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HYPOTHALAMIC RELEASING HORMONES AND FERTILITY REGULATION WITH EMPHASIS ON OVULATION INDUCTION BEFORE PUBERTY*

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SUMMARY

The nature, mode of action, and neuroendocrine mechanisms of hypothalamic releasing hormones have been reviewed, LH releasing hormones can be used for (a) basic research in neuroendocrinology, (b) diagnostic tool to differentiate hypothalamic and pituitary disorders in clinical gynecology; and (c) to induce ovulation in mature and immature females.

Prepubertal female rats of 28, 32 and 39 days of age were treated with a single dose 0–300 μg of LRF, and examined for oviductal ova 20 h later. Ovulation was not induced in all the females within 24 h. Doses less than 300 μg had no effect on ovarian and uterine weight. In 28 and 32 day old rats treated with 300 μg of LRF, an increase in ovarian and uterine weight was equivalent to the increase resulting from 1 mg of LH. To determine the effective dose of LRF for ovulation, 23 day old rats were primed with 5 I.U. of PMD, ovulation occurred 2 h following LRF treatment, as in rats injected with 1 mg of LH. In another experiment, rats 18–23-days of age were injected with 0–80 μg of LRF about 56 h following 5 i.u. of PMS injection. The response was age-dependent: 5μg of LRF caused ovulation in 21-day-old rats, whereas 10 μg evoked this response in 18-day-old rats.

A possible relationship between folliculogenesis and LRF-induced ovulation was examined. Twenty-one and 25-day-old rats were primed with 5 I.U. of PMS and a single injection of LRF (40 μg) at 24, 30, 36 and 42 h after PMS treatment. The females were examined for ovulation 20 h later. Ovulation occurred only in a few 25-day olds when LRF treatment was given after 24 h of priming, whereas all ovulated when priming was done for 36 h after PMS treatment; however, ovulation was induced by 1 mg of LH. Only 20 and 50% of the rats showed ovulation when primed 30 and 36 h respectively. Estradiol has a stimulating effect on the release of LH in adult rats. Twenty-five-day-old rats were primed first with 10 μg of estradiol, and a single dose of LRF (0–100 μg) about 48 h, then autopsied 24 h later. Vaginal opening was observed in all of the rats but ovulation did not occur in all. The possible interaction between various doses of estrogen and the time of LRF injection after estrogen priming will be examined. Sexual maturity in the female involves an interaction between the synthesis and the release of hypothalamic releasing hormones and functional integrity of the hypothalamo-hypophysealovarian axis.

I NATURE AND MODE OF ACTION OF RELEASING HORMONES

Hypothalamic neurons release neurohormones into the hypothalamic-hypophyseal portal system which links the brain with the pars distalis. These neurohormones, chemically identified, are generally referred to as releasing (or inhibiting) hormones or as hypothalamic hypophysiotropic hormones. However, little is known about the anatomical relationships of the neurons responsible which secrete these releasing hormones.

Neurosecretory cells are distributed throughout the hypothalamus, with a highest frequency in the supraoptic and paraventricular nuclei and in the loci anterior to the nucleus intercalatus. Various regions of the median eminence as well as the hypophysiotrophic region, adjacent to the nucleus intercalatus are rich in hypothalamic releasing hormones, particularly LRH. This was confirmed by physiological, biochemical, immunochemical and immunofluorescent techniques [1–6]. Studies on functional anatomy of the hypothalamus indicate that the action of the hypothalamic releasing hormones are mediated through the adenohypophysis, and possibly cyclic AMP. Different cell types of anterior pituitary glands posses two types of membrane receptors which selectively bind specific hypothalamic neurosecretion [7–10]. This binding phenomenon seems to regulate the biosynthesis and secretion of the pituitary hormones. Membrane adenyl cyclase is stimulated thereby raising intracellular cyclic AMP levels which, in turn, activate protein kinases. Protein kinases appear to be associated in the control of synthesis and release of pituitary hormones [11–13].

The catecholamines, dopamine and noradrenaline, present in distinct neuronal pathways in the brain substance is thought to act as a neuro-transmitter. The abundant presence of catecholamines in the hypothalamus affect hypothalamic mechanisms which

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control the pars distalis of the hypophysis. It is possible that there are axo-axonic synapses between the adrenergic and peptidergic axons. It is also possible that the ependymal cells of the median eminence may be under adrenergic neural control. The release of hypothalamic hormones is controlled by several exteroceptive stimuli, and is affected by the administration of steroid hormones and biogenic amines.

