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INTERACTION OF NEUROPEPTIDES WITH GONADAL FUNCTION

CATHERINE RIVIER
The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

INTRODUCTION

The secretion of the gonadotropins LH and FSH is controlled by the interaction of factors from brain and peripheral origin [1–4]. The decapeptide gonadotropin-releasing hormone (GnRH), originally isolated and characterized in the mammalian hypothalamus [5, 6], represents a major physiological regulator of reproductive functions, but its pituitary effects are modulated by sex steroids and non-steroidal gonadal proteins. The secretion of GnRH itself is under the multifactorial influence of opiates, bioamines and steroids [3, 7]. LH and FSH are secreted concomitantly under a variety of physiological and pathophysiological circumstances, but there are also several well-defined conditions of dissociated LH and FSH release. These include portions of the rodent estrous cycle [8], acute gonadectomy [9] and, in the male, Sertoli cell dysfunction [10, 11]. There is now evidence that non-steroidal gonadal compounds, including a 32 kDa protein called inhibin [12, 13], may be involved in mediating the non-parallel release of gonadotropins [14–17]. This paper will discuss some aspects of the physiological role and pharmacological effects of inhibin in the female rat.

EXPERIMENTAL

Female Sprague–Dawley rats were kept under standard laboratory conditions, with rat chow and water *ad libitum*. Ovariectomy, when necessary, was performed under metofane anesthesia. Blood samples were also obtained in anesthetized animals. Separated plasma were snap-frozen and kept at −20°C until assayed. Plasma LH and FSH levels were measured by RIA, using reagents provided by the National Pituitary and Hormone Distribution Program of the NIDDK. Results are expressed in terms of the RP-2 LH or FSH standards. Recombinant human inhibin was prepared as described previously [18]. Following ANOVA, differences between treatments were analyzed by the multiple range test of Duncan.

RESULTS AND DISCUSSION

Inhibin is a protein secreted, in particular, by granulosa cells [19], which is found in follicular fluid (FF) [20] and which specifically interferes with FSH secretion [21, 22]. The physiological role of inhibin has been demonstrated by experiments showing that immunoneutralization of endogens inhibin increases plasma FSH, but not LH, in female rats aged 20 days and older (Fig. 1) [23–26]. This suggests that FSH release is under the tonic inhibitory influence of endogenous inhibin. Further support for the role of inhibin in mediating FSH secretion came from the simultaneous measurement of plasma inhibin and FSH levels in the female rat. Until day 17 of age, the circulating levels of both FSH and inhibin increase, and immunoneutralization of endogenous inhibin does not measurably alter FSH values [24, 26]. At day 17 however, plasma FSH levels show an abrupt decrease, accompanied by a concomitant rise in inhibin values [24], and from that time, polyclonal antibodies raised against the N-terminal portion of the α-chain of inhibin [27] increase radioimmunoassayable FSH levels [28]. These results suggest the existence of a functional relationship between inhibin and FSH in female rats older than 17 days and supports the hypothesis that endogenous inhibin plays a physiological role in regulating FSH secretion.

The estrous cycle of the rat is characterized by a proestrous surge of LH and FSH (the primary

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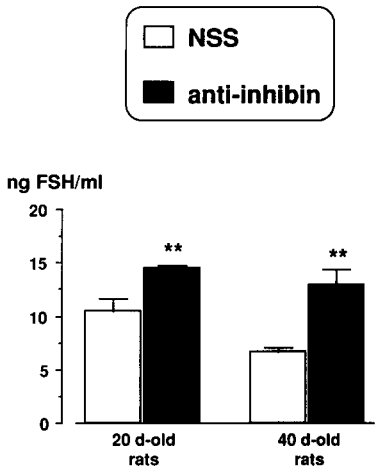

Fig. 1. Effect of immunoneutralization of endogenous inhibin on FSH secretion in the female rat. Normal sheep serum (NSS) or polyclonal antibodies against the N-terminal portion of the α-chain of inhibin were injected i.v. to intact rats. Blood samples were obtained 6 h later. Each bar represents the mean ± SEM of 5 animals. $^{**}P \leq 0.01$.

