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THE PARTICIPATION OF LUTEINIZING HORMONE RELEASING HORMONE (LRH) IN THE PROCESS OF SEXUAL MATURATION[1]

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Summary

Precocious pubertal ovulation and premature steroid-induced gonadotropin surges were triggered in 23-day old rats by unilateral electrolytic lesions placed in the basal hypothalamus. Attempts were made to reproduce both aspects of precocious sexual maturation by repeated injections or prolonged infusion of Luteinizing Hormone Releasing Hormone (LRH) or by repeated administration of the potent and long-acting analog (D-Leu^{6}, des-Gly-NH_2 ^{10})-LRH-ethylamide. Neither effect of the brain lesion was reproduced by LRH or its analog. It is concluded that brain lesions advance puberty in this species by mechanisms not involving the discharge of LRH. The role of the adrenal cortex in providing estrogen precursors required for the induction of ovarian gonadotropin receptors is discussed.

Zusammenfassung

Vorzeitige pubertale Ovulation und steroid-indizierte Gonadotropin-Ausschüttung konnten bei 23 Tage alten Ratten durch unilaterale elektrolytische Läsionen im basalen Hypothalamus erzeugt werden. Durch wiederholte Injektionen oder protrahierte Infusion von LRH oder durch wiederholte Zuführung des stark und lange wirkenden LRH Analogs (D-Leu^{6}, des-Gly-NH_2 ^{10})-LRH-Ethylamid, versuchten wir beide Aspekte der Pubertas praecox experimentell zu reproduzieren, jedoch ohne Erfolg. Daraus ziehen wir den Schluss, dass die durch Hirnläsionen induzierte Pubertas praecox vom Mechanismus der LRH-Ausschüttung unabhängig ist. Die Nebennierenrinde als Entstehungsort von Oestrogen-Vorstufen könnte bei der Induktion der ovariellen Gonadotropin-Rezeptoren eine Rolle spielen.

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Introduction

From the very beginning of the modern era of endocrinology, two classes of hormones have become firmly associated with sexual maturation in female mammals: ovarian estrogens (ALLEN and DOISY 1923) and pituitary gonadotropins (SMITH, 1926). Following the identification of LRH (SCHALLY et al. 1971), it appeared plausible to postulate that the pubertal activation of the pituitary-gonadal axis might be triggered by increased secretion of this decapeptide (GRUMBACH et al. 1974). Experimental support in favor of this hypothesis, however, has been slow to accumulate. In two cases of precocious puberty caused by hamartomas of the tuber cinereum, BIERICH et al. (1967) reported increased concentrations of "Luteinizing Hormone Releasing Factor" (as determined by bioassay) in cerebrospinal fluid. JUDGE et al. (1977) described the presence of immunoreactive LRH in a similar tumor. According to ROOT et al. (1977), normal sexual maturation in children is accompanied by increased urinary excretion of LRH which correlates well with rising concentrations of LH and FSH. However, in view of the methodological problems which still plague the radioimmunological determination of biologically active LRH in body fluids (JEFFCOATE, 1977), this finding can hardly be considered as final. Consequently, the participation of LRH in the initiation of puberty cannot presently be considered established.

Attempts to induce precocious sexual maturation in laboratory or domestic animals by administration of exogenous LRH have met with limited success. In immature female rats, the chronic administration of the decapeptide induces pubertal ovulation only in the period immediately preceding spontaneous puberty (SCHRÖDER et al. 1973) or after pretreatment with sub-ovulatory amounts of Pregnant Mare Serum Gonadotropin (PMSG) (HAFEZ and SUGAWARA 1975; HIRSCH and GIVNER 1975). Sexually immature rats exposed to constant light ovulate in response to LRH, but littermates reared under normal lighting conditions do not (STEGER et al. 1975). One feature which is common to these particular experimental conditions is an advanced state of follicular development. Since immature rats readily release FSH and LH in response to minute amounts of LRH (JOHNSON and MALLAMPATI 1975 a, b), it must be assumed that the gonad, rather than the anterior pituitary gland, is the rate-limiting factor at this age. In immature male rats, the chronic administration of LRH induces neither precocious spermatogenesis nor maturation of Leydig cells (GARCIA-HJARLAS and VILAR 1975). Similar negative results have been obtained in sexually immature bulls (MONGKONPUNYA et al. 1975; HAYNES et al. 1977) and ewes (TYRELL et al. 1975). Finally, VALDEZ-MARTINES and PEDROSA-GARCIA (1977a) have reported that immature female rats treated with LRH-antiserum show only modest delays in the onset of

