Median Eminence Lesions Reveal Separate Hypothalamic Control of Pulsatile Follicle-Stimulating Hormone and Luteinizing Hormone Release (44356)

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Abstract. **The effects of hypothalamic lesions designed to destroy either the anterior median eminence (ME) or the posterior and mid-ME on pulsatile release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined in castrated male rats. In sham-operated animals, mean plasma FSH concentrations rose to peak at 10 min after the onset of sampling, whereas LH declined to a nadir during this time. In the final sample at 120 min, the mean FSH concentrations peaked as LH decreased to its minimal value. In rats with anterior ME lesions, there was suppression of LH pulses with continuing FSH pulses in 12 of 21 rats. On the other hand, in animals with posterior to mid-ME lesions, 3 out of 21 rats had elimination of FSH pulses, whereas LH pulses were maintained. Fifteen of 42 operated rats had complete ME lesions, and pulses of both hormones were abolished. The remaining 12 rats had partial ME lesions that produced a partial block of the release of both hormones. The results support the concept of separate hypothalamic control of FSH and LH release with the axons of the putative FSH-releasing factor (FSHRF) neuronal system terminating primarily in the mid- to caudal ME, whereas those of the LHRH neuronal system terminate in the anterior and mid-median eminence. We hypothesize that pulses of FSH alone are mediated by release of the FSHRF into the hypophyseal portal vessels, whereas those of LH alone are mediated by LHRH. Pulses of both gonadotropins simultaneously may be mediated by pulses of both releasing hormones simultaneously. Alternatively, relatively large pulses of LHRH alone may account for simultaneous pulses of both gonadotropins since LHRH has intrinsic FSH-releasing activity.**

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Gonadotropin secretion is controlled by the release of hypothalamic peptides that enter the hypophyseal portal vessels and cause the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the gonadotropes (1, 2). With the discovery of the structure of LH-releasing hormone (LHRH) and the knowledge that it would release FSH as well as LH (1–3), albeit with a higher dose and the release of lesser amounts of FSH that LH, the name LHRH was changed to gonadotropin (Gn)RH (3). It was thought that the known dissociations between FSH and LH release could be accounted for by changes in gonadal steroid release, which either increased or decreased the response of the pituitary to GnRH with regard to FSH or LH release (1–3). Other dissociations in FSH from LH release were thought to be accounted for by inhibin secreted from the gonads, which has a preferential effect to inhibit FSH, but not LH release (1–5). With the discovery of the structure of two inhibins, one a 32-kDa glycoprotein heterodimer (4) and the other, a 92-amino-acid peptide (5), another protein, activin, was soon identified that selectively releases FSH (6).

In our earlier studies in female rats, stimulation of the

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medial preoptic area produced LH but not FSH release, whereas stimulation more caudally in the dorsomedial anterior hypothalamic area produced FSH but not LH release (7, 8). Conversely, lesions in the suprachiasmatic region caused a preferential suppression of steroid-induced LH, but not FSH release (9), whereas lesions in the dorsal anterior hypothalamic area produced selective suppression of FSH and not LH release (10) and abolished pulsatile FSH but not LH release in castrated male rats (11). Lesions of the anterior and mid-median eminence region in male rats produced a suppression of basal LH release, whereas lesions of the posterior and mid-median eminence produced a preferential suppression of basal FSH release (12). Furthermore, the posterior portion of the median eminence of male rats contained more FSH releasing activity than could be accounted for by the content of LHRH (13). Consequently, we postulated that the FSH-controlling area is localized in the dorsomedial anterior hypothalamic area, with cell bodies of the postulated FSH-releasing factor (FSHRF) neurons localized there and their axons projecting preferentially to the mid- and caudal median eminence (3). Since in our previous study using male rats (12), plasma FSH and LH were only measured at sacrifice, it was important to study the pulsatile release of both hormones. Castrated male rats were employed since removal of gonadal negative feedback resulted in increased gonadotropin release and readily measurable pulsatile release of both FSH and LH. Therefore, in the present experiments, lesions designed to destroy either the anterior or posterior median eminence (ME) were produced and the effects on pulsatile release of both gonadotropins were determined. A preliminary report of this work has appeared (14).

Materials and Methods

Animals. Adult male Sprague-Dawley rats, 280–300 g (Holtzman Co., Madison, WI) were used in all experiments. They were housed under controlled conditions of lighting (lights on from 0500–1900 hr) and temperature (24–26°C). Rat chow and tap water were available to all animals *ad libitum.*

All the animals were castrated while anesthetized with ether, and the hypothalamic lesions were placed 1 week later.

