Behavioral Effects of LH-RH

MICHAEL D. MAUK, GAYLE A. OLSON, ABBA J. KASTIN* AND RICHARD D. OLSON

Department of Psychology, University of New Orleans, New Orleans, LA 70122

and

*Veterans Administration Medical Center and Tulane University School of Medicine, New Orleans, LA 70146

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MAUK, M. D., G. A. OLSON, A. J. KASTIN AND R. D. OLSON. Behavioral effects of LH-RH. NEUROSCI. BIOBEHAV. REV. 4(1) 1–8, 1980.—Brief summaries of the structure, localization, and physiological effects of luteinizing hormone-releasing hormone (LH-RH) are followed by a review of behavioral and clinical research with this hypothalamic peptide. Facilitation of mating behavior in rats after administration of LH-RH provided the basis for clinical studies in human sexual function. The clinical results of LH-RH in behavior are inconclusive thus far and further studies of clinical applications are awaited.

Behavior LH-RH Peptides Separation

SINCE the early 1960’s a great deal of research has been devoted to the elucidation of the structure, localization, physiological, and endocrine effects of luteinizing hormone-releasing hormone (LH-RH). Only recently have the behavioral effects of this decapeptide been systematically investigated. Some reasons for the delay in behavioral investigation were the questions concerning the short half-life of the peptide and the problems concerning the use of peripheral administration [77]. Recently however, the effectiveness of peripheral administration of hypothalamic peptides has been well established [46, 54, 55, 57, 59, 67, 112]. These CNS-dependent behavioral activities occur independently of the the pituitary and primarily involve effects on sexual behavior [104]. Though the following review includes abbreviated summaries on localization, structure, and physiological effects of LH-RH, as well as its analogs, major emphasis will be placed on the behavioral effects of LH-RH.

LOCALIZATION

The first direct evidence that the hypothalamus contains a substance which releases luteinizing hormone (LH) was reported by McCann in 1960 [85]. Using a bioassay method, McCann examined hypothalamic extracts and found the greatest concentration of LH-RH to be in the median eminence with lesser amounts in the basal hypothalamus. Subsequent microdissection followed by radioimmunoassay produced similar results and demonstrated small amounts in the arcuate and ventromedial nuclei [113]. Workers generally agree the median eminence of the rat, mouse, hamster, and guinea pig are rich in LH-RH [6,114]. Several have reported as well that LH-RH is present as far forward as the preoptic area and extends in fine processes towards the arcuate nucleus-eminence complex [1, 10, 105, 137, 147].

LH-RH has been found in extrahypothalamic areas as well, including the mesencephalon of the dog and rabbit [36] and rat cerebral cortex [116]. The presence of LH-RH has also been detected in the organum vasculosum of the lamina terminalis [147,150] as well as the para-olfactory complex [1]. The presence of LH-RH in these anterior locations is particularly significant in the light of behavioral findings that LH-RH affects sexual activity in both male and female rats maintained at constant gonadal status [33, 94, 101, 115]. White et al. [148] reported that ovine, bovine, and porcine pituitary glands contain levels of LH-RH 20 times higher than in respective hypothalami. No LH-RH however, was found in the pineal of the rat, or monkey [2]. Morris and Knigge [88] reported that male rats showed a marked rise of LH-RH in the cerebrospinal fluid (CSF) as well as in the blood after ether stress, although Cramer and Barraclough [25] were unable to find LH-RH in the third ventricle CSF of the rat under a variety of experimental conditions.

The presence of LH-RH has also been demonstrated in the peripheral blood of rats [107] and monkeys [31]. Seyler and Reichlin [138–140] have reported LH-RH activity in the peripheral plasma in what appears to be short-lived bursts. Studies investigating the presence of LH-RH in the blood show considerable discrepancies. Several investigators have found LH-RH concentrations in the blood of women at midcycle [5,80] while others failed to detect any mid-cycle surge [75, 109, 110] and one group detected no LH-RH in the blood at all [52].