puberty, inasmuch as passive immunization does not interfere with vaginal opening and subsequent estrous cycles in fully 1/3 of animals so treated. On the assumption that the negative results obtained with native LRH might be due to its short biological half-life (REDDING and SCHALLY, 1973), attempts have been made to induce puberty with LRH analogs which are less prone to rapid enzymatic degradation. After repeated administration of the potent LRH agonist (D-Leu^{6}, des-Gly-NH$_2$ ^{10})-LRH-ethylamide, RIPPEL and JOHNSON (1976) observed a small but significant advancement of the timing of vaginal opening in rats, but pronounced antigonadotropic effects were also seen. In other studies the same investigators (JOHNSON et al. 1976), obtained a clear-cut inhibition of ovarian steroid output resulting in delayed puberty. It is now clear that this paradoxical effect is a property of many potent LRH agonists and is a consequence of massive or prolonged stimulation with native LRH as well. The phenomenon appears to be due primarily to the desensitization ("down-regulation") of gonadal gonadotropin receptor sites (CATT and DUFAU 1976).

In immature female rats, precocious sexual maturation is readily induced by small unilateral electrolytic lesions placed in the mediobasal hypothalamus (RUF et al. 1974). Prominent effects of such lesions are an increase in LH and FSH concentrations in the blood and an activation of ovarian steroidogenesis leading to increased concentrations of circulating estrogen and marked uterine growth (RUF et al. 1974; YOUNGLAI et al. 1976). In the majority of rats subjected to a unilateral brain lesion on day 23 of life, precocious pubertal ovulation is induced within 4 or 5 days; the remaining animals show lesser degrees of sexual development. The efficiency of the procedure is enhanced by simultaneous injection of a small dose of estrogen (RUF et al. 1976). Moreover, the brain lesion is able to replace estrogen in priming an immature rat for subsequent progesterone-induced gonadotropin release (RUF et al. 1975). Taken together, these findings indicate that brain lesions hasten the maturation of ovarian follicles and induce the development of positive feedback mechanisms which trigger an ovulatory gonadotropin surge. Both components are required for precocious pubertal ovulation to occur. The experiments described below were designed to evaluate whether either of these two effects of brain lesions could be mimicked by the application of exogenous LRH. Since the lesion site is particularly rich in LRH containing nerve endings terminating on the primary plexus of the hypophysial portal system (KRULICH et al. 1977; ZIMMERMANN, 1977) such lesions could conceivably induce precocious sexual maturation by acutely or chronically discharging the decapeptide from disrupted axons.

Table I. Reproductive Changes Induced by brain lesions placed on day 23

Age (days)	N	Uterine Weights (mg) (mean ± S.E.)	Vagina	Number of Ova (mean ± S.E.)
23	5	24.0 ± 1.5	closed	
24	5	30.0 ± 0.5[a]	closed	
25	5	105.0 ± 9.1[b]	closed	
26	5	115.6 ± 10.0[b]	closed	
27	5	149.8 ± 13.1[b]	open (estrous smear)	10.0 ± 0.7 (4/5)

a = $p < 0.01$, b = $p < 0.001$ as compared to untreated controls (t-test).

