Development of a quotidian increase in pituitary responsiveness to GnRH in prepubertal female rats

M. Wilkinson and W. H. Moger

*Department of Physiology & Biophysics, Faculty of Medicine, Dalhousie University,
Halifax, Nova Scotia, Canada B3H 4H7*

Summary. Pituitaries from immature male and female rats (5, 13, 21, 27 and 30 days of age) were removed at 08:00 or 16:00 h and stimulated with GnRH *in vitro*. In female rats more LH was released from pituitaries taken at 16:00 h than at 08:00 h on Days 21, 27 and 30, but not on Days 5 and 13. There were no differences in male rats in responsiveness of pituitaries taken at these two times. The ability of GnRH to induce release of more LH in the late afternoon may help to synchronize hormone output to coincide with first ovulation.

Introduction

Studies of man (Boyar, 1978; Chipman, 1980; Grumbach, 1980) and a variety of animals have produced compelling evidence that the brain provides ultimate control over the onset of female sexual maturation (Ramaley, 1979; Döcke & Dörner, 1979; Foster & Ryan, 1979; Wildt, Marshall & Knobil, 1980). In the mature female rat the hypothalamo-pituitary unit receives a daily neural signal essential for the maintenance of reproductive cyclicity (see Everett, 1964, 1977). MacKinnon, Puig-Duran & Laynes (1978) have drawn attention to the possible ontogenetic appearance of this circadian event as an important developmental correlate of puberty. A further aspect of the hypothalamo-pituitary unit of mature animals, which may also be relevant to younger animals, is the ability of oestradiol to induce diurnal variations in pituitary responsiveness to gonadotrophin-releasing hormone (GnRH). The pituitaries of oestrogen-primed ovariectomized rats become more responsive to GnRH late in the light phase (Ruf & McElhone, 1979). In the immature female rat a spontaneous increase in blood and tissue levels of unbound (biologically active) oestradiol takes place about 21 days after birth (Germain, Campbell & Anderson, 1978; Puig-Duran, Greenstein & MacKinnon, 1979). The present study investigated whether this increase in blood oestrogen in the intact immature rat induces detectable fluctuations in pituitary responsiveness to GnRH of a type comparable to those noted by Ruf & McElhone (1979) in the adult.

Materials and Methods

Sprague–Dawley rats of both sexes were obtained from Canadian Breeding Farm and Laboratories, St Constant, Québec. The young, 0, 9 and 18 days old, arrived as litters of 6 with their mothers and were housed under fixed lighting conditions (lights on 07:00–19:00 h). Vaginal opening in this strain normally occurs between 35 and 40 days after birth.

Culture technique

Groups of 6 female rats were killed at 08:00 and 16:00 h on Days 5, 13, 21, 27 and 30 after birth. Male rats were killed at 5, 13, 27 and 65 days of age. Under a binocular microscope the anterior pituitary glands were carefully dissected free of the neural lobe, and bisected *in situ* (except those from the 5-day-old rats which were used whole to reduce tissue damage). The pituitary tissue was placed in organ culture as previously described (Wilkinson, de Ziegler, Cassard & Ruf, 1977). Briefly, individual pituitary halves were placed on a stainless-steel grid in an organ culture dish (Falcon No. 3010) which contained 0.5 ml Medium 199 (Gibco; cat. no. 330-1180) modified to contain HEPES buffer (0.01 M; Sigma), glucose (500 mg/100 ml), sodium pyruvate (0.001 M), 50 i.u. penicillin/ml and 50 µg streptomycin/ml. Cultures were incubated at 37°C in a water-saturated atmosphere of 95% air/5% CO₂ for two separate 60-min periods to allow basal secretion to stabilize. The medium was discarded after each period except for a 200 µl sample at 120 min which was used to determine basal (unstimulated) secretion of LH. At this point the medium was replaced with fresh medium which contained 500 µg bacitracin/ml (Sigma) and 1.7×10^{-9} M GnRH. The bacitracin was included to prevent degradation of the GnRH over the extended incubation periods used in these experiments (McKelvy *et al.*, 1976). The amount of LH secreted into the medium was determined in 50-µl aliquots removed at 60, 120 and 180 min after the addition of the GnRH. Each aliquot was replaced with 50 µl of fresh medium. Culture medium was diluted with buffer (0.01 M-sodium phosphate, pH 7.6, in 0.15 M-sodium chloride which also contained 0.1% gelatin and 0.01% Thimerosal) and frozen at -20°C. LH content in 20–40 µl aliquots of the diluted medium was assessed by radioimmunoassay with NIAMDD LH kits (rat NIAMDD-LH-RP1 reference preparation). The intra- and inter-assay coefficients of variation of the assay were 7.2% ($n = 10$) and 15.5% ($n = 6$) respectively (Wilkinson, Moger & Selin, 1980). The sensitivity was 1 ng/ml.