STRUCTURE

LH-RH is a simple, linear decapetide with the amino acid sequence Ipr-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂ [17,82]. Hundreds of analogs have been synthesized providing useful structure/function information [23] as well as providing substances with greater activity and prolonged effect. Analogs with modifications of each particular amino acid residue in LH-RH have been synthesized and

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*Please address reprint requests to Michael D. Mauk, Department of Psychology, University of New Orleans, New Orleans, LA 70122.
tested for agonist and antagonist activity [22,86]. Early analogs showed increased potency, but little prolongation of gonadotropin release was found [60,106]. Analogos synthesized a little later (D-Ala-6-des-Gly-10-LH-RH-9-ethylamide and D-Leu-6-des-Gly-10-LH-RH-9-ethylamide) induced a more prolonged release of gonadotropins [43, 53, 142]. In fact, the latter analog proved effective by oral administration in humans at high doses (10 mg) [43,44]. Modifications at the sixth, seventh, and tenth positions have produced particularly potent analogs [19-22, 23, 87]. These more potent analogs have great potential in behavioral research due to their increased activity as well as their more prolonged effects, but it is also likely that there might be dissociation between the structural requirements for behavioral effects and those for endocrine effects. A review of LH-RH analogs has been presented elsewhere [133].

PHYSIOLOGICAL EFFECTS

Physiologically LH-RH has been shown to release both FSH and LH, induce ovulation, and elevate sperm counts, as well as elevate production of gonadal steroids [14, 47, 121, 130]. LH-RH plays a key physiological role in maintaining secretion of LH and FSH as demonstrated by lowered levels of both gonadotropins in either normal or castrated rats treated with anti-LH-RH serum [3]. Similar effects have been reported with LH-RH inhibitory analogs [28,29]. Furthermore, the active immunization of rabbits and rats with LH-RH results in infertility and gonadal atrophy in both males and females [7,143]. Most workers have reported no other releasing agent for FSH besides LH-RH [132,134] while a few others have reported evidence of a distinct FSH releasing agent [16,141]. The existence of substances which differentially affect the release of LH and FSH peripherally [41, 45, 74, 78] have led some investigators to believe LH-RH may be the only hypothalamic secreting agent of both gonadotropins [145] and differentiation may take place extrahypothalamically, probably at the pituitary.

Information from endocrinological and physiological research has direct implications for the behavioral effects as well [55]. For example, although LH-RH has a half life in human blood of only four minutes, maximum gonadotropin release does not occur until 20 minutes after injection [62,68]. In fact, levels of LH-RH become undetectable before endocrine effects are observed [4, 55, 121]. LH-RH effects have been shown to persist two to six hours post injection in lordosis behavior [101,115], dopa potentiation [117] and electrophysiological changes [76].

BEHAVIORAL RESEARCH

Moss and his associates have outlined three observations [104] that led them to investigate the effects of LH-RH on sexual activity. The first observation was the temporal relationship between the release of LH induced by LH-RH and the onset of sexual receptivity. A preovulatory surge of gonadotropins, apparently triggered by LH-RH release, occurs in the normal female rat the afternoon of proestrus [37,84] and is followed two to three hours later by behavioral heat [15, 108, 135]. Secondly, there is an apparent anatomical overlap of the neural tissue responsible for producing LH-RH controlling gonadotropin secretion, and regulating mating behavior [1, 10, 105, 137, 147]. Thirdly, LH-RH responsive neurons are present in the areas responsible for reproductive activities [33, 71, 72, 81, 98-100, 119, 123, 124].

Motivated by these observations both Moss and McCann [101] and Pfaff [115], working independently, demonstrated the effects of LH-RH on sexual behavior in rats.

