Alteration of Hypophyseal LH and FSH Release by Exogenous LH and FSH in Orchidectomized Mice* (35599)

CLAUDE DESJARDINS
(Introduced by N. Kaliss)
The Jackson Laboratory, Bar Harbor, Maine 04609

Implanting luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the median eminence of intact or gonadectomized rats of both sexes suppresses hypophyseal LH and FSH secretion, respectively (1, 2). Placing LH and FSH in the median eminence also reduces the concentration of their respective hypothalamic releasing factors (3, 4). These findings support the theory that the secretion of LH and FSH may be regulated, in part, via an "internal" feedback mechanism by which LH and FSH control the production of their own releasing factors and hence their respective hypophyseal secretions. Inherent in this theory is the concept that the hypothalamus contains feedback receptors that respond specifically to changing hypophyseal hormone titers (5–8). The present experiment was designed to examine the specificity of the "internal" feedback system for LH and FSH. This was done by measuring hypophyseal and plasma concentrations of both LH and FSH following systemic injections of either LH or FSH in orchidectomized mice. The results show that exogenous LH suppressed hypophyseal LH release independently of FSH and that exogenous FSH suppressed hypophyseal FSH release independently of LH.

Materials and Methods. Male C57BL/6J mice were weaned at 21–25 days of age and housed in cages (15 × 15 × 30 cm) in groups of six at 20 ± 2° with 14 hr of light every 24 hr. Mice were orchidectomized at 70–75 days of age and assigned to treatment groups 21 days later using a 3 × 3 completely randomized block design. The experiment was replicated three times with 25 mice/block. Subcutaneous injections of either (i) LH (83.3 μg of NIH-LH-S11), (ii) FSH (83.3 μg of NIH-FSH-S3), or (iii) a control solution containing 100 μg of ovine serum glycoprotein was administered at 8-hr intervals in 0.25-ml aliquots of 0.85% sodium chloride for either 1, 3, or 6 days.

Mice were decapitated 8 hr after the last injection to obtain blood and hypophyses. Oxalate-treated blood was centrifuged and the supernatant plasma was stored at —25°. Plasma from each replicate block was pooled within treatment (75 mice) and assayed for LH and FSH activity once. Hypophyses were weighed, and pooled homogenates were prepared (10 mg/ml in 0.85% sodium chloride) from each block of 25 mice. Homogenates were centrifuged at 5000*g* for 15 min at 3° and the supernatant fluid was stored at —25°. The supernatant fluid from each block was assayed for LH and FSH activity.

The LH was determined with the 1 ovary, 4-hr ovarian ascorbic acid depletion method (9), employing 5 rats (Sprague-Dawley) for each dose level of standard or unknown. The LH potencies of unknown hypophyses were measured with 0.2 and 0.8 mg-equivalents of fresh tissue, and these were compared with 0.4 and 1.6 μg of NIH-LH-S11. Plasma LH was similarly determined except that 1 and 4 ml of blood plasma were used as unknowns for the low and high doses. Since previous research (10, 11) cautioned that the ovarian ascorbic acid depletion assay may not be specific for plasma LH, a pilot experiment was designed to determine whether or not this assay could be used to quantitate plasma LH in mice. The ovarian ascorbic acid depleting ability of 0.85% sodium chloride was compared with three types of mouse blood plasma: (i) normal plasma, (ii) normal plasma incubated at 98° for 15 min to destroy LH

* Supported by an allocation from a U. S. Public Health Service General Research Support Grant, FR-00545-05, to The Jackson Laboratory. The principles of laboratory animal care as promulgated by the Council of the American Physiological Society are observed in this Laboratory.

activity, and (iii) plasma from hypophysectomized male mice. The concentration of ovarian ascorbic acid recorded after administering incubated plasma, plasma from hypophysectomized mice, and 0.85% sodium chloride to suitably prepared rats (9) was not significantly different ($p>0.10$). Only normal plasma depleted ovarian ascorbic acid ($p<0.03$).

The FSH was measured according to the ovarian weight augmentation assay (12), employing 5 rats (Sprague-Dawley) for each dose level of standard and unknown. The FSH potencies of unknown hypophyses were measured with 3 and 6 mg-equivalents of fresh tissue and these were compared with 60 and 120 μg of NIH-FSH-S3 and with 50 IU of human chorionic gonadotropin alone in 21-day-old rats. Plasma FSH was similarly determined except that 7.5 and 15 ml of blood plasma was used for the low and high dose, respectively.