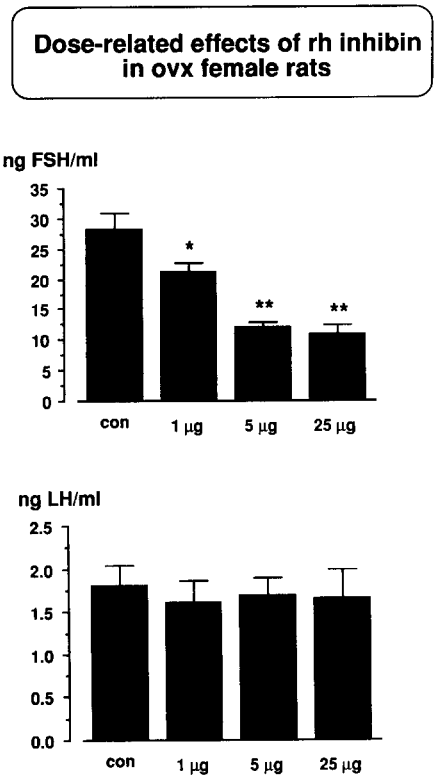

Fig. 2. Effect of the i.v. injection of rh inhibin on FSH and LH secretion in adult ovariectomized rats. Each bar represents the mean ± SEM of 5 animals. Blood samples were obtained 6 h after treatment. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

surge), while only FSH secretion increases during the early morning of estrous (the secondary surge) [8]. Studies showing that injections of purified FF, but not GnRH antagonists, interferes with this secondary surge [29–34], indicated that the estrous secretion of FSH was at least in part modulated by inhibin. We [35] and others [36] have provided support for this hypothesis by demonstrating that plasma radioimmunoassayable inhibin levels significantly decrease during late proestrous in rats showing a secondary FSH surge [35], that experimental manipulations which would prevent the decrease in inhibin release (such as a blockade of the primary LH rise) abolish the estrous changes in FSH release, and that exogenous LH, which accentuates the fall in inhibin values of GnRH-antagonists treated rats [37], also restores the secondary FSH surge [32, 37–39].

While many valuable studies have been carried out with purified, charcoal-extracted FF as a source of inhibin, we now know that such preparations contain not only inhibin, but also activin (which specifically stimulates FSH release) and follistatin (which inhibits this release) [20, 22, 40]. Therefore, the net FSH stimulating or inhibiting activity of FF depends on the interaction of several compounds, and does not represent the true biological action of inhibin. It was therefore of great interest to examine the ability of recently available preparations of recombinant human (rh) inhibin [18], to alter FSH and LH secretion. We have recently reported that the i.v. injection of rh inhibin causes dose-dependent decreases in plasma radioimmunoassayable FSH levels in the absence of measurable changes in LH secretion [41]. This effect can be observed in female rats as early as day 14 of age, as well as in both intact or ovariectomized adult animals (Fig. 2). In our hands, both mean plasma FSH levels and all parameters of pulsatile FSH secretion, are inhibited by rh inhibin [41].

These studies indicate that in addition to GnRH, sex steroids, and possibly follistatin and activin, inhibin represents an important regulator of FSH secretion in the female rat.

