PHYSIOLOGICAL, VETERINARY AND CLINICAL EFFECTS OF HYPOTHALAMIC RELEASING HORMONES (RH), ESPECIALLY LH-RH

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ABSTRACT

Several polypeptide hormones have been isolated in pure state from hypothalamic tissue. These hypothalamic hormones stimulate or inhibit the release of pituitary hormones. The determination of their primary structure was followed by large scale synthesis and extensive physiological, clinical and veterinary studies on natural and synthetic hormones as well as their synthetic analogues. The tripeptide (pyro)Glu-His-Pro-NH_2 (TRH) stimulates the release of thyrotropin and prolactin in laboratory and domestic animals and humans and can be used clinically for diagnostic tests. The tripeptide Pro-Leu-Gly-NH_2 (MIF) inhibits the release of melanocyte stimulating hormone (MSH) in frogs and may have direct effects upon the brain. Further work is needed for the isolation and determination of structure of physiological growth hormone-releasing hormone, which would be of major clinical value. The structure of prolactin release-inhibiting factor (PIF) has not yet been elucidated. The decapeptide (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LH-RH) stimulates the release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) in rats, hamsters, rabbits, sheep, pigs, cattle, monkeys, chickens and humans, but whether this decapeptide is also the physiological FSH-RH, remains to be established. LH-RH/FSH-RH also stimulates biosynthesis of LH and FSH in rats and induces ovulation in rats, rabbits, hamsters, sheep and women. LH-RH/FSH-RH stimulates spermatogenesis in rats, and based on preliminary results, also in men. Sex steroids appear to be responsible in part for the preferential release of LH and FSH in response to LH-RH/FSH-RH. Examination of the biological properties of a large number of decapeptide analogues of LH-RH/FSH-RH shows that (pyro)Glu, His and Trp residues may be necessary for the release of LH and FSH. Analogues of LH-RH in which other amino acids such as Tyr, Ser, Arg and Leu have been replaced still possess major biological activity, suggesting that these amino acids may be required only for binding to receptors. There has been no dissociation of LH-RH activity from FSH-RH activity in any of the analogues examined. Tripeptide to nonapeptide analogues of LH-RH have little, if any, activity. The synthesis of a competitive analogue of LH-RH/FSH-RH could lead to the development of new methods of birth control. Another such approach could be obtained eventually by the use of antisera to LH-RH.

The existence of hypothalamic substances regulating anterior pituitary gland was postulated many years ago[1]. However, it is only during the past few years that sufficient progress has been made to prove this concept and establish the practical significance of hypothalamic hormones. The isolation, determination of structure, and synthesis of several hypothalamic hormones have been accomplished and these hormones are being evaluated now for diagnostic and therapeutic uses. It is likely that additional hypothalamic hormones will be structurally identified and synthesized during the next few years. Thus, through hypothalamic hormones, we may be able one day eventually to exert complete control over much of the endocrine system.

Because the hypothalamus is the part of the brain nearest to the pituitary gland, it was reasonable to postulate that neural components were involved in the control of the secretion of the pituitary hormones. The median eminence, the part of the hypothalamus closest to the pituitary, is connected to the pituitary by means of a stalk. Harris[1] suggested that a portal system of blood vessels between the median eminence and pituitary is the pathway for the hypothalamic regulation of pituitary function[1]. After cutting the portal blood vessels between the hypothalamus and pituitary, changes occurred in the function of the endocrine glands (ovary, testis, adrenal cortex and thyroid) acted upon by anterior pituitary hormones. The best explanation of the relationship between the portal blood supply and anterior pituitary function was that hypothalamic nerve fibres liberate hormonal substances into the capillaries in the median eminence, and then these substances are carried by the portal vessels to the pituitary gland where they stimulate or inhibit the release of various anterior pituitary hormones[1]. Direct evidence for the existence of specific hypothalamic neurohormones involved in the release of anterior pituitary hormones was lacking for many years. Demonstration of the existence of a corticotropin-releasing factor by Saffran and Schally in 1955[2] opened the way for subsequent discoveries of other hypothalamic regulatory substances. The existence of at least nine hypothalamic hormones regulating the pituitary gland is now reasonably well established (*Table 1*).