Moss and co-workers [95, 101, 102] found that 500 ng subcutaneous injections facilitated mating behavior, as measured by the lordosis response, in estrone-primed ovarioctomized female rats. Effects are independent of the gonadotropins and the pituitary-adrenal axis since LH-RH administered to ovarioctomized, adrenalectomized [90, 95, 102, 103] as well as ovarioctomized, hypophysectomized rats [115] is effective. Supportive are experiments demonstrating that facilitation of lordosis responsiveness by vaginal stimulation is independent of anterior and posterior pituitary gland hormones [125].

LH-RH effects on sexual behavior are not limited to female rats. Though not of the magnitude as was first demonstrated in female rats, LH-RH administration can apparently facilitate sexual behavior in male rats. Moss [95] treated sexually-experienced male rats with subcutaneous injections of synthetic LH-RH or saline two hours before a 15 minute testing period. LH-RH significantly reduced mean latency to first intromission and first ejaculation but had no significant effect on number of mounts or intromissions. Subsequent testing of sexually experienced castrated males primed with testosterone propionate in doses too low (11 μg) to initiate consistent copulatory behavior showed significant decreases only in latency to first ejaculation [104].

Phylogenetic generality of sexual facilitation by LH-RH has not been well demonstrated despite the presence of immunoreactive LH-RH in a wide variety of species including chickens [50], amphibia [50], and sheep [81] hypothalamus as well as LH-RH activity in cockerel [42], amphibia [144] and fish [144]. Facilitation of sexual behavior by LH-RH in mice [79] has been demonstrated, but only slight facilitation of lordosis was initiated in guinea pigs, and none at all in hamsters [18]. Moss et al. [104] have reported a personal communication from Doering, McGinnis, Kraemer, and Hamberg stating a long acting analog of LH-RH had no effect of the initiation of sexual behavior in adult male chimpanzees.

It is important to note that LH-RH alone is not sufficient to facilitate sexual behavior in rats. Ovarioctomized female and castrated male rats must be primed with estrone and testosterone respectively to affect sexual behavior.

Further evidence that LH-RH facilitates sexual behavior was obtained with administration of substances known to release LH-RH. The prostaglandins (PG's) have been shown to facilitate LH-RH release, particularly PGE2 [111]. PGE2 facilitated lordosis behavior in ovarioctomized and adrenalectomized rats with [32, 48, 126] and without [127] estrogen priming. It has been suggested that PGE2 acts indirectly via the release of LH-RH [32] in the light of evidence suggested that PG2 modulates firing of preoptic-hypothalamic LH-RH neurons [118].

Findings presented thus far suggest LH-RH might facilitate sexual behavior by acting directly on neural tissue as would be expected from the extra-pituitary effects initially described for hypothalamic peptides [62,63]. Furthermore, Moss and Moss tested this possibility by studying the effects on lordosis of infusions of LH-RH into the medial preoptic area, arcuate nucleus, lateral hypothalamic area, and the cerebral cortex [38, 39, 97, 100]. Small quantities (50 ng) of LH-RH infused into the medial preoptic and arcuate nuclei facilitated lordotic behavior in estrone-primed, ovarioctomized female rats. No change in lordosis was found after microinfusions
into the cerebral cortex or lateral hypothalamus. Similar infusion of TRH into the medial preoptic and arcuate nuclei decreased sexual behavior. In subsequent experiments in rats primed with enough estrone to elicit high mating activity, TRH but not LH-RH suppressed mating [40]. Also, addition of FSH or LH to LH-RH infusions reduced the effects on sexual behavior [40].

These findings of LH-RH facilitation of TRH suppression of sexual activity correlate with neurophysiological findings of respective excitation and depression of neuronal activity in the septum, medial preoptic area, and the arcuate-ventromedial complex [33, 91–93, 96, 100, 122–124]. Furthermore, LH infusions into the medial preoptic area and arcuate-ventromedial regions inhibit lordotic behavior in female rats [40]. Taken together, these findings suggest that these peptides are acting directly on neural tissue, with LH acting by a feedback mechanism on the hypothalamus in the coordination of both male and female reproductive responses [104].

