar R, Batt M, McCann SM. Separation of FSH-releasing factor from LH-releasing factor. Endocrinology **76:**290–294, 1965.
3. Dhariwal APS, Watanabe S, Antunes-Rodrigues J, McCann SM. Chromatographic behavior of follicle stimulating hormone releasing factor on Sephadex and carboxymethyl cellulose. Neuroendocrinology **2:**294–303, 1967.
4. Mizunuma H, Samson WK, Lumpkin MD, Moltz JH, Fawcett CP, McCann SM. Purification of a bioactive FSH-releasing factor (FSHRF). Brain Res Bull **10:**623–629, 1983.
5. Schally AV, Arimura A, Kastin AJ, Matsuo H, Baba Y, Redding TW, Nair RM, Debeljuk G, White WF. The gonadotropin-releasing hormone: A single hypothalamic polypeptide regulates the secretion of both LH and FSH. Science **173:**1036–1037, 1971.
6. McCann SM, Marubayashi U, Sun H-Q, Yu WH. Control of follicle stimulating hormone and luteinizing hormone release by hypothalamic peptides. Ann N Y Acad Sci **687:**55–59, 1993.
7. Schally AV, Saito T, Arimura A, Muller EE, Bowers CY, White WF. Purification of follicle-stimulating hormone-releasing factor (FSH-RF) from bovine hypothalamus. Endocrinology **79:**1087–1094, 1966.
8. Matsuo H, Arimura A, Nair RMG, Schally AV. Synthesis of the porcine LH- and FSH-releasing hormone by the solid phase method. Biochem Biophys Res Commun **45:**822–827, 1971.
9. Burgus R, Butcher M, Amoss M, Ling N, Monahan M, Rivier J. Primary structure of the ovine hypothalamic luteinizing hormone-releasing factor (LRF). Proc Natl Acad Sci U S A **69:**278–282, 1972.
10. Libertun C, Orias R, McCann SM. Biphasic effect of estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LRF). Endocrinology **94:**1094–1100, 1974.
11. Zeballos G, McCann SM. Alterations during the estrous cycle in the responsiveness of the pituitary to subcutaneous administration of synthetic LH-releasing hormone (LHRH). Endocrinology **96:**1377–1385, 1975.
12. Schally AV, Arimura A, Redding TW, Debeljuk L, Carter W, DuPont A, Vilche-Martinez JA. Re-examination of porcine and bovine hypothalamic fractions for additional luteinizing hormone and follicle stimulating hormone–releasing activities. Endocrinology **98:**380–391, 1976.
13. Lumpkin MD, Moltz JH, Yu WH, Samson WK, McCann SM. Purification of FSH-releasing factor: Its dissimilarity from LHRH of mammalian, avian, and piscian origin. Brain Res Bull **18:**175–178, 1987.
14. Yu WH, Karanth S, Walczewska A, Sower SA, McCann SM. A hypothalamic follicle stimulating hormone-releasing decapeptide in the rat. Proc Natl Acad Sci U S A **94:**9499–9503, 1997.
15. Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci U S A **94:**1023–1028, 1997.
16. Sower SA, Chiang YC, Lovas S, Conlon JM. Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. Endocrinology **132:**1125–1131, 1993.
17. Sherwood NM, Sower SA, Marshak DR, Fraser BA, Brownstein MJ. Primary structure of gonadotropin-releasing hormone from lamprey brain. J Biol Chem **261:**4812–4819, 1986.
18. Dees WL, Hiney JK, Sower SA, Yu WH, McCann SM. Localization of immunoreactive lamprey gonadotropin-releasing hormone in the rat brain. Peptides **20:**1503–1511, 1999.
19. Ojeda SR, Jameson HE, McCann SM. Hypothalamic areas involved in prostaglandin (PG)-induced gonadotropin release. II. Effect of PGE_2 and $PGF_2\alpha$ implants on follicle stimulating hormone release. Endocrinology **100:**1595–1603, 1977.
20. Lumpkin MD, McCann SM. Effect of destruction of the dorsal anterior hypothalamus on selective follicle stimulating hormone secretion. Endocrinology **115:**2473–2480, 1984.