Statistical analysis was by Student's *t* test.

Results

Female rats

Stimulation of anterior pituitary tissue with GnRH from rats aged 5 and 13 days revealed no differences between the amount of LH released at 08:00 and 16:00 h (Text-fig. 1). However, at Days 21, 27 and 30 there was a significant increase in LH release from pituitary halves removed at 16:00 h when compared with those stimulated at 08:00 h (Table 1). In tissue from rats older than 21 days the overall response at 08:00 and 16:00 h began to decline; the age-related variation in pituitary response to stimulation with GnRH is shown in Text-fig. 2.

Table 1. LH released (ng/ml/3 h) from rat pituitaries incubated with GnRH (1.7×10^{-9} M)

Sex	Age (days)	Time killed	
		08:00 h	16:00 h
Female	21	13 200 ± 1100	16 800 ± 1200*
	27	9800 ± 700	14 400 ± 1000**
	30	4000 ± 450	5800 ± 450*
Male	5	1100 ± 200	1600 ± 150
	13	2300 ± 200	4300 ± 300**
	27	9500 ± 1000	7500 ± 550
	65	10 500 ± 1000	12 000 ± 1200

Values are mean ± s.e.m. for 10–12 dishes.

Significantly different from value at 08:00 h; * $P < 0.05$, ** $P < 0.001$.

Text-fig. 1. Time course of LH released into culture medium from GnRH stimulation of pituitary tissue obtained from female rats (aged 5 and 13 days) at 08:00 h (●) and 16:00 h (○). Values are mean $\pm$ s.e.m. for 10–12 culture dishes.

Text-fig. 2. Age-related changes in pituitary responsiveness to stimulation with GnRH (1.7×10^{-9} M). Each point (10–12 dishes) represents total LH released ($\pm$ s.e.m.) in a 3-h period from pituitary tissue obtained from female rats at 08:00 h. The responses are parallel for the two methods of calculation.

Additional experiments were performed at 12:00 h with animals aged 15 and 25 days (data not shown). At Day 15, there was no difference between the amount of LH released at 08:00 and at 12:00 h, but at Day 25, the pituitary halves removed at 12:00 h released significantly more LH in the first 2 h of incubation than did those obtained at 08:00 h (+62% at 60 min, $P < 0.001$, and +50% at 120 min, $P < 0.02$). However, total LH released over a 3-h period from pituitary tissue stimulated at 08:00 and 12:00 h was not significantly different.

Male rats

Anterior pituitary tissue from male rats was less responsive in terms of total LH release (Table 1). Only at Day 13 was a difference in response observed at 16:00 h compared to that at 08:00 h. However, pituitaries taken at 16:00 h from Day-65 rats were considerably more responsive ($P < 0.001$) at 60 and 120 min after addition of the GnRH than were those stimulated at 08:00 h from rats of the same age (data not shown). At 3 h the difference was no longer statistically significant (Table 1), suggesting that the readily releasable pool of LH is greater at 16:00 h.

Discussion

The onset of puberty in women is associated with a sleep-induced augmentation of pulsatile gonadotrophin secretion (Boyar, 1978; Grumbach, 1980) and is indicative of central nervous system maturation. The development of pulsatile LH secretion and its importance in sexual maturation has also been described for the monkey (Wildt *et al.*, 1980) and the sheep (Ryan & Foster, 1980). In the immature rat, fluctuating levels of gonadotrophins have been reported but appear to be restricted to rats younger than 21 days (Döhler & Wuttke, 1976; MacKinnon *et al.*, 1978; Frawley & Henricks, 1979; Ramaley, 1979). Grumbach (1980) has speculated that with the approach of puberty an increased pulsatile discharge of GnRH acts to augment pituitary responsiveness by a self-priming effect. Our results for prepubertal female rats could be interpreted in a similar way. Hypothalamic content of GnRH increases with the approach of puberty (Araki, Toran-Allerand, Ferin & Vande Wiele, 1975; Goomer, Saxena & Sheth, 1977; Watanabe, 1980), as does the pituitary content of gonadotrophins (Ramírez, 1974). The increase in responsiveness of the prepubertal female rat pituitary to GnRH could therefore be consistent with the existence of an endogenous pulsatile release of GnRH. An alternative possibility for the variable responsiveness which we have observed is a hormonal mechanism which de-sensitizes–re-sensitizes the pituitary to GnRH. In the adult female rat dexamethasone is able to abolish the diurnal changes in pituitary sensitivity (Ruf & McElhone, 1979). The circadian pituitary–adrenal rhythm matures in rats between 18 and 24 days after birth, depending on strain (Ader, 1969; Campbell & Ramaley, 1974; Ramaley, 1978). We are confident that daily or age-related changes in degradative enzymes are not contributing to the stimulatory efficacy of the GnRH noted in the present study because inclusion of bacitracin in the culture medium prevents this (McKelvy *et al.*, 1976; Kuhl, Rosniatowski & Taubert, 1978; Griffiths & Kelly, 1979).