As previously stated, LH-RH affects ovarian and testicular function through the release of LH and FSH [47,130]. Paradoxically, LH-RH or its super acting analogs can inhibit follicular development and testicular function [8,9]. Recent evidence suggests LH-RH and its analogs accomplish this inhibitory action by a direct effect on the gonads [49,149]. This contrasts with the facilitatory action on sexual behavior in which LH-RH acts directly on neural tissue.

CLINICAL RESEARCH

Considering the numerous findings of the effects of LH-RH on sexual behavior in animals, subsequent study of the applications of LH-RH in human sexual function and pathology was only natural. The possibility of treatments for infertility, impotence, sex hormone related disorders, as well as possibilities of new birth control methods has provided motivation for a great deal of clinical research. Data collected on endocrine mechanisms, effects of direct administration, as well as the development of analogs with greater potency and duration of action all add to the knowledge of the clinical applications of LH-RH.

Critical to all clinical and behavioral research was the demonstration that LH-RH has endocrine effects in humans. Kastin et al. [62] in 1969 found that LH-RH extracted from porcine hypothalami stimulates not only the release of LH but FSH as well. At the time whether this dual effect was due to impurities in the porcine extract or was simply an intrinsic quality of the peptide was not known. When in 1971 the structure of LH-RH was determined and pure LH-RH synthesized [82,134], confirmation that LH-RH releases both gonadotropins in man was obtained [61]. In all tests, LH-RH induced release of LH is greater than that of FSH in normals and affects both gonadotropins in a dose related manner [64].

During the early studies, Kastin and his co-workers also tested LH-RH induced release of LH and FSH in humans of differing sexual status [63]. LH-RH was found to release LH and FSH in menopausal women which was somewhat surprising in that at menopause the ovaries stop secreting the steroid hormones which are negative feedback factors for LH and FSH, so that levels of both gonadotropins were thought to be at a maximal level [58]. Two genetic disorders in which gonadotropin levels in the blood are unusually high are Klinfelter's and Turner's syndromes. In both conditions, highly purified porcine LH-RH induced further release of LH and FSH [56]. Men in whom high gonadotropin levels had been induced by clomiphene also showed further LH-RH induced release of LH and FSH [65].

Some clinically oriented research has provided useful information on physiological process as well. For example, excess exogenous sex steroid given as oral contraceptive drugs result in depressed gonadotropin levels. Whether the decreased LH and FSH levels are caused by the sex steroids suppressing the release of LH-RH at the hypothalamus or by directly acting upon the pituitary to suppress gonadotropin was not established and it was shown that women pretreated with oral contraceptives were able to release LH and FSH in response to LH-RH [62] suggesting that the gonadotropic suppressing action of the steroids occurred mainly at the hypothalamus or higher CNS centers [56], even though an action at the pituitary is important for modulating the release [45].

Katz [70] reported patients suffering from anorexia nervosa whose LH levels were prepubertal responded with normal LH release from a single injection of LH-RH. The data suggested that normal pituitary function may occur in patients with anorexia nervosa and that these patients may have disturbed hypothalamic function.

LH-RH research has also provided information on the mechanisms involved in the onset of puberty. At this time changes occur which result in an increased release of sex steroids. It is unlikely that the gonads change their sensitivity to the sex steroids at puberty since both the ovaries and testes of children have been shown to be responsive to gonadotropins. If the pituitary changes at puberty the LH-RH responsiveness in children would be unexpected. What Kastin et al. [66] found was children's pituitaries do in fact release LH and FSH upon administration of porcine LH-RH. Many researchers subsequently found different responses for FSH and LH before and during puberty [11, 51, 128, 129]. Others reported FSH responses to be similar in prepubertal and adult males [73], yet the responsiveness of prepubertal pituitaries to LH-RH remains certain. Responsiveness of children's pituitaries to LH-RH suggests the onset of puberty is triggered at the hypothalamus or higher CNS centers. Also the differential responses of prepubertal and pubertal children may provide information concerning the mechanisms of puberty, but no satisfactory explanation has yet been presented.