Bliss's (13) procedure for calculating relative potencies was followed. Replicate potency estimates were combined by the procedure of Sheps and Moore (14). Hypophyseal potency estimates were subjected to analysis of variance and orthogonal contrasts were used to evaluate treatment differences.

Results. Hypophyseal LH concentrations were lower in LH treated than in control mice (Table I), when LH was administered for either 3 or 6 days ($p<0.05$), but not for 1 day ($p>0.10$). Mice receiving exogenous LH for 1, 3, and 6 days contained an average of 51, 77, and 88% less hypophyseal LH, respectively, than mice receiving control injections at these same times. Plasma LH concentrations were 14, 39, and 68% less than their respective control values when LH was administered for 1, 3, and 6 days, respectively. In contrast, FSH administration for 6 days did not measurably lower the concentration of LH in either the hypophyses ($p>0.15$) or plasma from their respective control values (Table I).

Mice receiving exogenous FSH for either 3 or 6 days, but not for 1 day, contained significantly less hypophyseal FSH than control mice (Table II) treated on these same days ($p<0.05$). The mean concentration of hypophyseal FSH was 29, 62, and 85% less than their respective controls when FSH was administered for 1, 3, and 6 days. Plasma FSH concentration decreased 8, 29, and 52% below their respective control values after exogenous FSH was administered for 1, 3, and 6 days, respectively. Again, in contrast, 6 days of LH administration failed to measurably reduce the concentration of FSH in ei-

TABLE I. Effect of Exogenous LH and FSH on the Concentration of Hypophyseal and Plasma LH in Orchidectomized Mice.

Exogenous hormone	No. of days treated	Hypophyseal LH[a] Potency (95% limits)	Plasma LH[b] Potency (95% limits)
Control	1	6.77 (1.54– 9.88)	3.31 (1.92–6.91)
LH	1	3.27 (1.37– 5.58)	2.83 (1.51–4.99)
FSH	1	6.46 (3.34– 8.71)	2.96 (1.83–5.37)
Control	3	7.33 (3.45–12.68)	2.97 (1.61–6.77)
LH	3	1.62 (0.49– 4.57)	1.81 (0.67–4.22)
FSH	3	6.28 (3.64– 9.03)	3.27 (1.88–5.69)
Control	6	7.29 (3.48–12.90)	3.45 (1.75–6.65)
LH	6	0.86 (0.34– 1.87)	1.07 (0.34–3.76)
FSH	6	6.70 (3.03– 9.11)	2.63 (1.74–5.18)

[a] Each value represents the combined potency and 95% limits of three replicate assays expressed in μg-equivalents of NIH-LH-S11/mg of wet hypophysis. Combined index of precision was 0.28.

[b] Each value represents the potency and 95% limits of one assay expressed in μg-equivalents of NIH-LH-S11/100 ml of blood plasma. Combined index of precision was 0.32.

TABLE II. Effect of Exogenous LH and FSH on the Concentration of Hypophyseal and Plasma FSH in Orchidectomized Mice.

Exogenous hormone	No. of days treated	Hypophyseal FSH[a] Potency (95% limits)	Plasma FSH[b] Potency (95% limits)
Control	1	3.95 (1.76–7.07)	6.41 (3.31–9.36)
LH	1	3.54 (1.67–5.32)	6.13 (3.17–9.25)
FSH	1	2.79 (1.08–4.84)	5.87 (3.02–8.53)
Control	3	4.01 (1.88–7.16)	5.97 (3.11–8.91)
LH	3	3.74 (1.63–5.15)	6.22 (3.20–9.24)
FSH	3	1.49 (0.59–3.17)	4.21 (1.94–6.30)
Control	6	4.87 (1.49–7.43)	6.33 (3.28–9.08)
LH	6	3.78 (1.48–5.44)	6.01 (3.15–8.78)
FSH	6	0.73 (0.21–2.21)	3.01 (1.83–4.89)

[a] Each value represents the combined potency and 95% limits of three replicate assays expressed in μg-equivalents of NIH-FSH-S3/mg of wet hypophysis. Combined index of precision was 0.31.

[b] Each value represents the potency and 95% limits of one assay expressed in μg-equivalents of NIH-FSH-S3/100 ml of blood plasma. Combined index of precision was 0.36.

ther the hypophyses ($p > 01.5$) or plasma below control values (Table II).