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REFERENCES

1. McNeilly A. S.: The control of FSH secretion. *Acta Endocr. Copenh.* **288** (1988) 31–40.

2. McCann S. M.: Regulation of secretion of follicle-stimulating hormone and luteinizing hormone. In *Handbook of Physiology* (Edited by E. Knobil and W. H. Sawyer). Am. Physiol. Soc., Washington, DC, Vol. 4 Sect. 7 (1974) pp. 489–517.
3. Kalra S. P. and Kalra P. S.: Neural regulation of luteinizing hormone secretion in the rat. *Endocrine Rev.* **4** (1983) 311–351.
4. Chappel S. C.: Neuroendocrine regulation of luteinizing hormone and follicle stimulating hormone: a review. *Life Sci.* **36** (1985) 97–103.
5. Burgus R., Butcher M., Amoss M., Ling N., Monahan M., Rivier J., Fellows R., Blackwell R., Vale W. and Guillemin R.: Primary structure of the ovine hypothalamic luteinizing hormone-releasing factor (LRF). *Proc. Natn. Acad. Sci. U.S.A.* **69** (1972) 278–282.
6. Matsuo H., Baba Y., Nair R., Arimura A. and Schally A. V.: Structure of the porcine LH- and FSH-releasing hormone. I. Proposed amino acid sequence. *Biochem. Biophys. Res. Commun.* **43** (1971) 1334–1339.
7. Barraclough C. A. and Wise P. M.: The role of catecholamines in the regulation of pituitary luteinizing hormone and follicle-stimulating hormone secretion. *Endocrine Rev.* **3** (1982) 91–119.
8. Schwartz N. B.: A model for the regulation of ovulation in the rat. *Rec. Prog. Horm. Res.* **25** (1969) 1–55.
9. Srtz N. B., Milette J. J. and Cohen I. R.: Animal models which demonstrate divergence in secretion or storage of FSH and LH. In *Inhibin—Non-steroidal Regulation of Follicle Stimulating Hormone Secretion* (Edited by H. G. Burger, D. M. DeKretser, J. K. Findlay and M. Igarashi). Serono Symposia, Raven Press, New York, Vol. 42 (1987) p. 239.
10. Au C. L., Robertson D. M. and DeKretser D. M.: Relationship between testicular inhibin content and serum FSH concentrations in rats after bilateral efferent duct ligation. *J. Reprod. Fert.* **72** (1984) 351–356.
11. Au C. L., Robertson D. M. and DeKretser D. M.: Changes in testicular inhibin after a single episode of heating of rat testes. *Endocrinology* **120** (1987) 973–977.
12. Mason A. J., Hayflick J. S., Ling N., Esch F., Ueno N., Ying S. Y., Guillemin R., Niall H. and Seeburg P. H.: Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor-β. *Nature* **318** (1985) 659–663.
13. Mayo K. E., Cerelli G. M., Spiess J., Rivier J., Rosenfeld M. G., Evans R. M. and Vale W.: Inhibin α-subunit cDNAs from porcine ovary and human placenta. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 5849–5853.
14. Charlesworth M. C., Grady R. R., Shin L., Vale W. W., Rivier C., Rivier J. and Schwartz N. B.: Differential suppression of FSH and LH secretion by follicular fluid in the presence or absence of GnRH. *Neuroendocrinology* **38** (1984) 199–205.
15. Grady R. R., Charlesworth M. C. and Schwartz N. B.: Characterization of the FSH-suppressing activity in follicular fluid. *Recent Prog. Horm. Res.* **38** (1982) 409–456.
16. Channing C. P., Gordon W. L., Liu W. K. and Ward D. N.: Physiology and biochemistry of ovarian inhibin. *Proc. Soc. Exp. Biol. Med.* **178** (1985) 339–361.
17. DeJong F. H.: Inhibin. *Physiol. Rev.* **68** (1988) 555–607.
18. Mason A. J., Schwall R., Renzy M., Rhee L. M., Nikolics K. and Seeburg P. H.: Human inhibin and activin: structure and recombinant expression in mammalian cells. In *Inhibin—Non-steroidal Regulation of Follicle-stimulating Hormone Secretion* (Edited by H. G. Burger, D. M. DeKretser, J. K. Findlay and M. Igarashi). Raven Press, New York (1987) pp. 77–88.
19. Bicsak T. A., Tucker E. M., Cappel S., Vaughan J. M., Rivier J., Vale W. and Hsueh A. J. W.: Hormonal regulation of granulosa cell inhibin synthesis. *Endocrinology* **119** (1986) 2711–2719.
20. Vale W., Hsueh A., Rivier C. and Yu J.: The inhibin-activin family of hormones and growth factor. In *Peptide Growth Factors and Their Receptors, Handbook of Experimental Pharmacology* (Edited by M. A. Sporn and A. B. Roberts). Springer, New York (1990) 211–248.
21. DeJong F. H., Grootenhuis A. J. and Klaij I. A.: Inhibin and related proteins: localization, regulation and effects. In *Circulating Regulatory Factors and Neuroendocrine Function* (Edited by J. C. Porter and D. Ježovó) (1990) p. 271.
22. Vale W., Rivier C., Hsueh A., Campen C., Meunier H., Bicsak T., Vaughan J., Corrigan A., Bardin W., Sawchenko P., Petraglia F., Yu J., Plotsky P., Spiess J. and Rivier J.: Chemical and biological characterization of the inhibin family of protein hormones. In *Proceedings of the Laurentian Conference*. Academic Press, San Diego, CA, Vol. 44 (1988) pp. 1–34.
23. Rivier C., Rivier J. and Vale W.: Inhibin-mediated feedback control of follicle-stimulating hormone secretion in the female rat. *Science* **234** (1986) 205–208.
24. Rivier C. and Vale W.: Inhibin: measurement and role in the immature female rat. *Endocrinology* **120** (1987) 1688–1690.
25. Culler M. D. and Negro-Villar A.: Endogenous inhibin suppresses only basal FSH secretion but suppresses all parameters of pulsatile LH secretion in the diestrous female rat. *Endocrinology* **124** (1989) 2944–2953.
26. Culler M. D. and Negro-Villar A.: Passive immunoneutralization of endogenous inhibin: sex-related differences in the role of inhibin during development. *Molec. Cell. Endocr.* **58** (1988) 263–273.
27. Vaughan J. M., Rivier J., Corrigan A. Z., McClintock R., Campen C. A., Jolley D., Voglmayr J. K., Bardin C. W., Rivier C. and Vale W.: Detection and purification of inhibin using antisera generated against synthetic peptide fragments. In *Methods in Enzymology* (Edited by P. M. Conn). Academic Press, Orlando, FL, Vol. 168 (1988) pp. 588–617.
28. Rivier C., Rivier J. and Vale W.: Inhibin: measurement and role in the rat. *I. S. O. R. VII Colloque Int. de Lyon*, Lyon France (April 1987).
29. Hasegawa Y., Miyamoto K., Yazaki C. and Igarashi M.: Regulation of the second surge of follicle-stimulating hormone; effects of antiluteinizing hormone- releasing hormone serum and pentobarbital. *Endocrinology* **109** (1981) 130–135.
30. Blake C. A. and Kelch R. P.: Administration of anti-luteinizing hormone-releasing hormone serum to rats: effects on periovulatory secretion of luteinizing hormone and follicle-stimulating hormone. *Endocrinology* **109** (1981) 2175–2179.
31. Condon T. P., Heber D., Stewart J. M., Sawr C. H. and Whitmoyer D. I.: Differential gonadotropin secretion: blockade of periovulatory LH but not FSH secretion by a potent LHRH antagonist. *Neuroendocrinology* **38** (1984) 357–361.
32. Ashiru O. A. and Blake C. A.: Stimulation of endogenous follicle-stimulating hormone release during estrus by exogenous follicle-stimulating hormone of luteinizing hormone at proestrus in the phenobarbital-blocked rat. *Endocrinology* **105** (1979) 1162–1167.
33. Shander D., Anderson L. D., Barraclough C. A. and Channing C. P.: Interactions of porcine follicular fluid with ovarian steroids and luteinizing hormone-releasing hormone on the secretion of luteinizing hormone and follicle-stimulating hormone by cultured pituitary cells. *Endocrinology* **106** (1980) 237.
34. Schwartz N. B. and Channing C. P.: Evidence for ovarian "inhibin": suppression of the secondary rise in serum follicle stimulating hormone levels in proestrous rats by injection of porcine follicular fluid. *Proc. Natn. Acad. Sci. U.S.A.* **74** (1977) 5721–5724.