Table 1. Hypothalamic hormones known to control the release of pituitary hormones

Hypothalamic hormone (or factor)	Abbreviation
Corticotropin (ACTH)-releasing hormone	CRH or CRF
Thyrotropin (TSH)-releasing hormone	TRH or TRF
Luteinizing hormone (LH)-releasing hormone	LH-RH or LH-RF
Follicle stimulating hormone (FSH)-releasing hormone	FSH-RH or FSH-RF
Growth hormone (GH)-releasing hormone	GH-RH or GH-RF
Growth hormone (GH) release-inhibiting hormone	GH-RIH or GIF
Prolactin release-inhibiting hormone	PRIH or PIF
Prolactin-releasing hormone	PRH or PRF
Melanocyte stimulating hormone (MSH) release-inhibiting hormone	MRIH or MIF
Melanocyte stimulating hormone (MSH)-releasing hormone	MRH or MRF

The abbreviation -RH could represent releasing hormone or regulating hormone, since some hypothalamic hormones appear to affect the synthesis

as well as release of respective anterior pituitary hormones. For three pituitary hormones, there is a dual system of hypothalamic control, one system being inhibitory and one being stimulatory. The existence of hypothalamic inhibitors, as well as stimulators of growth hormone, prolactin and melanocyte-stimulating hormone may be explained by the absence of negative feedback products from their target tissues. In the case of corticotropin, thyrotropin, luteinizing hormone and follicle-stimulating hormones, hormones (corticosteroids, thyroid hormones and sex steroids) from the target glands inhibit secretion of these anterior pituitary hormones by negative feedback action exerted on the pituitary, hypothalamus or both[3]. Before describing in detail the most recent physiological, biochemical and clinical findings relating to each of the known hypothalamic hormones, we would like to present some general information about these hormones which may be of particular interest to chemists.

These hypothalamic hormones are polypeptides and appear to be present in the hypothalamus in microscopic quantities, e.g. in the case of the LH-releasing hormone (LH-RH), its content is about 50 nanogrammes per one pig or sheep hypothalamus. Consequently, large quantities of hypothalami amounting to 250000–1000000 or more fragments have to be used for purification. Because of the instability of some hypothalamic hormones, especially CRH, as well as the presence of hundreds or thousands of other peptides in the hypothalamic tissue, the most modern methods of protein and peptide chemistry are used for the purification. Frequently, even a maximal recovery of activity may not yield enough material for structural work. The latter was carried out by conventional methods but on a micro-scale and was based on the Edman–Dansyl stepwise degradation, occasionally supplemented by mass spectroscopy and selective tritiation of the C-terminus[4]. The syntheses were carried out by solid-phase as well as classical methods. Natural and synthetic hypothalamic hormones can exert their effects *in vivo* and *in vitro* in nanogramme or even picogramme doses, testifying to the extremely high biological activity of these substances. For veterinary use in large domestic animals and for clinical use in human beings for diagnosis and therapy, dosages of a few tens or hundreds of microgrammes are adequate in spite of dilution in general blood circulation after intravenous, intramuscular or subcutaneous administration.