Materials and methods

Female rats of a Sprague-Dawley substrain weighing 50-55 g on day 21 were commercially obtained and housed under conditions of controlled illumination (lights on from 0500 - 1900 h). Unilateral brain lesions were stereotaxically placed on day 23 via insulated stainless steel electrodes as previously described (RUF et al. 1974). Native LRH (Ayerst AY-24,031) and the LRH analog (D-Ala^6, des-Gly-NH_2^{10})-LRH-ethylamide (AY-24,204) were dissolved in 0,9 % saline/0,1 % gelatine (BANIK and GIVNER 1976) and injected s.c. in a volume of 0,2 ml. Alzet minipumps (Alza Corporation, Palo Alto, Calif.) were filled with various concentrations of native LRH (Beckman) dissolved in propylene glycol (BOWERS and FOLKERS 1976); the osmotic pumps were inserted beneath the dorsal skin under light ether anaesthesia. PMSG was administered s.c. in 0,2 ml saline; phenoxybenzamine (2,5 mg/100 g b.w. in saline) and DL-propranolol (2 mg/100 g b.w. (KALRA et al. 1972) were given i.p. Progesterone was administered in 0,2 ml sesame oil s.c. Animals were either observed for vaginal opening, estrous smears and ovulation over a 5 - 7 day period or sacrificed at regular 24 h intervals. Uterine weights were determined after rapid dissection on blotting paper; ovulation rates were assessed by disrupting Fallopian tubes under a dissecting microscope. Plasma LH and FSH were determined by radioimmunoassay using NIAMDD kits; results are expressed in terms of RP-1 standards.

Results

I. Induction of precocious ovulation by brain lesions: A representative experiment involving 25 animals is summarized in Table 1. Rats subjected to a brain lesion on day 23 of life showed a significant increase in uterine weights (indicative of enhanced estrogen production) from day 24 onwards. On day 27, all animals exhibited vaginal opening accompanied by estrous smears (presence of cornified cells). A full adult-size complement of tubal ova was found in 4 out of 5 rats.

Table II. Reproductive Changes Induced by Daily Injections of (D-Ala^6, des-Gly-$NH_2{}^{10}$)-LRH-Ethylamide

Pretreatment	N	Dose/24 h	Duration	Uterine Weights (mg) (day 28)	Ovulation
none	3	1 ng	days 23 - 28	75.3 ± 1.8	none
	3	10 ng	days 23 - 28	79.7 ± 1.3	none
	3	100 ng	days 23 - 28	79.3 ± 5.5	none
Estradiol-benzoate, 125 ng, day 22	3	1 ng	days 23 - 28	86.3 ± 2.2	none
	3	10 ng	days 23 - 28	85.0 ± 1.7	none
	3	100 ng	days 23 - 28	83.0 ± 2.0	none
	3	1 ng	days 23 - 28	85.3 ± 3.2	none
	3	10 ng	days 23 - 28	88.3 ± 3.6	none
	3	100 ng	days 23 - 28	85.7 ± 2.2	none
Untreated Controls	8			81.0 ± 2.5	none

Table III. Reproductive Changes Induced by continuous LRH infusion (days 23 - 27)

Rate of Delivery (ng/24 h)	N	Immature	Reproductive state on day 30 Anovulatory	Ovulatory
30	5	4	1	0
10	5	1	3	1
3	5	2	2	1
1	5	2	0	3
0.3	5	3	2	0

II. Induction of precocious ovulation by native LRH or by a LRH-analog: In preliminary experiments, a variety of treatment schedules (such as 1 or 5 ng LRH injected hourly between 3 p.m. and 8 p.m. on days 23 and 24 or, alternatively, a bolus injection of 250 ng LRH on day 26 after pretreatment with PMSG (8 I.U.) or estrogen benzoate (1-10 ug) on day 23) were tried. The only protocol which reliably induced ovulation on day 27 was the combination of PMSG and LRH-bolous, thus confirming the observation by HAFEZ and SUGAWARA (1975) and HIRSCH and GIVNER (1975) that a certain degree of follicular development is a prerequisite for the LRH-induction of ovulation at this age. In view of the limited efficiency of the decapeptide applied in this way, animals were either injected with the more potent analog (D-Ala^6,-des-Gly-$NH_2{}^{10}$)-LRH-ethylamide or continuously infused with native LRH delivered by implanted Alza minipumps. As shown in Table II, daily injections of various doses of the analog were equally ineffective in stimulating uterine growth or inducing ovulation. Likewise, constant infusion of LRH for 96 h (Table III) had a very limited stimulatory effect. Although infusion rates of 1-10 ng/24 h did cause precocious pubertal ovulation in a few animals, others remained sexually immature, and the success rate of this treatment fell far short of that obtained with unilateral brain lesions (Table 1).