Discussion. The present findings demonstrate the systemic injections of LH unequivocally depressed the concentration of hypophyseal and plasma LH without measurably altering the concentration of either hypophyseal or plasma FSH. Additionally, systemic injections of FSH markedly reduced hypophyseal and plasma FSH concentrations without effectively lowering the LH concentration of either the hypophyses or plasma. These observations demonstrate for the first time that systemic injections of relatively "pure" LH and FSH specifically suppressed the release and possibly the synthesis of their own hypophyseal counterparts.

The decrease in endogenous LH and FSH release following LH and FSH injections was cumulative and parallel throughout the time these tropins were administered. The ability of exogenous LH and FSH to suppress their respective hypophyseal secretions may be explained on the basis of a direct effect of each gonadotropin or by one of their metabolites acting on the hypothalamus. This speculation appears warranted since LH implants placed in the hypothalamus inhibited LH secretion while those placed in either the hypophysis or cortex did not (2).

Although the concentrations of hypophyseal LH and FSH were less than control values when the other hormone was administered these differences were not significant. These observations may be explained on the basis of contaminants or by either intrinsic LH activity in FSH or of an intrinsic FSH activity in LH (15). The failure to observe a measurable interaction between LH and FSH in the present experiments coincides with the observation that implants of LH and FSH in the median eminence specifically suppressed hypophyseal LH and FSH secretion, respectively (3, 4). The finding that exogenous LH and FSH may specifically suppress the release of their own hypophyseal counterparts supports the claim of others that the hypothalamus contains sensitive feedback receptors capable of differentiating between LH and FSH (2, 5).

Whether the changes in endogenous LH and FSH release elicited by exogenous LH and FSH has physiological significance in terms of "internal" feedback mechanisms or was simply caused by the pharmacological levels of LH and FSH employed here remains to be shown. Nevertheless, recent observations indicate that the unit firing rate of cells in the rat hypothalamus may be low-

ered within minutes after injecting physiological doses of LH (16).

Summary. The concentration of LH and FSH in the hypophyses and plasma of ochidectomized mice was determined after injecting LH, FSH, or a control protein for 1, 3, and 6 days, respectively. Exogenous LH reduced the concentration of hypophyseal and plasma LH but FSH release was not affected in these same animals. Similarly, exogenous FSH reduced the concentration of hypophyseal and plasma FSH but LH release was not affected. This evidence suggests that exogenous LH and FSH can suppress their respective secretions specifically.

The author expresses appreciation to the Endocrine Study Section of the National Institutes of Health for supplies of NIH-LH-S11 and NIH-FSH-S3.

1. Corbin, A. and Cohen, A. I., Endocrinology **78**, 41 (1966).

2. David, M. A., Fraschini, F., and Martini, L., Endocrinology **78**, 55 (1965).

3. Corbin, A., Endocrinology **78**, 893 (1966).

4. Corbin, A. and Story, J. C., Endocrinology **80**, 1006 (1967).

5. Motta, M., Mangili, G., and Martini, L., Endocrinology **77**, 392 (1965).

6. Katz, S., Moltich, M., and McCann, S. M., Program 49th Meeting Endocrine Soc. **1967**, 23.

7. Clemens, J. A. and Meites, J., Endocrinology **82**, 878 (1968).

8. Mangili, G., Motta, M., and Martini, L., *in* "Neuroendocrinology" (L. Martini and W. F. Ganong, eds.); Vol. 1, p. 297. Academic Press, New York (1966).

9. Parlow, A. F., *in* "Human Pituitary Gonadotropins" (A. Albert, ed.) p. 300. Thomas, Springfield, Illinois (1961).

10. Schwartz, N. B. and Caldarelli, D., Proc. Soc. Exptl. Biol. Med. **119**, 16 (1965).

11. de Groot, C. A., Acta Endocrinol. **55**, 611 (1967).

12. Steelman, S. L. and Pohley, F. M., Endocrinology **53**, 604 (1953).

13. Bliss, C. I., "The Statistics of Bioassay." Academic Press, New York (1952).

14. Sheps, M. C. and Moore, E. A., J. Pharmacol. Exptl. Therap. **128**, 99 (1960).

15. Gemzell, C. and Roos, P., *in* "The Pituitary Gland" (G. W. Harris and B. T. Donovan, eds.) Vol. 1, p. 492. Univ. of California Press, Berkeley, California (1966).

16. Ramirez, V. D., Komisaruk, B. R., Whitmoyer, D. I., and Sawyer, C. H., Am. J. Physiol. **212**, 1376 (1967).

Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.