CORTICOTROPIN RELEASING HORMONE (CRH)

The central nervous system (CNS), and the hypothalamus mediate the response to 'stress'. Thus, external environmental factors, sensory stimuli and emotions can result in the liberation of corticotropin-releasing hormone (CRH) which stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary. In turn, ACTH augments the secretion by the adrenal cortex of steroids necessary for survival. CRH was the first hypothalamic hormone to be demonstrated by Saffran and Schally[2], but its instability and the difficulty with its assays have delayed the isolation of adequate amounts for elucidation of its structure. In our early attempts to purify CRF, we utilized posterior pituitary powders, since hypothalami were not readily available. A

tentative, partial amino acid sequence of a corticotropin-releasing factor (CRF) purified from powdered porcine posterior pituitary tissue was reported[5] as Ac-Ser-Tyr-Cys-Phe-His-[Asp-NH_2, Glu-NH_2]-Cys-(Pro, Val)-Lys-Gly-NH_2. It is not known whether the physiological CRH from the hypothalamus is related to the proposed neurohypophysical CRF. Polypeptides synthesized by coupling the dipeptides Ser-His or His-Ser to the free amino-terminal group of lysine vasopressin have some CRF activity[6]. The clinical usefulness of CRH may be limited to diagnostic tests.

THYROTROPIN RELEASING HORMONE (TRH)

Hypothalamic thyrotropin-releasing hormone (TRH) stimulates TSH release, and thyroid hormones inhibit it[3]. In 1966 we reported that porcine TRH, the first of the hypothalamic hormones to be isolated, contained three amino acids, histidine, proline and glutamic acid, in equimolar ratios[7]. This constituted the first proof that hypothalamic hormones are polypeptides. In subsequent investigations, we determined the amino acid sequence and the structure of porcine TRH and achieved its synthesis[8-12]. The molecular structure of porcine TRH is shown in *Figure 1*.

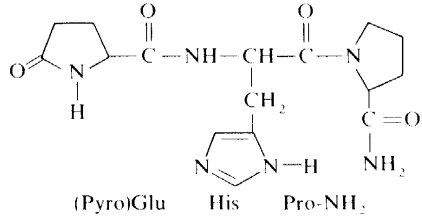

Figure 1. Molecular structure of thyrotropin-releasing hormone (TRH)

Similar experiments conducted by Burgus and co-workers established that the structure of ovine TRH was also (pyro)Glu-His-Pro-amide[13]. It is probable that bovine and human TRH have the same structure[14, 15]. Many analogues of TRH have been synthesized in an attempt to study the relationship between structure and activity[3, 16]. Most analogues have little TRH activity but one, with a methyl group in the 3-N position of the imidazole ring of histidine, has much greater activity than natural TRH itself[16].

The results of various physiological studies conducted since 1963 with natural preparations of TRH[14, 15] have been confirmed and extended by using synthetic TRH, which has the same activity as natural TRH[3]. TSH release into the plasma occurs when TRH is administered intravenously, subcutaneously, intraperitoneally or orally. *In vitro*, TRH in picogramme dose releases TSH from the pituitaries of rats, sheep and goats[3]. A dose-response relationship, both *in vivo* and *in vitro*, is readily demonstrable – that is, increasing doses of TRH cause a progressively greater release of TSH. The thyroid hormones thyroxine and triiodothyronine can block the stimulatory effect of TRH by an action exerted directly on the pituitary gland[15].

After administration of tritiated or ^{14}C-labelled TRH to rats and mice, the radioactivity accumulates in the pituitary[17, 18]. Some radioactivity also becomes concentrated in kidney and liver, probably because of their role in the inactivation and excretion of TRH. Rapid inactivation of TRH by rat and

human plasma[19] is caused mainly by the enzymatic cleavage of the amide group at the prolyl end. The half-life of TRH in the blood of the rat is about four minutes[18].

Cyclic AMP may be the mediator of the action of TRH on the pituitary cell[3]. The studies of the metabolism, distribution and mechanism of action of TRH have been important because of the increasing use of the hormone. The initial demonstration of the effectiveness of TRH in releasing TSH in human beings was made with a natural preparation of porcine TRH[3,15]. The elucidation of the structure and large scale synthesis of TRH made possible extensive clinical trials with this hormone in many countries in which it was demonstrated that TRH is a powerful, safe and nontoxic stimulant of TSH release in men, women and children[3,15,20]. It is also a useful diagnostic compound for testing pituitary TSH reserve and distinguishing pituitary from hypothalamic hypothyroidism. The pattern of rise of TSH in the plasma, after the administration of TRH, may also shed some light on thyroid disease. Recent studies suggest that TRH may relieve some types of mental depression. Synthetic TRH will also release prolactin in sheep, cows and human beings[3,21], but it remains to be established whether TRH is the main prolactin releasing hormone.