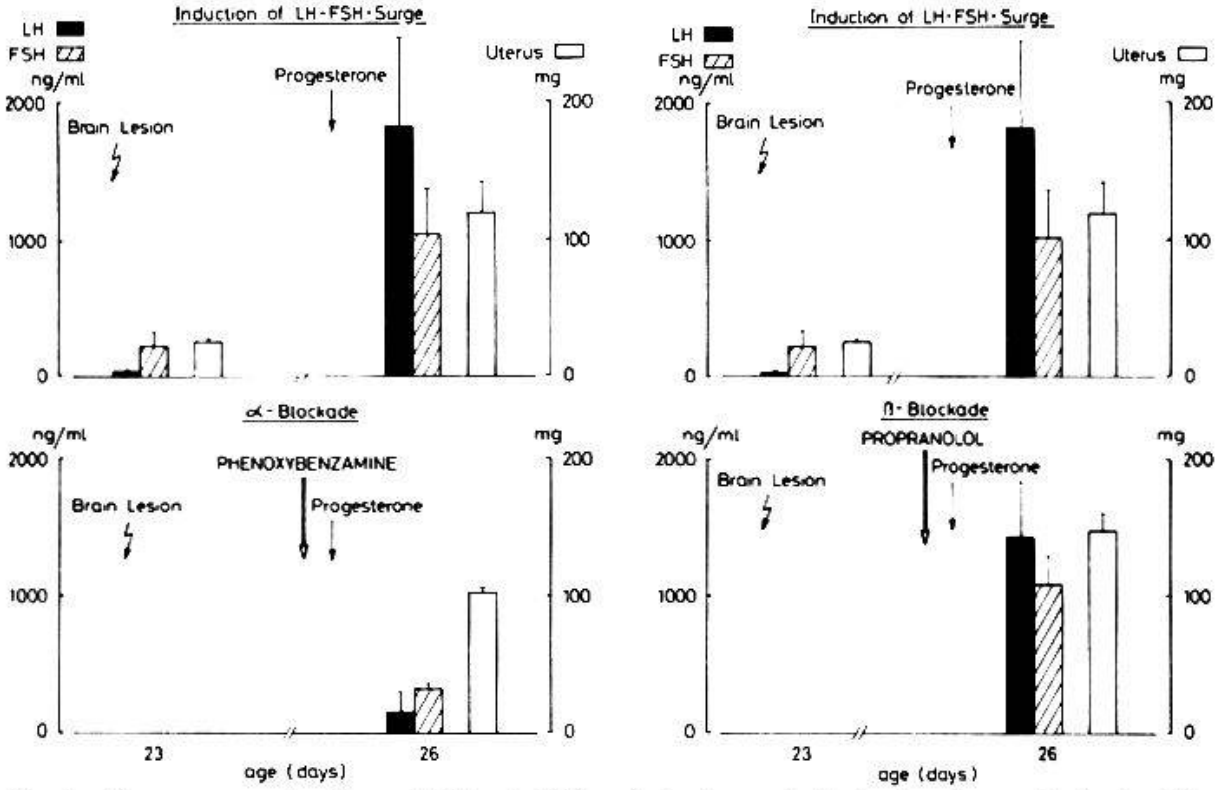

Fig. 1. Plasma concentrations of LH and FSH and uterine weights in immature rats "primed" with a brain lesion and challenged with progesterone. Note abolition of the gonadotropin surge by phenoxybenzamine but not by propranolol.

III. Induction of the stimulatory feedback effect of progesterone by unilateral brain lesions:

Sexually immature rats treated with a brain lesion on day 23 of life and challenged with progesterone on day 26 exhibited large LH and FSH surges 5 h later (Fig. 1). At the time of sacrifice, uteri were hypertrophic, reflecting again increased estrogen production caused by the brain lesion. In order to ascertain whether the mechanism underlying the steroid-induced gonadotropin surge was comparable to the one operative in the estrogen-treated adult castrate (KALRA et al. 1972), adrenergic blocking agents were administered 1 h prior to the steroid injection. The surge of both gonadotropins was abolished by phenoxybenzamine, but not by propranolol. It appears likely that the premature activation of estrogen synthesis caused by the brain lesion activates alpha-adrenergic synapses with pharmacological properties similar to those required for cyclic gonadal function in adult life.