Three hypothalamic hypophysiotropic hormones have been isolated, characterized structurally and synthesized: Gonadotropin Releasing Hormone (LRH; GnRH; or LH/FSH-RH); *Thyrotropin Releasing Hormone* (TRH or TRF); and *Somatostatin* (GR-IH; or SR-IF). GnRH activates the secretion of hypophyseal LH, and to a lesser degree the secretion of hypophyseal FSH in immature and mature females and males. However Jonsson and his associates could not detect a midcycle peak of GnRH to coincide with LH peak of the menstrual cycle. Bjorklund *et al.*[14] studied the physiological mechanisms controlling the release of gonadotropins of pituitaries in organ cultures. Human fetal pituitaries exposed to extracts of ovine hypothalamus or to GnRH released more LH than FSH.

II RELEASING HORMONES AND FERTILITY REGULATION

GnRH may be used for fertility regulation in animals and man. GnRH can be used to promote timed ovulation in women with irregular menstrual cycles who require insemination therapy. LHR is administered as a single or multiple subcutaneous dose which may be preceded by small doses of human menopausal gonadotropins. In patients with hypothalamo-hypophyseal-gonadal anomalies, the response of anterior pituitary to exogenous GnRH seems to be related to the secretion pattern and the previous exposure to endogenous LRH [15–17].

A single dose of synthetic GnRH was used to induce ovulation in prepubertal rodents, with and without PMS and estradiol. It is concluded that sexual maturity in the female involves an interaction between the synthesis and the release of hypothalamic releasing hormones, and functional integrity of the hypothalamo-hypophyseal-ovarian axis.

III RELEASING HORMONES AS DIAGNOSTIC TOOLS

The administration of LHR is used as a diagnostic pituitary function test to recognize the hypothalamic-pituitary disorders; *e.g.* oligomenorrhea, delayed puberty, pre-menarchal Stein Leventhal syndrome, persistent hypothalamine immaturity, gonadal agenesis, insensitive ovary syndrome, and hypogonadotropism of both hypothalamic or hypophyseal origin. Following the intravenous injection of one dose of GnRH serum, LH and FSH is measured at intervals using radioimmunoassay. The different patterns of LH and FSH response to LRH, as compared to the response in normal control subject, is a useful tool for hypothalamic-pituitary deficiencies.

TRH stimulates the secretion of hypophyseal TSH and prolactin in immature and adult patients. The administration of TRH may in some cases induce a prolactin secretory response which blocks neither the LH surge nor ovarian responsiveness to endogenous gonadotropins in normally menstuating women. Somatostatin inhibits the secretion of hypophyseal growth hormone in normal men and in patients with acromegaly. Somatostatin can be used clinically to control giantism, acromegaly and juvenile diabetes mellitus.

LRH rabbit antiserum was produced for use in physiological studies, and to develop two different types of radioimmunoassay for GnRH. Dermody developed more appropriate techniques RIA, dependent on physical and chemical characteristics of the peptide antigen, such as cross-reactivity of peptide analogs, thin layer chromatography, disc gel electrophoresis at different pH's and gel densities, and column chromatography.

The hypothalamic releasing hormone GnRH is a polypeptide (pGlu-His-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) and several of its synthetic derivatives have a wide spectrum of biological activity. Several GnRH agonists have been designed based on subtle changes at the C-terminus. Rippel and his associates have replaced the Gly-NH_2 structure by substituted amides on the proline residue in position 9, resulting in several analogs more active than LRH both *in vitro* and in ovulation induction. The most active member of these derivatives (des-Gly-$NH_2{}^{10}$ Pro-ethylamide[9]) are much more active than GnRH.

IV RELEASING HORMONES AND PREPUBERTAL INDUCTION OF OVULATION

Ovulation was not induced in 28-, 31- and 32-day-old rats, when injected with dosages varying from 10–300 μg of LRH. Ovulation was induced when 35-day-old rats were injected with 300 μg of LRH. Dosages less than 300 μg of LRH had no effect on ovarian and uterine weight. The response to 300 μg of LRH was equivalent to 1 mg of LH.