GROWTH HORMONE (GH)-RELEASING HORMONE (GH-RH) AND GROWTH HORMONE-RELEASE INHIBITING HORMONE (GH-RIH, GIF)

Progress in the isolation of GH-RH has been hampered by the difficulty of the methods used for its detection. For following GH-RH activity in the course of its purification, we have conducted tests *in vitro* and *in vivo*. The *in vitro* assay is based on stimulation of release of bioassayable GH[22] from rat pituitaries[23]. The materials to be tested are added to the incubation medium. For the test *in vivo*, we use depletion of bioassayable GH in the rat pituitary after intracarotid injection as an index for GH-RH activity[24]. A decapeptide active in these test systems was isolated in pure form from pig hypothalami[25]. It was structually characterized as Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala[26] and synthesized[27]. This amino acid sequence is similar to that of the amino-terminal sequence of the β-chain or porcine haemoglobin[27]. The pure porcine material and the synthetic decapeptide corresponding structurally to the proposed natural GH-RH were both active in these assay systems *in vitro* and *in vivo*.

However, discrepancies between the results obtained by bioassay[22] and radioimmunoassay[28] for GH soon became evident. Preparations of the natural porcine decapeptide proposed as GH-RH[25,26], or of the corresponding synthetic decapeptide[27], did not stimulate the release of immunoreactive GH when administered to rats, pigs, sheep, monkeys and men[3]. Consequently, it is unlikely that the decapeptide Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala is the physiological GH-RH. The search for a GH-RH which releases immunoreactive GH was continued and we found, in Sephadex fractions or porcine hypothalamic extracts, GH-RH activity which augmented the release of GH as measured by radioimmunoassay. Subsequent purification of GH-RH was achieved by the use of phenol extraction, chromatography on carboxy-

methyl cellulose (CMC) and countercurrent distribution (CCD). This work confirms the existence of a GH-RH capable of stimulating the release of immunoreactive GH[3]. Further work is needed for the isolation, determination of structure, and synthesis of this GH-RH. This GH-RH might provide a material of possible therapeutic use in inducing normal growth in children and perhaps acting as an anabolic agent free of androgenic effects.

Tests of other partially purified and highly purified fractions of porcine hypothalami revealed the presence of materials capable, in microgramme or nanogramme doses, of inhibiting the release of immunoreactive GH[3]. The existence of a GH release-inhibiting hormone (GH-RIH or GIF) was first suggested by Krulich *et al.*[29]. It is likely that many of the problems encountered in the assays for GH-RH were caused by the presence of GH-RH and GIF in the same fractions. The net result was that GIF decreased the stimulation of release of GH or abolished it altogether. Thus, GIF and GH-RH can cancel out their respective effects in some fractions. The detection of GIF in sheep hypothalami can be made more readily, and Brazeau *et al.* reported the isolation and structure of ovine GIF[30] and its synthesis. The structure of ovine GIF can be seen in *Figure 2*. We have also synthesized this substance and found it to inhibit GH release in rats, sheep and in men[31,32]. Porcine hypothalamic extracts appear to contain several substances which inhibit the release of GH[33] and the structure of one of them appears to correspond to ovine GIF. It is conceivable that GIF could be used in the control of some types of diabetes and perhaps even some pituitary tumours associated with acromegaly.