Lesions. The rats were anesthetized with ether and placed in a David Kopf stereotaxic apparatus (David Kopf, Inc., Tujunga, CA). An insulated nichrome wire, exposed and flattened at the tip, was lowered through burr holes in the calvarium to the base of the brain at coordinates designed to destroy either the anterior or caudal ME. The electrode was constructed from 21-gauge stainless steel wire and was coated with epoxylite for insulation. Direct cathodal current of 5 milliamps for 20 sec for large lesions and 2 milliamps for 10 sec for small lesions was used (12). Afterwards, the animals were housed individually with free access to food and water.

Experimental Procedures. After 1 week, the rats were lightly anesthetized with ether, and a catheter was placed into the right external jugular vein and advanced to the right atrium using the technique of Harms and Ojeda (15). The following day, heparinized (50 U heparin sulfate) blood samples (0.3 ml) were withdrawn every 5 min for 2 hr. The volume of blood withdrawn was replaced immediately by an equal volume of a suspension of washed red cells from normal male donors. The cells were diluted with an equal volume of 0.9% NaCl before injection.

Histology. At the end of the experiment, the rats were decapitated, and the brains were stored in 4% formaldehyde. Frontal sections were cut on a freezing microtome (−10°C) at a tissue thickness of 20 μm and stained with cresyl violet to verify lesion localization. The sections were examined microscopically and the extent of each lesion was mapped with the aid of the rat brain atlas of De Groot (16).

Gonadotropin Assays. Plasma FSH concentrations were measured according to the directions supplied with the NIADDK Radioimmunoassay (RIA) kit and were expressed in terms of the RP-1 reference preparation. The intraassay coefficient of variation ranged between 4.5%–7.8%, and the cumulative interassay coefficient of variation was 8.7%. For LH measurements, the RIA method of Niswender *et al.* (17) was employed using the NIADDK, RP-2 rat pituitary LH reference preparation, but the results were expressed in terms of the NIH LH-S1 standard, so as to be comparable with previous data from this laboratory. Antiovine LH serum (GND no. 15) was generously supplied by Dr. G. Niswender (Colorado State University), and purified LH for radioiodination was kindly supplied by Dr. L. E. Reichert (Albany Medical College, Albany, NY). The intraassay coefficient of variation for the LH RIA ranged from 4.0–7.3%, and the cumulative interassay coefficient of variation was 8.9%. All plasma samples were measured in duplicate aliquots. Plasma samples within each of the four experiments were measured in the same FSH or LH assay. The four groups were as follows: 1) Large anterior; 2) posterior; 3) smaller anterior; and 4) posterior ME lesions, with seven sham-operated controls interspersed within the four groups.

Statistics. Significant differences between the means of three or more treatment groups were determined by analysis of variance with repeated measure, and significance was determined by the Student-Newman-Keuls test. Pulse analysis for FSH and LH was determined in each animal by both the method of Cahoreau *et al.* (18) and the pulsar program of Merriam modified for rats by Gitzen and Ramirez (19).

Results

Sham-Operated Castrated Rats. These animals were prepared by exactly the same procedures as the animals with ME lesions except for the lowering of the electrode bilaterally to a spot 2 mm above the base of the brain. The pooled results of the seven animals illustrated a remarkable phenomenon; the animals initially pulsed both hor-

mones as a group, but the results were dissimilar initially between FSH and LH (Fig. 1). Plasma FSH rose dramatically 5 min after onset of sampling, reaching a peak at 10 min, which was followed by a precipitous decline in the next 5 min, reaching a nadir at 35 min, after which the values rose again reaching a peak at 45 min, followed by another peak at 65 min, and finally a dramatic rise to a peak at 120 min.

The initial response of plasma FSH was the mirror image of that of LH concentrations that plummeted within 5 min in these same animals and reached a minimum at 10 min, at which time the FSH values had reached their highest point in the entire 120 min of sampling. LH peaked for the first time at 25 min, while FSH values were continuing to plummet to reach a nadir at 35 min, at the same time as LH.

Then, the situation changed and there were simultaneous pulses of FSH and LH at 45 min, followed by a decline of both hormones and another simultaneous pulse of both gonadotropins at 65 min. There were two small pulses of LH following this time, but LH tended to decline and reached minimum values between 105 and 120 min, in contrast to FSH values that rose dramatically between 115 and 120 min.

An individual example of the pattern of FSH and LH pulses in one of these sham-operated animals is illustrated in Figure 2. This animal had an initial FSH pulse peaking at 10 min, whereas LH had decreased during this time (Fig. 2). In this animal, only two out of six FSH pulses were simultaneous with an LH pulse.