In rats, 23 days of age, primed with 5 I.U. of PMSG, ovulation was induced in 1/5th of the animals without the administration of LRH. With the administration of 10 μg of LRH all females ovulated. In rats primed with PMSG, ovulation was induced within 12 h after LRH administration; a period similar to that of rats injected with 1 mg of LH. The number of ovulation points increased with the increase of the dose of LRH beyond 10 μg.

Ovulatory response to LRH is related to the age of the female. The administration of 2·5 μg of LRH induced ovulation in 19-day-old rats whereas the administration of 10 μg evoked this response in 18-day-old rats. In 18-day-old rats, 40 μg of LRH caused ovulation in 71% of treated females. Ovulation

occurred in all 21-day-old rats injected with 10 μg or more of LRH, although in 71% of the rats 2·5 μg of LRH caused ovulation.

Respective of the age of the females, the number of ova shed in LRH-treated females was less than those treated with 1 mg of LH. Ovulation rate in 21-day-old rats was twice as high than that of other groups. The administration of 1 mg of LH seemed to induce superovulation. The weight of the ovaries and uteri did not seem to be affected by the dose of LRH.

The injection of immature rats with one dose of LRH is not effective in induction of ovulation near puberty, whereas higher doses evoked increased ovarian and uterine weight. The effective doses of LRH to induce ovulation in immature rats primed with PMS is higher than that for adult rats and hamsters whose LH-surge was blocked by Nembutal (Arimura *et al.*, 1967, 1972; Humphrey *et al.*, 1973). The effective doses of LRH on ovulation are age-dependent. The induction of ovulation in immature rats by HCG alone was also dependent upon the age of the animals [21]. When LRH was given at various times after priming PMSG primed rats, ovulatory effectiveness of LRH was closely related to the degree of follicular maturation.

Arimura *et al.*[22] demonstrated that pre-treatment with estrogen augumented the response to LRH in the rat. In addition, estradiol has a stimulating effect on the release of LH evoking ovulation in adult pseudopregnant rats (Takeuchi *et al.*[22]).

In immature rats near puberty, various doses of estradiol did not induce ovulation. Ovulation did not occur in a rat primed with estradiol, when LRH was injected 32 and 48 h after priming. These findings may indicate that hypothalamus and pituitary responsiveness to steroid and exogeneous LRF in immature rats could be related to differences in the degree of maturation of the hypothalamo-pituitary-ovarian axis.

The effect of natural and synthetic luteinizing Releasing Hormone (LH, RH, LRF or GnRH) on ovarian response has been extensively studied in experimental animals and man [24, 25]. The responsiveness of the pituitary gland to a releasing hormone in immature animals and in prepubertal children [26, 27] with the administration of LRH causes a sudden increase in serum LH levels.

Multiple administration of synthetic LRH did not induce ovulation in 26- to 42-day-old rats [28]. The treatments of LRF released both the hormones (LH and FSH) implicated in the ovulatory process.

This investigation is designed to examine the efficacy of synthetic LRH in ovulation induction in immature rats.

A single dose of 50 μg of LRH caused superovulation in estrous rabbits [29]. In immature rats primed with 5 I.U. of PMSG, and a higher dose of LRH, the number of the shed ova increased twofold. Further experiments are needed to determine whether a higher dose of LRH causes superovulation.

Sexual maturity in the female involves:

(a) an interaction between the synthesis, storage and release of a hypothalamic releasing hormone, and

(b) the functional integrity of the hypothalamo-hypophyseal-ovarian axis.

V FUTURE RESEARCH

Future research is needed (a) to elucidate the anatomical relationships between the adrenergic neurones and those secreting the releasing factors, (b) to localize the releasing hormones in the median eminence, (c) to characterize the specific type of neurons responsible for the biosynthesis and release of GnRH, and (d) to find out whether each RH is elaborated by a particular type of neuron or whether a single neuron can release several releasing hormones. Hypothalamic releasing hormones, with a short half-life in the systemic circulation, are active by oral, buccal, and intranasal routes. However, the biological potency by these routes is only 1/50 to 1/1000 that of parental routes. Future research is needed to develop more convenient delivery systems to extend the duration or retard the absorption of these hormones, *e.g.* use of gel formulations, or zinc complexes [30]. Analogs for hypothalamic releasing hormones with agonist or antagonist activity should be developed for clinical application in fertility regulation in animals and man.

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