IV. Induction of the stimulatory effect of progesterone by native LRH and by a LRH-analog:

Sexually immature rats injected daily with 1-100 ng of LRH-analog (cf. Table II, N = 5) or infused with 1 ng/24 h of the native decapeptide (cf. Table III, N = 5) were challenged with progesterone on day 26. At the time of sacrifice, 5 h later, uterine weights were similar to those of non-treated controls, and LH and FSH concentrations in plasma were not significantly different from base-line values.

Discussion

The results indicate that neither the stimulatory effect of brain lesions on uterine growth nor the lesion-induced maturation of the stimulatory feedback system underlying the preovulatory gonadotropin surge are easily duplicated by the administration of exogenous LRH. While the prolonged infusion of native LRH caused precocious pubertal ovulation in a few cases, repeated daily injections of the decapeptide or of one of its long-acting and potent analogs (BANIK and GIVNER 1975) proved entirely ineffective. Our report, therefore, joins a growing list of papers emphasizing the inability of LRH to advance the physiological process of puberty in several species (for references see Introduction) and challenges the notion that increased secretion of LRH is the primum movens for sexual maturation (GRUMBACH et al. 1974).

For rats of the age used in this study, it has been shown that FSH induces the formation of LH receptors in the ovarian follicle (ZELEZNIK et al. 1974) and that this process is enhanced in the presence of estrogen (RICHARDS et al. 1976). Under the influence of FSH, granulosa cells are able to form estrogen from androgen precursors (DORRINGTON et al. 1975), but the synthesis of androgens by the theca cells is critically dependent on LH which mediates the conversion of cholesterol to pregnenolone (HALL and YOUNG 1968). It is to be expected, therefore, that the LH pulses released by the doses and types of LRH used in this study (JOHNSON et al. 1976; VALDEZ-MARTINES et al. 1976b) are not perceived by the ovary and that increments in FSH may not be sufficient to induce synthesis of LH binding sites in the absence of aromatizable substrate. Prolonged stimulation with PMSG overcomes this block and renders the immature ovary responsive to a LRH-induced gonadotropin surge (HAFEZ and SUGAWARA, 1975; HIRSCH and GIVNER 1975; STEGER et al. 1975).

If, as suggested above, the immature ovary is unable to respond to LH, how can brain lesions precipitate premature ovulation? One possibility which is presently being explored is that such lesions lead to an activation of adrenal androgen production and that these androgen precursors can serve as substrate for aromatization by the ovary. In separate experiments (KRAULIS et al. 1977), the aromatizable androgens dehydroepiandrosterone, androstenedione and testosterone have proven to be excellent replacements for estrogen in terms of the obligatory estrogen priming required in immature female rats for the triggering of a gonadotropin surge by progesterone (CALIGARIS et al. 1972). Increased concentrations of dehydroepiandrosterone and its sulfate have recently been described in women suffering from hyperprolactinaemia (BASSI et al. 1977; VERMEULEN et al. 1977), and increments in circulating prolactin concentrations in immature female rats subjected to hypothalamic lesions have

been documented (WUTTKE and GELATO 1976). Whether such prolactinergic stimulation of adrenal androgen production can account for the process of "adrenarche" or whether a specific "cortico-adrenal-stimulating hormone" (CASH, PARKER and ODELL, 1977) of pituitary origin needs to be invoked is presently unclear.

It has recently been reported that immature female rats undergoing precocious sexual maturation in response to PMSG exhibit a two-fold increase in axodendritic synapses in the arcuate nucleus within 72 h. This change is most likely mediated by estrogen since it is abolished by ovariectomy (MATSUMOTO and ARAI, 1977). Synaptogenic effects of estrogen have been described by several investigators (for review see NAFTOLIN and BRAWER, 1977). The possibility that estrogen produced in response to the brain lesions is responsible for the apparent maturation of alpha-adrenergic synapses (Fig. 1) merits further study.

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