H-Ala-Gly-CyS-Lys-Asp(NH_2)-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-CyS-OH

Figure 2. Amino acid sequence of growth hormone-release inhibiting hormone (GH-RH, GIF)

PROLACTIN RELEASING HORMONE (PRH) AND PROLACTIN RELEASE-INHIBITING HORMONE (PRIH, PIF)

The hypothalamus can both stimulate and inhibit prolactin secretion[3,34]. The galactorrhea seen in human patients after surgical cutting of the pituitary stalk or administration of certain tranquillizers may result from removal of the inhibitory influence of the hypothalamus on prolactin secretion. Similarly, in rats, transplantation of the pituitary results in increased prolactin secretion[3].

That the inhibition of prolactin secretion in rats is mediated by a hypothalamic substance was first shown by studies conducted *in vitro*[35,36]. Subsequent studies revealed the presence of PRIH or PIF in hypothalamic extracts of sheep, cattle and pigs[37]. We have utilized the techniques of Sephadex gel filtration, chromatography on CMC and CCD for purification of PIF from pig hypothalamic extractions and have obtained it in a virtually homogeneous state. The structural work is in progress. It is possible that PIF will be of clinical significance in inhibiting undesired lactation. Its possible use for treatment of breast cancer is based only on speculation.

Frequently, the contamination of hypothalamic extracts with prolactin-releasing hormone (PRH) obscures their PIF effect. PRH was thought, at

first, to be present only in bird hypothalami[34], but recent studies indicate that hypothalamic extracts of rats and pigs contain this activity in addition to PIF[3]. A part of this prolactin-releasing activity can be attributed to TRH. Synthetic TRH has been shown to stimulate release of prolactin as well as TSH in rats, human beings, cows and sheep[3,21]. Nevertheless, TRH may not be the physiological PRH. Recent studies indicate that purified pig hypothalamic fractions, from which TRH was removed, still stimulate prolactin secretion *in vitro*, but the structure of PRH is still unknown. The usefulness of TRH in increasing milk production in cows is being evaluated.

MELANOCYTE-STIMULATING HORMONE (MSH)-RELEASING HORMONE (MRH) AND MSH-RELEASE INHIBITING HORMONE (MRIH, MIF)

Melanocyte-stimulating hormone (MSH) darkens the skin of amphibia, but its role in mammals is not clearly established. The hypothalamus exerts an inhibitory influence upon the release of MSH in lower vertebrates and in mammals[3,38]. This effect is exerted by a hypothalamic hormone[3,39]. Two peptides with MIF activity were isolated from bovine hypothalami and identified as Pro-Leu-Gly-NH_2 and Pro-His-Phe-Arg-Gly-NH_2[3,40]. The first of these, which happens to be the carboxyl terminal tripeptide sidechain of oxytocin, had greater activity in frogs. Celis *et al.*[41] observed that Pro-Leu-Gly-NH_2 can be formed by incubating oxytocin with an enzyme found in hypothalamic tissue and found that this compound inhibits MSH release in the rat also. However, Bower and co-workers[42] suggest that tocinoic acid, Cys-Tyr-Ile-Gln-Asn-Cys-OH, the cyclic pentapeptide ring of oxytocin, or its amide, tocinamide, is more likely to be MIF.

There is also evidence for an MSH-releasing hormone (MRH)[39]. It was suggested that the opened NH_2-terminal ring portion of oxytocin, H-Cys-Tyr-Ile-Gln-Asn-Cys-OH, constitutes MRH[43]. Neither tocinoic acid nor its reduced open form has yet been identified in hypothalamic tissue. Pro-Leu-Gly-NH_2 has been shown to be effective in several animal models of Parkinsonism and depression[44] as well as in a few patients with Parkinson's disease. Further clinical trials with this MIF are in progress.

LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE RELEASING HORMONE (LH-RH/FSH-RH)

It has been known for many centuries that reproduction is influenced by seasonal, environmental and emotional factors. Studies conducted during the past 40 years have proved the role of the CNS in the regulation of reproductive cycles and involvement of the hypothalamus in the control of secretion of the gonadotropins LH and FSH[1,3,45]. The hypothalamus controls the secretion of LH and FSH from the pituitary and through LH and FSH, regulates ovarian and testicular function. In turn, sex hormones (oestrogen, progesterone and testosterone) secreted by the gonads, exert a predominantly inhibitory feedback action on the hypothalamus and the pituitary. In animals, an accurately timed signal from the brain causes a massive discharge of LH which results in ovulation. This impulse for the

discharge of LH is mediated by the LH-releasing hormone (LH-RH) which was first demonstrated in the early 1960s in hypothalamic extracts of rats[46] and subsequently found in similar extracts from domestic animals and human beings[3]. Hypothalamic extracts were also found to contain an FSH-releasing hormone (FSH-RH)[3,14,45].

Initially, it was thought that LH-RH and FSH-RH activities were due to two different substances. In several laboratories, attempts were made to isolate both of these substances. Our own efforts were intensified when we demonstrated that highly purified LH-RH of porcine origin unequivocally stimulated both LH and FSH release in men and women under a variety of conditions[3,45]. Our work resulted in the isolation from porcine hypothalami of a decapeptide with both LH-RH and FSH-RH activity and the determination of its amino acid composition and sequence[47,48,4,49]. The structure of LH-RH is shown in *Figure 3*.

This decapeptide was then synthesized[50]. After the announcement of its structure, LH-RH was synthesized by workers in many other laboratories because of its anticipated medical importance[3,45]. Assays in our laboratory showed that not only the decapeptide synthesized by us but also the LH-RH preparations made by others according to the structure we proposed[4,49] had the same LH-RH and FSH-RH activity as natural LH-RH[3,45].

Because both natural LH-RH and the synthetic decapeptide corresponding to its structure possessed major FSH-RH as well as LH-RH activity in rats, domestic animals and human beings, we proposed that one hypothalamic hormone, designated LH-RH/FSH-RH, could be responsible for stimulating the release of both FSH and LH from the anterior pituitary gland[3,45]. The time courses of the release of LH and FSH *in vitro*, induced by the synthetic or natural hormone, are identical.

Occasional divergence of LH and FSH release in the human menstrual cycle, the oestrous cycle in other animals, and in certain pathological conditions can be explained in part by interactions with sex steroids as oestrogens stimulate LH release and inhibit FSH release in response to LH-RH, and some androgens have the opposite effect. The possibility that another hormone which releases only or predominantly FSH is present in hypothalamic tissue cannot be excluded at present. Nevertheless, the ovulation which can be induced in rats, golden hamsters, rabbits, sheep[3], and amenorrhoeic women[51] after treatment with natural and synthetic LH-RH demonstrate that this decapeptide may release enough FSH to cause follicular ovarian maturation. In any case, the detection by radioimmunoassay of a peak of LH-RH during the preovulatory surge of LH and FSH in sheep and women suggests that this decapeptide is most probably the hypothalamic mediator responsible for stimulating the release of the ovulatory quota of LH and FSH[3].

Natural and synthetic LH-RH/FSH-RH can stimulate the synthesis as well as the release of LH and FSH[52]. This was proved by using modified organ cultures of rat anterior pituitary glands. The addition of nanogramme amounts of LH-RH to the incubation medium daily for five days augmented the total content of LH and FSH in the stimulated tissue and medium, and increased the incorporation of ^{3}H-glucosamine into LH and FSH[52]. Hypophysectomized male rats bearing pituitary grafts, after two months, showed