The age-related changes in female pituitary response (Text-fig. 2) are readily correlated with previous in-vitro data (Dullaart, 1977) and also with the work of Chan, Clayton & Catt (1980) that showed pituitary GnRH receptors to be maximal at 20 days of age, but falling rapidly between Days 20 and 50. In males the correlation is also reasonably good, the highest number of receptors occurring at 30 days, but at 65 days pituitary responsiveness remains high although receptor levels have fallen by 60%. Aubert, Conne & Sizonenko (1979) used a different radioligand to measure GnRH receptors and found age-related changes in receptor number different from those reported by Chan *et al.* (1980). There is also a discrepancy between the

in-vitro and in-vivo results for the time of day at which peak response is obtained; Ruf & McElhone (1979) noted that the greatest response to GnRH *in vivo* occurred at 12:00 h whereas our results in the immature, intact females show this peak to be later in the day. Preliminary observations (unpublished) with the adult preparations used by Ruf & McElhone (1979) show that *in vitro* the maximum response changes from 12:00 to 17:00 h, suggesting that possibly some additional, circulating, factor is exerting an influence in the in-vivo experiments.

Our results are suggestive of a putative quotidian increase in pituitary response to GnRH, possibly through the pulsatile release of endogenous GnRH, which may assist in the synchronization of gonadotrophin output at first ovulation.

We thank Alice Giles and Dale Grovestine for their help with the LH assays: Dr S. L. Dombey (Hoechst, U.K.) for the GnRH; and Dr Catherine Wilson for her interest in the preliminary stages of this work. The reagents for the LH assays were supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Financial support was from the M.R.C. of Canada, Grant Nos MA-7131 (M.W.) and MA-5401 (W.H.M.).