Rats with Anterior-ME Lesions. We only partially

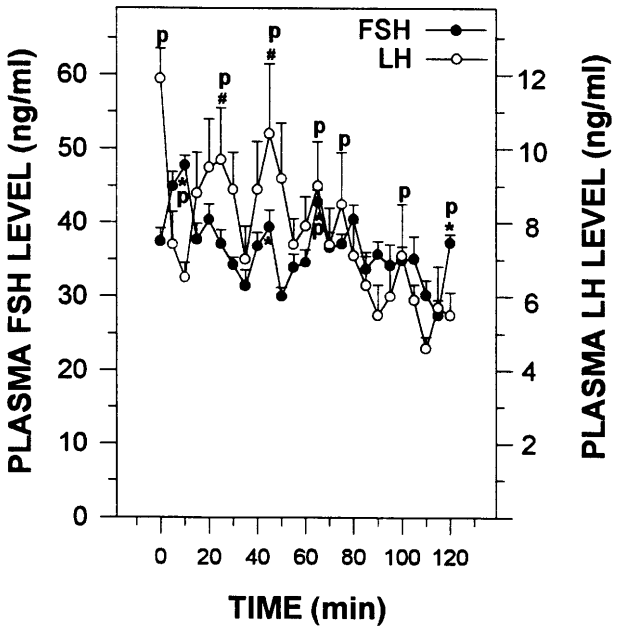


Figure 1. Pattern of fluctuations of plasma FSH and LH during 120 min in seven bilaterally castrated rats bearing sham ME lesions (MEL) (n = 7). Values are mean + SEM. In this and subsequent figures, asterisks above or below the values denote pulses of FSH release by Cahoreau analysis and # above open circles denote pulses of LH release by Cahoreau; p = pulses of FSH or LH release by Pulsar analysis.

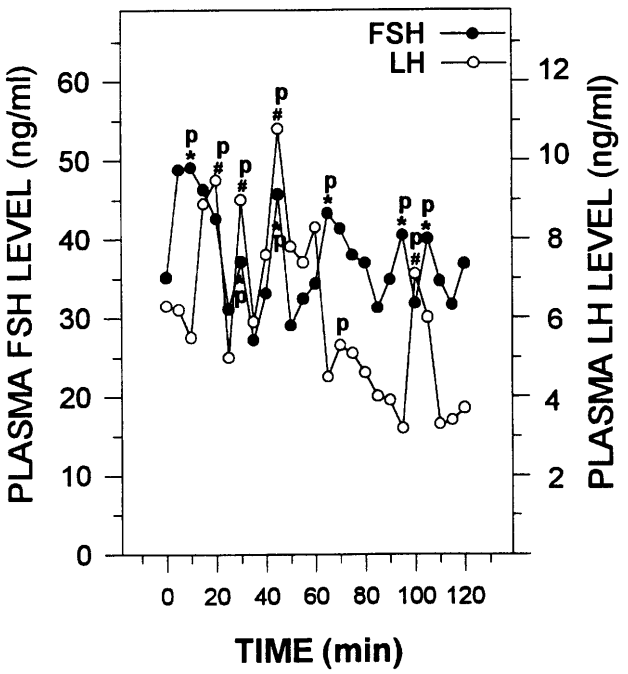
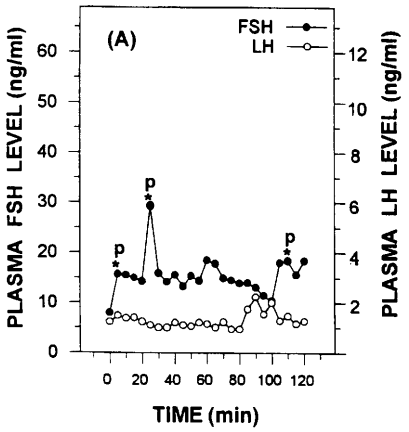


Figure 2. Profiles of pulsatile fluctuations of plasma FSH and LH in a castrated rat bearing a sham MEL.

achieved the aim of obtaining lesions that were specifically localized only to the anterior ME on the one hand, or to the posterior ME on the other. Therefore, many of the lesions included the whole ME, probably because the separation of the coordinates was too small and the size of the lesions too large. However, in general the anterior ME lesions interfered more or less selectively with pulsatile LH release and to a lesser degree with pulsatile FSH release, although in most instances both gonadotropins were affected.

In rat 114, with an exclusively anterior ME lesion (Fig. 3B), there were three pulses of FSH and none of LH (Fig. 3A). The concentration of LH was significantly lowered below levels in sham-operated rats ($p < 0.01$), but still readily detectable ($p < 0.01$). The mean level of FSH was also lowered significantly ($p < 0.01$), but this animal clearly showed a selective abolition of LH pulses, since the mean number of LH pulses in sham-operated animals was 4.0 ± 0.53. In rat 111 (Fig. 4A,B), the dissociation was even more drastic with four pulses of FSH and no detectable LH pulses. The mean level of LH was decreased ($p < 0.01$), but the mean level of FSH was only slightly decreased below that of sham-operated animals.