35. Rivier C. and Vale W.: Immunoneutralization of endogenous inhibin modifies hormone secretion and ovulation rate in the rat. *Endocrinology* **125** (1989) 152–157.
36. Hasegawa Y., Miyamoto K. and Igarashi M.: Changes in serum concentrations of immunoreactive inhibin during the oestrous cycle of the rat. *J. Endocr.* **121** (1989) 91–100.
37. Rivier C., Roberts V. and Vale W.: Possible role of LH and FSH in modulating inhibin secretion and expression during the estrous cycle of the rat. *Endocrinology* **125** (1989) 876–880.
38. Ashiru O. A. and Blake C. A.: Variations in the effectiveness with rat follicle-stimulating hormone can stimulate its own secretion during the rat estrous cycle. *Endocrinology* **106** (1980) 476–480.
39. Schwartz N. B. and Talley W. L.: Effects of exogenous LH of FSH on endogenous FSH, progesterone and estradiol secretion. *Biol. Reprod.* **17** (1978) 820–828.
40. Ling N., Ueno N., Ying S. Y., Esch F., Shimasaki S., Hotta M., Cuevas P. and Guillemin R.: Inhibins and activins. *Vit. Horm.* **44** (1988) 1–46.
41. Rivier C., Schwall R., Mason A., Burton L. and Vaughan J.: Effect of recombinant inhibin on LH and FSH secretion in the rat. *Endocrinology* **128** (1991) 1548–1554.