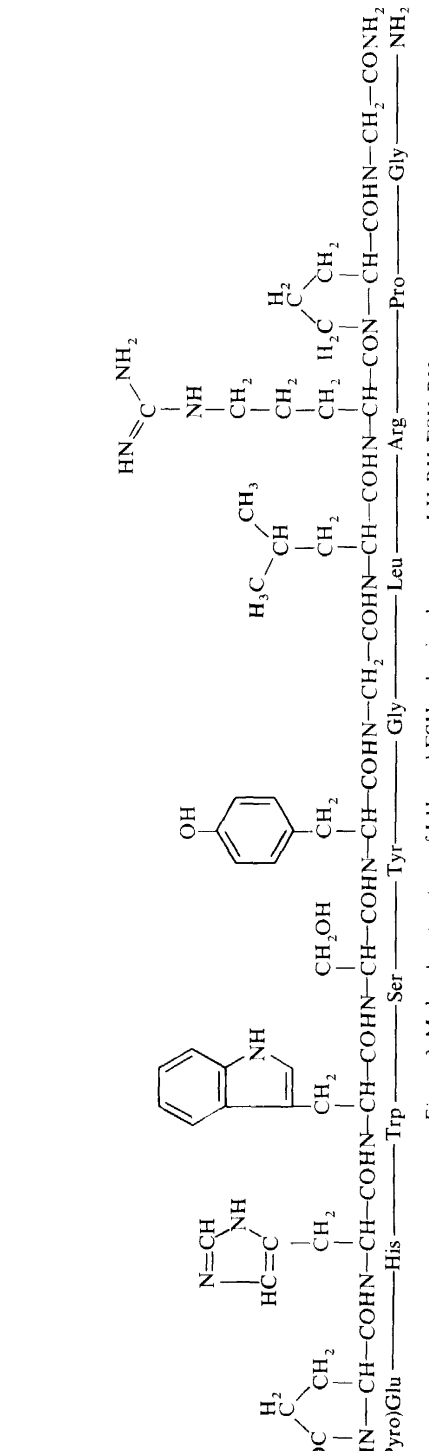

Figure 3. Molecular structure of LH- and FSH-releasing hormone (LH-RH, FSH-RH)

a severe regression of spermatogenesis, but similar rats injected with LH-RH exhibited a striking stimulation of the spermatogenesis. In hypophysectomized female rats with pituitary grafts, long-term treatment with synthetic LH-RH stimulated follicular development[3].

It was demonstrated by electron microscopy that LH-RH induces the extrusion of secretory granules from LH gonadotrophs in rats[3]. The mechanism of action of LH-RH/FSH-RH in inducing LH and FSH release is not known, but cyclic AMP may be the mediator of the action of LH-RH on the anterior pituitary[3].

Natural and synthetic preparations of LH-RH/FSH-RH have also been studied in other species of mammals, in chickens and in fish[3]. In golden hamsters, the administration of LH-RH induces ovulation and increases the concentration of LH in the plasma: LH-RH administered to rabbits produces similar effects. When LH-RH is given to sheep, it increases the concentration of LH and FSH in the plasma and induces ovulation in the ewes. Bulls and prepubertal pigs injected with synthetic LH-RH similarly show increased LH concentrations in the plasma. Premature ovulation is also induced in chickens given LH-RH. Studies *in vivo* and *in vitro* suggest that fish respond to LH-RH. The effect of LH-RH in domestic animals, fish and chickens indicates a possible application of LH-RH/FSH-RH in animal husbandry.

It is well documented that LH-RH/FSH-RH of natural or synthetic origin releases LH and FSH in human beings[3,45]. Maximum increases in the concentration of LH and FSH in the plasma were observed 15 to 60 minutes after intravenous or subcutaneous injection of LH-RH. It has been shown that LH-RH does not stimulate the release of growth hormone, thyrotropin and ACTH, nor does it inhibit the release of prolactin in rats or humans. In studies in Mexico, LH-RH given by intravenous infusion or intramuscularly induced ovulation, confirmed by pregnancy in several women with hypothalamic amenorrhoea[51]. Similar observations were reported from Chile and Germany. This indicates that LH-RH may be useful in the treatment of sterility. Because LH-RH/FSH-RH is active in a number of laboratory and domestic animals, primates including man, bird and fish, species specificity probably does not exist for this hormone. The structure of ovine LH-RH was found to be identical to that of the previously announced porcine hormone[53]. Immunological studies supplemented by physicochemical and bioassay data indicate that bovine, human and rat LH-RH are identical with the porcine and ovine hormone[54].