Rat 116 (Fig. 5) was designed to have an anterior ME lesion; however, sections revealed that it had a massive preoptic lesion that extended quite far laterally and quite far dorsally, and as far caudally as the anterior median eminence, but ended just rostral to the separation of the pituitary stalk, but didn't injure the ME directly. Interestingly, even though this lesion did not reach the posterior hypothalamus, it abolished LH but not FSH pulsatility and drastically decreased the concentration of both hormones. This lesion probably cut most of the axons coming from the postulated



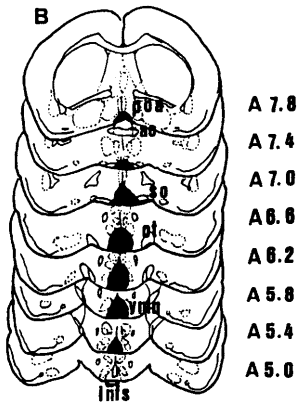


Figure 3. (A) Profiles of pulsatile fluctuations of plasma FSH and LH release in a castrated rat bearing an anterior MEL that blocked LH but not FSH pulses. (B) Frontal brain sections through the hypothalamus of this rat. Abbreviations in this and subsequent figures: ac = anterior commissure; ot = optic tract; poa = preoptic area; so = supraoptic nucleus; vmh = ventromedial hypothalamic nucleus; infs = infundibular stem. The black area represents the extent of the lesion.

FSHRF-containing neurons descending caudally from the dorsal anterior hypothalamic area, but left terminals that were in the posterior ME intact (Dees WL *et al.*, unpublished data), thereby permitting pulsatile FSH release, albeit of reduced amplitude (Fig. 5A). The lesion cut the axons of the LHRH neurons arising in the preoptic region and destroyed their axons as they entered the anterior ME. This may account for the complete blockade of LH release.

Posterior ME Lesions. Most of the posterior lesions extended forward to destroy also the anterior ME and abolished the pulsatile release of both hormones; however, there were three exceptions to this. For example, rat 122, whose lesion destroyed the posterior and mid- but not the anterior ME (Fig. 6B), had a large defect in FSH secretion, and no pulses were recorded, whereas this animal exhibited four sharp and large LH pulses (Fig. 6A).

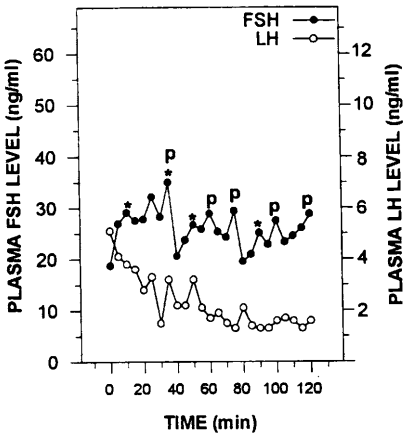
Mean Plasma FSH and LH Values in Animals with ME Lesions. The mean levels of FSH and LH were lowered in all of the animals (Fig. 7). Twelve out of the 42 animals tested showed an equivalent partial block of the release of both gonadotropins. Fifteen showed a dramatic inhibition of the release of both with blockade of pulses for both LH and FSH, and all of these animals had complete ME lesions.

Twelve of the animals had a block of LH, but not FSH pulses, although an equivalent lowering of mean LH values occurred in animals with blockade of both types of pulses. These rats had predominately anterior ME lesions (Fig. 7). Even though FSH continued to pulse in these animals, there was a reduction in mean FSH values, but the FSH concentrations were 30% higher ($p < 0.05$) than those in the animals in which pulses of both hormones had been blocked.

Finally, there was a much smaller group of animals (3) in which there was blockade of only FSH but not LH pulses. These rats had low mean levels of both hormones.