References

- Ader, R. (1969) Early experiences accelerate maturation of the 24-hour adrenocortical rhythm. *Science, N.Y.* **163**, 1225–1226.
- Araki, S., Toran-Allerand, C.D., Ferin, M. & Vande Wiele, R.L. (1975) Immunoreactive GnRH during maturation in the rat: ontogeny of regional hypothalamic differences. *Endocrinology* **97**, 693–697.
- Aubert, M.L., Conne, B.S. & Sizonenko, P.C. (1979) Developmental changes in pituitary LHRH receptors during puberty in the male rat. *Endocrinology* **104**, Suppl. p. 198, Abstr. 501.
- Boyar, R.M. (1978) Control of the onset of puberty. *Ann. Rev. Med.* **29**, 509–520.
- Campbell, C.B.G. & Ramaley, J.A. (1974) Retino-hypothalamic projections: correlation with onset of the adrenal rhythm in infant rats. *Endocrinology* **94**, 1201–1204.
- Chan, V., Clayton, R.N. & Catt, K.J. (1980) Pituitary GnRH receptors and gonadotropin secretion during development. *Proc. 6th Int. Congr. Endocrinology, Melbourne*, p. 295, Abstr. 172.
- Chipman, J.J. (1980) Pubertal control mechanisms as revealed from human studies. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **39**, 2391–2394.
- Döcke, F. & Dörner, G. (1979) Experimental studies on the neurohormonal control of female puberty. *Zentbl. Gynak.* **101**, 1082–1088.
- Döhler, K.D. & Wuttke, W. (1976) Circadian fluctuations of serum hormone levels in prepubertal male and female rats. *Acta endocr., Copenh.* **83**, 269–279.
- Dullaart, J. (1977) Immature rat pituitary glands *in vitro*: age and sex-related changes in luteinizing hormone releasing hormone-stimulated gonadotrophin release. *J. Endocr.* **73**, 309–319.
- Everett, J.W. (1964) Central neural control of reproductive function of the adeno-hypophysis. *Physiol. Rev.* **44**, 373–431.
- Everett, J.W. (1977) The timing of ovulation. *J. Endocr.* **75**, 3P–13P.
- Foster, D.L. & Ryan, K.D. (1979) Endocrine mechanisms governing transition into adulthood: a marked increase in inhibitory feedback action of estradiol on tonic secretion of LH in the lamb during puberty. *Endocrinology* **105**, 896–904.
- Frawley, L.S. & Henricks, D.M. (1979) Mode of gonadotropin secretion in infantile female rats and the role of estrogen in feedback regulation. *Endocrinology* **105**, 1064–1072.
- Germain, B.J., Campbell, P.S. & Anderson, J.N. (1978) Role of the serum estrogen-binding protein in the control of tissue estradiol levels during postnatal development of the female rat. *Endocrinology* **103**, 1401–1410.
- Goomer, N., Saxena, R.N. & Sheth, A.R. (1977) Correlation of hypothalamic LHRH, pituitary LH and plasma LH in developing rats. *Endokrinologie* **70**, 131–141.
- Griffiths, E.C. & Kelly, J.A. (1979) Mechanisms of inactivation of hypothalamic regulatory hormones. *Mol. cell. Endocr.* **14**, 3–17.
- Grumbach, M.M. (1980) The neuroendocrinology of puberty. In *Neuroendocrinology*, pp. 249–258. Eds D. T. Krieger & J. C. Hughes. Sinauer Associates Inc., Sunderland, Massachusetts.
- Kuhl, H., Rosnatiowski, C. & Taubert, H.-D. (1978) Activity of an LHRH degrading enzyme in the anterior pituitary during the rat oestrus cycle and its alteration by injections of sex hormones. *Acta endocr., Copenh.* **87**, 476–484.
- MacKinnon, P.C.B., Puig-Duran, E. & Laynes, R. (1978) Reflections on the attainment of puberty in the rat: have circadian signals a role to play in its onset? *J. Reprod. Fert.* **52**, 401–412.
- McKelvy, J.F., LeBlanc, P., Laudes, C., Perrie, S., Grimm-Jorgensen, Y. & Kordon, C. (1976) The use of bacitracin as an inhibitor of the degradation of TRF and LRF. *Biochem. Biophys. Res. Commun.* **73**, 507–515.
- Puig-Duran, E., Greenstein, B.D. & MacKinnon, P.C.B. (1979) Effects of serum oestrogen-binding components on the unbound oestradiol-17 β fraction in the serum of developing female rats and on inhibition of [3 H]oestradiol uptake by uterine tissue *in vitro*. *J. Reprod. Fert.* **56**, 707–714.

- Ramaley, J.A.** (1978) The adrenal rhythm and puberty onset in the female rat. *Life Sci.* **23**, 2079–2088.
- Ramaley, J.A.** (1979) Development of gonadotropin regulation in the prepubertal mammal. *Biol. Reprod.* **20**, 1–31.
- Ramírez, V.D.** (1974) Endocrinology of puberty. In *Handbook of Physiology*, Section 7. Endocrinology, Vol. II, Part 1, pp. 1–28. Eds R. O. Greep & E. B. Astwood. Am. Physiol. Soc., Washington, D.C.
- Ruf, K.B. & McElhorne, J.** (1979) Diurnal variation in pituitary sensitivity to LHRH. *Soc. Neurosci. Abstr.* **5**, 457.
- Ryan, K.D. & Foster, D.L.** (1980) Neuroendocrine mechanisms involved in onset of puberty in the female: concepts derived from the lamb. *Fedn Proc. Fedn Am. Socs exp. Biol.* **39**, 2373–2377.
- Watanabe, K.** (1980) Regional differences in the development of LHRH nerve endings in the rat. *Endocrinology* **106**, 139–144.
- Wildt, L., Marshall, G. & Knobil, E.** (1980) Experimental induction of puberty in the infantile female rhesus monkey. *Science, N.Y.* **207**, 1373–1375.
- Wilkinson, M., de Ziegler, D., Cassard, D. & Ruf, K.B.** (1977) In vitro studies of the effects of oestrogen pretreatment on the sensitivity of the immature female rat pituitary gland to stimulation with GnRH. *J. Endocr.* **74**, 11–21.
- Wilkinson, M., Moger, W.H. & Setin, L.K.** (1980) An unexpected increase in pituitary sensitivity to GnRH after treatment with porcine follicular fluid (inhibin) in immature hemicastrate rats. *Can. J. Physiol. Pharmacol.* **58**, 220–222.

Received 21 July 1980