One of the most important potential therapeutic uses of LH-RH is the induction of ovulation. In 1971 LH-RH was infused IV into a woman with secondary amenorrhea for one 24 hour period. Subsequently she became pregnant and delivered a healthy baby [69]. Theoretically multiple births often associated with gonadotropin administration should not occur after LH-RH because of the negative feedback of LH-RH at the pituitary, and none have occurred yet. LH-RH could also be used in conjunction with clomiphene to induce ovulation in patients who are clomiphene non-responders.

Considering the profound effects of LH-RH on sexual behavior in animals, coupled with the knowledge that LH-RH is active in man, one would expect great promise for LH-RH facilitation of libido in man. After some promising early reports, evidence of LH-RH facilitation of libido in humans has been mediocre at best. In 1974 Mortimer et al. [89] reported that 500 µg injections of LH-RH every 8 hours for several weeks increased sexual potency in six men with hormonal deficiencies. Similar results were reported with one month continuous treatment of LH-RH [136]. Benkert et al. [13] reported increased sexual activity in previously im-
potent men four to six weeks after completion of treatment with 1.0 mg per day LH-RH administered via nasal spray. Moss et al. has also published reports of increased sexual activity in men capable of masturbation after administration of LH-RH [104].

Despite these promising reports, more recent investigation has been much less optimistic. Ehrenising and Kastin [34] found no effect with IV injection of LH-RH in normal adult men in a double-blind study. Davies et al. [26] found even though the improvement of libido after LH-RH administration in men suffering secondary impotence was statistically significant, treatment effects were small and of little apparent clinical significance.

In a very recent double-blind study, Ehrenising, Kastin, and Schally [35] tested the effects of large doses of LH-RH on nine married men with no apparent physiological or psychiatric disorders, who were suffering from decreased ability to sustain and maintain penile erections. A one week, single-blind baseline period was recorded in which all subjects injected themselves with a placebo. Two 4-week periods followed in which subjects injected themselves with 3 mg of LH-RH or placebo in a double-blind crossover design.

The results indicate that at the dose and regimen used, LH-RH had virtually no clinically significant effects in men with decreased sexual ability, particularly considering that no beneficial effects were measured during a two-month follow-up period.

It appears then that though LH-RH has relatively profound effects on sex behavior in animals, therapeutic usefulness for human sexual dysfunction is not yet feasible. Most animal studies dealing with LH-RH facilitation of sexual behavior in animals have been documented in females. In human studies however, most tests have been with male subjects. This may be a partial explanation for the differential findings [55].

LH-RH effects on mood in humans has been recently documented by McAdoo et al. [83]. Though no immediate effects on mood and behavior were noted, LH-RH was found to increase alertness, decrease anxiety and fatigue, as well as increase levels of testosterone induced by LH-RH. Benkert et al. [12] found no improvement after LH-RH and TRH administration to depressive patients.

Though therapeutic usefulness of LH-RH in psychogenic sexual dysfunction and other pathologies appears questionable at best, other problems of perhaps even greater social importance may eventually be aided by LH-RH research. An excellent example is the possibility of LH-RH inhibitory analogs used as contraceptives. Several such analogs have been synthesized which not only inhibit gonadotropin release but inhibit ovulation as well [27,146]. LH-RH and its agonistic analogs also have a paradoxical inhibitory effect on ovulation. With increasing world population a growing problem, one or both of these approaches have the potential to be the most significant result of LH-RH research.

Both the well documented role of LH-RH in reproductive behavior as well as the positive behavioral results obtained from studies of this peptide with animals in experimental paradigms suggest a strong clinical potential. Although clinical research thus far has been inconclusive, further research of the clinical potentials of LH-RH and its analogs is eagerly awaited.

REFERENCES