The Effect of ME Lesions on the Number of LH and FSH Pulses. In the animals in which LH pulses were selectively blocked, the number of FSH pulses was reduced by roughly 65% from a mean of five in sham-operated animals to slightly less than two per rat (Fig. 8). Similarly, in the animals in which only FSH pulses were blocked, the number of LH pulses was significantly reduced and were only about 40% of the number present in sham-operated controls. In the case of the animals with partial blockade, the FSH pulses were not significantly reduced in numbers, but there was still a significant, approximately 40%, reduction in the number of LH pulses (Fig. 8)

Distribution of FSH and LH Pulse Frequency and Amplitude in Rats with Lesions. Twelve animals had no LH pulses, but only three had no FSH pulses. In the animals with no LH pulses, FSH pulses ranged up to the normal amplitude and frequency (Fig. 9). The three rats that



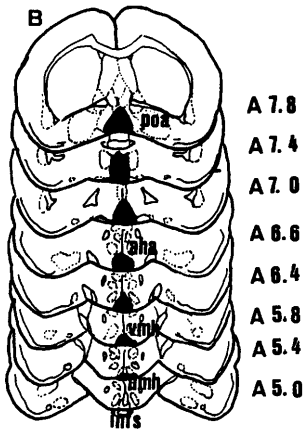


Figure 4. (A) Profiles of pulsatile fluctuations of plasma FSH and LH release in another rat with anterior MEL showing only FSH pulses. (B) Frontal brain sections through the hypothalamus of this rat; aha = anterior hypothalamic area; dmh = dorsomedial hypothalamic area (dmh).

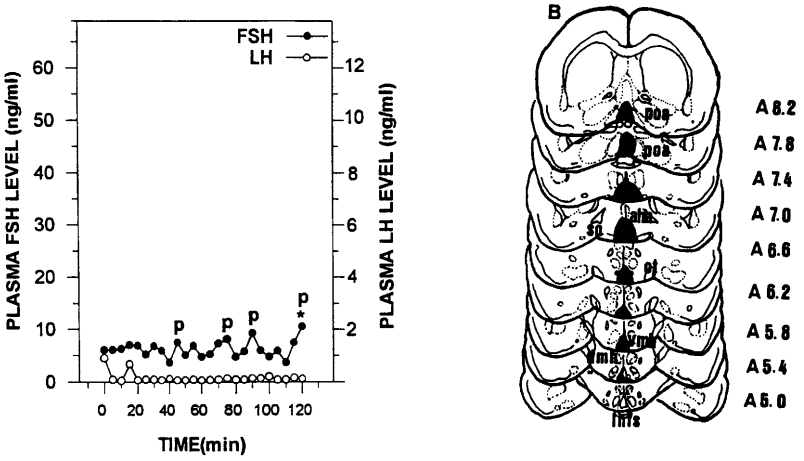


Figure 5. (A) Patterns of FSH and LH plasma levels in a rat with a large preoptic lesion that blocked both LH and FSH secretion except for small FSH pulses. (B) Frontal brain sections through the hypothalamus of this rat. Although this lesion approaches the base of the brain in the region of the anterior ME, it did not damage this structure directly.

had no FSH pulses had LH pulses within the normal range. The rats with partial blockade of both FSH and LH release had pulses of both hormones of normal amplitude.

Body Weights of the Animals. The mean body weights of all of the groups of animals with lesions and various degrees of impaired gonadotropin release were slightly but significantly ($p < 0.05$) reduced as compared to those of sham-operated animals (with the exception of those that had only a partial block of both FSH and LH release) whose weight was not reduced. There was no significant difference in this reduction between the various groups that lost weight and no relation between the degree of weight loss and the impairment in gonadotropin secretion (data not shown).

Pituitary Weights of the Animals. When expressed in terms of body weight, the pituitary weight was reduced in all of the animals with lesions, but the greatest and highly significant reduction occurred in animals with blockade of both LH and FSH, or FSH alone. The animals with blockade of only LH release had significantly larger pituitaries than those with blockade of only FSH release. The pituitary weights of the animals with partial block were significantly smaller than those of sham-operated rats, but significantly larger than those of animals in which both LH and FSH, or only FSH, was blocked (data not shown).

Pituitary Gonadotropin Content in Animals with ME Lesions. In the rats with lesions, there were nonsignificant reductions in pituitary hormone content from those in the sham-operated animals in most groups. In the animals in which there was a blockade of both LH and FSH release, LH levels were slightly lower, but not significantly so, whereas FSH levels were significantly less than those of sham-operated animals ($p < 0.05$) (data not shown).

Localization of Lesions in the Animals with Anterior or Posterior ME Lesions. In general, anterior median eminence lesions commenced at or just caudal to the optic chiasm and destroyed the median eminence and overlying arcuate nucleus in the midline and extended bilaterally, in some cases as much as 0.5–0.75 mm. These lesions extended caudally and terminated at the point of separation of the pituitary stalk. We have diagrammed the lesions of rat 114b (Fig. 3B) and rat 111 (Fig. 4B). Their pattern of gonadotropin release was illustrated in Figs. 3A and 4A, respectively. Both of these rats had preferential suppression of LH and not FSH release. On the other hand, the lesions designed to destroy the posterior median eminence destroyed this structure caudal to the pituitary stalk and extended rostrally so as to destroy variable amounts of the mid- and anterior median eminence. The lesion is diagrammed in one case (Fig. 6B) in which a selective sup-

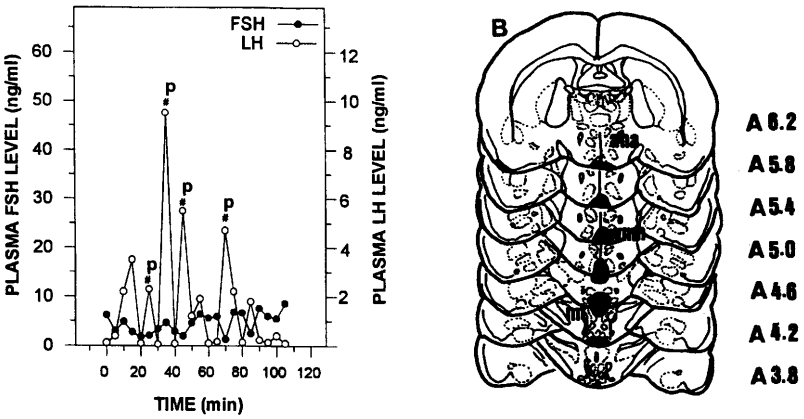


Fig 6. (A) Profiles of pulsatile fluctuations of plasma FSH and LH release in a rat with a mid-posterior MEL that abolished FSH pulses but had frequent large LH pulses. (B) Frontal brain sections through the hypothalamus of this rat: aha = anterior hypothalamic area; vmh = ventromedial hypothalamic nucleus; mt = mammillothalamic tract.

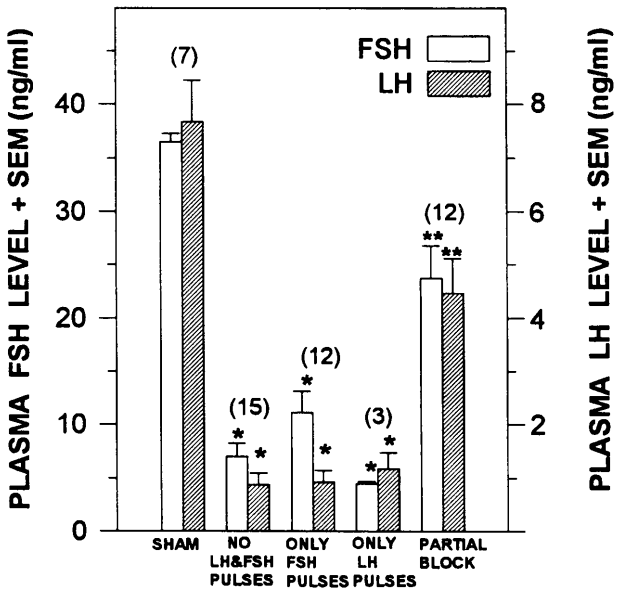


Figure 7. Mean plasma FSH and LH values plus SEM in rats with SHAM and MEL. *$p < 0.001$ versus SHAM and versus PARTIAL BLOCK; **$p < 0.05$ from SHAM; ( ) = number of animals.

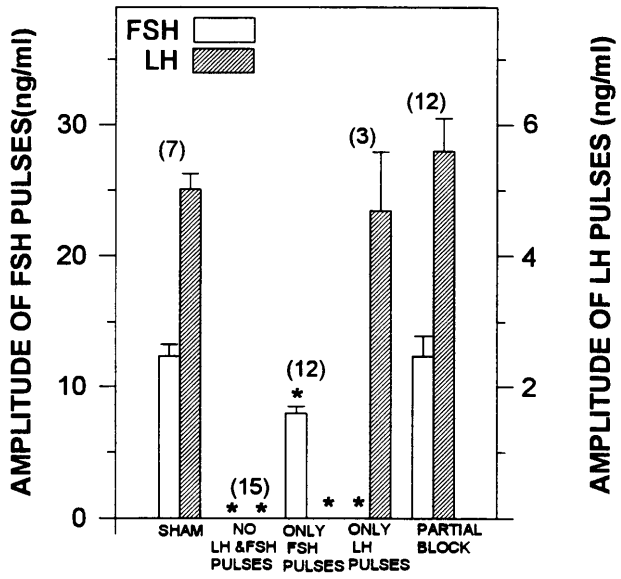


Figure 9. Mean amplitude of FSH and LH pulses in rats with SHAM or MEL. *$p < 0.001$ from SHAM; ( ) = number of animals.

pression of FSH and not LH was obtained as described in the results (Fig. 6A). Finally, the lesion in the rat with a lesion just above the ME that blocked LH but not FSH pulses (Fig. 5A) is shown (Fig. 5B).

Discussion

The results provide further compelling evidence for a separate hypothalamic control of FSH on a moment-to-moment basis presumably mediated by the elusive FSH-releasing factor. The sham-operated rats provided an excellent example of a complete dissociation of FSH and LH release. With onset of sampling, all of the animals showed an increase in FSH plasma, reaching a peak for the total group at 10 min, whereas at the same time, the LH levels were plummeting to reach a nadir at 10 min (Fig. 1). This could not be due to the action of LHRH, which was renamed GnRH many years ago. Interestingly, there was some synchronization of the pulses of FSH and LH as the collection period continued. There were two pulses of FSH in synchrony with LH, but the majority of the pulses of FSH were separate from those of LH. Even at the end of the experiment, FSH reached a peak at the same time that LH was at its minimal value. We hypothesize that the stress of the onset of blood sampling stimulated release of FSHRF and inhibited release of LHRH. Since the cumulative stress was considerable, this probably added to the initial stress as the experiment progressed. It appears that this type of stress has a differential effect on FSHRF and LHRH release, not altering the former but suppressing the release of the latter. In a similar experiment in estrogen-primed female rats, apparent FSHRF release was stimulated without alteration in LHRH release (Walczewska, *et al.*, unpublished data).

This is a most convincing case of dissociation of these pulses in brain-intact animals. Indeed, we previously showed the abolition of FSH pulses with maintenance of LH pulses in castrated rats with dorsal anterior hypothalamic lesions (10), and we found abolition of LH with maintenance of FSH pulses with treatment with a number of drugs; such as alcohol (20), δ^9-tetrahydrocannanabinol (21), and IL-1 (22). Many peptides produce selective release of LH but not FSH, and occasional ones stimulate FSH at the same time that LH is not stimulated or even inhibited (23). Finally, the release of LHRH is clearly stimulated by nitric

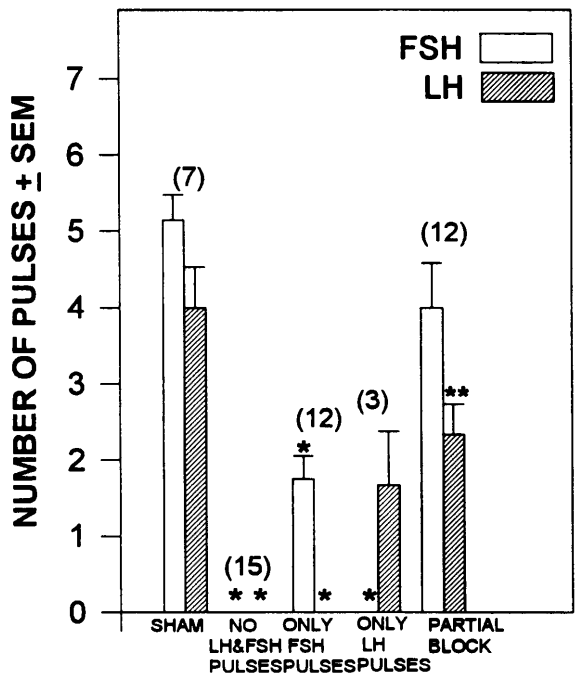


Figure 8. Number of FSH and LH pulses in SHAM and MEL *$p < 0.001$ from SHAM; **$p < 0.01$ from SHAM; ***$p < 0.05$ from SHAM, ( ) = number of animals.

oxide (NO), which does not seem to play any role in the release of the postulated FSHRH (24).

Since some of the pulses of LH and FSH, roughly 40%, coincide in castrated male rats (10, 20), it is possible that, in some instances, LHRH that has intrinsic FSH releasing activity, is generating the FSH pulses. Pulses of LH alone are almost certainly caused by LHRH. The asynchronous FSH pulses are presumably generated by FSHRF. This concept is supported by recent studies in sheep with scarified stalk and measurement of LHRH in portal blood together with peripheral levels of LH and FSH. Indeed, it was found that about half of the FSH pulses were separable from the LH pulses, and they were not accompanied by an increase in LHRH in portal blood, whereas, all the LH pulses were accompanied by an increase in LHRH (25).

Bioassaying for FSH- and LH-releasing activity, we demonstrated more FSHRF in the caudal ME than could be accounted for the content of LHRH but not in the anterior ME (13). This supports the concept that FSHRF is mainly distributed to the caudal and mid-ME. This concept was supported since in a number of animals with anterior mid-ME lesions, pulsatile LH release was completely blocked, yet pulsatile FSH release occurred, albeit with somewhat smaller and less frequent pulses. There were only three animals with caudal lesions that extended into the mid-ME that had a selective block of pulsatile FSH release and still maintained pulsatile LH release. In these rats, the postulated terminals of the FSHRF neurons in the mid- and posterior ME were presumably destroyed.

Therefore, the lesion studies support the view that LHRH and FSHRF neurons have an overlapping distribution in the ME, as previously hypothesized (26), with the predominant distribution of the FSHRF to mid- and caudal ME and LHRH to anterior and mid-ME (Fig. 10). In view of our previous stimulation and lesion experiments, we postulated that the cell bodies of the FSHRH neurons are in the dorsomedial, anterior-hypothalamic area in the region of the paraventricular nucleus, with axons that project caudally and ventrally to predominantly the mid- and posterior ME. Other axons project to the organum vasculosum lamina terminalis (OVLT) since OVLT extracts gave a dose-related preferential release of FSH (27) (Fig. 10). Since the anterior pituitary content of FSH and LH in the smaller pituitaries of the rats with ME lesions was usually lowered, it is clear that loss of hypothalamic drive by LHRH and the putative FSHRF is associated with decreased synthesis as well as decreased release of gonadotropins.

Recently, we discovered that lamprey (1) GnRH-III is a potent FSH-releasing peptide, devoid of LH-releasing activity except at doses 100 or more times greater than the MED for FSH release (28). We believe that FSHRH is either this peptide or a closely related one. Neurons immunostained by antibodies directed against GnRH-III have been localized to the dorsomedial preoptic area rostral to the anterior tip of the paraventricular nucleus and their axons

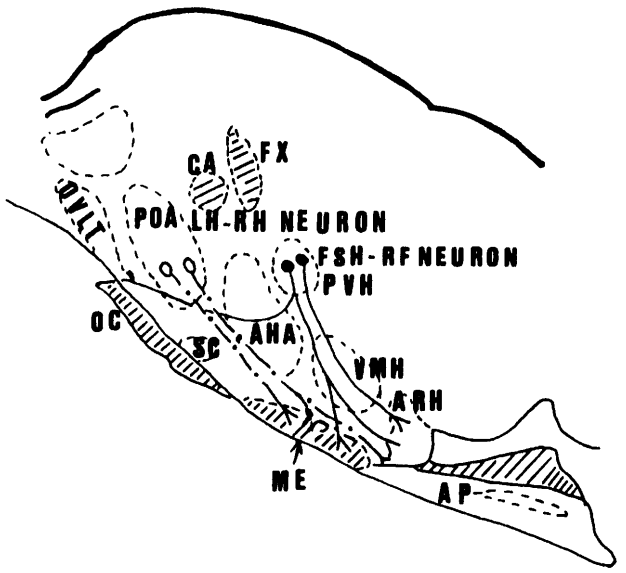


Figure 10. Postulated location of the FSHRF neuronal system on a parasagittal section through the hypothalamic-pituitary region of the rat. The known location of the LHRH neuronal system is also illustrated. Symbols: OC = optic chiasm; CA = anterior commissure; SC = suprachiasmatic nucleus; FX = fornix; PVH = paraventricular nucleus; AHA = anterior hypothalamic area; VMH = ventromedial nucleus; ARH = arcuate nucleus; ME = median eminence; AP = anterior pituitary.

traced to the ME (Dees WL *et al.*, unpublished data). Another population of GnRH-III neurons had cell bodies in the ventromedial preoptic area and axons that projected to the anterior ventral wall of the 3V and OVLT (Dees WL *et al.*, unpublished data), explaining the previous discovery of FSH-releasing activity in this region (27). The distribution of the GnRH-III immunoreactive neuronal system is consistent with the postulated distribution of the FSHRF neurons.

There are some similarities in the control of LHRH and FSHRH release. They both appear to be under noradrenergic and dopaminergic control (23); however, lesions of the zona incerta–dorsal hypothalamic dopaminergic tract, required for the preovulatory surge of LH, revealed a selective suppression of the LH, but not FSH, preovulatory surge, indicating a dopaminergic control of only the preovulatory LHRH but not the lesser preovulatory FSHRF surge (29). On the other hand, lesions of the locus coeruleus that blocked noradrenergic input to the hypothalamus interfered with the proestrous surge of both LH and FSH, although the effect on the LH surge was greater (30).

None of the results cited above can be explained on the basis of activin, a 30-kDa protein that also releases FSH selectively *in vitro* and *in vivo* (6). Because of its large size, it would not have the same elution position as the FSHRF following gel filtration (31). Furthermore, its minimal time to release FSH *in vivo* is at least 1 hr, whereas FSHRF increases plasma FSH within 10 min, the time course of elevation of FSH during FSH pulses *in vivo.* (32).

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