The use of LH-RH/FSH-RH, its structural analogues, and its other derivatives may lead to the development of new methods of birth control. The two main ways in which one could approach the control of fertility would be based on the development of specific antisera capable of neutralizing endogenous LH-RH and on the synthesis of competitive analogues of LH-RH[3,45].

Several types of antisera to LH-RH have been produced recently in rabbits and in guinea-pigs[3,54]. Of the male rabbits immunized against LH-RH, those which produced antibodies to LH-RH developed considerable testicular atrophy. For example, the weight of the two testes of one such rabbit was only 0.3 g, whereas the testes of a control rabbit weighed 5 g.

When rabbit antisera to LH-RH were given to female rats on the day of pro-oestrus, ovulation was blocked. These results indicate that antibodies to LH-RH can be produced in animals, but the clinical safety of purified animal antisera to LH-RH or of complexes containing conjugated LH-RH and which are used for immunizing animals is not known. The antisera to LH-RH have been used in radioimmunoassays of LH-RH[54]. By means of radioimmunoassays and studies with tritium labelled LH-RH, the half-life of exogenously administered LH-RH in the rat was measured and was found to be six to seven minutes. LH-RH administered to man is rapidly degraded in the blood. Its half-life in man is about four minutes. One of the principal mechanisms of inactivation of LH-RH in human blood is the cleavage of (pyro)Glu-His from the N-terminus.

The synthesis of a large number of structural analogues of LH-RH has helped establish the structure–activity relationship for this hormone[3,45,55]. This information has been used to guide attempts to create synthetic inhibitors of LH-RH. A contraceptive polypeptide must be devoid of significant LH-RH activity, but by competing with endogenous LH-RH for binding to the pituitary receptors, should lead to a decrease in the secretion of LH or FSH, or both. The synthesis of various analogues of LH-RH has been reported by several laboratories[3,55–60]. The results indicate that amino-terminal tripeptide and tetrapeptide fragments of LH-RH as well as the carboxyl-terminal nonapeptide and octapeptide of LH-RH have very little or no LH-RH activity[3,45,55]. This indicates that, unlike gastrin tetrapeptide amide, very active small fragments cannot be obtained from LH-RH. In contrast to early reports[58], the tetrapeptide (pyro)Glu-Tyr-Arg-Trp-NH_2, which is not a part of the LH-RH sequence, has only one part in 10000 of the activity of the LH-RH decapeptide and an equivalent FSH-RH activity. No dissociation of LH-RH activity from that of FSH-RH has been found for any LH-RH analogue. Certain amino acids can be replaced in the LH-RH molecule without major loss of activity. For instance, tyrosine can be replaced by phenylalanine. Replacement of the hydroxyl group by serine or by hydrogen, or of arginine by lysine or ornithine results in a 20- to 30-fold decrease in LH-RH potency, but still retains considerable activity[60]. Some modifications lead to an increase in LH-RH activity[56]. The first peptide with higher activity than LH-RH, Des-Gly-10-LH-RH-ethylamide, was recently synthesized by Fujino *et al.*[56]. It was found to be two to three times as potent as the parent hormone in releasing LH and FSH in rats, sheep and human beings. The increase in activity can be attributed to replacement of the C-terminal glycine amide of LH-RH by an ethylamide moiety, possibly resulting in an increased affinity for receptor sites, a decrease in enzymatic inactivation, or a combination of both factors. Also, replacement of glycine in position 6 by D-alanine increases the LH-RH activity.

Many modifications abolish LH-RH activity. Thus, most replacements for pyroGlu result in almost complete loss of LH-RH activity. The imidazole ring of His in position 2 and the indole ring of Trp in position 3 of the peptide appear to be crucial for biological activity and most changes in these positions result in virtually complete loss of LH-RH activity, such analogues having less than one part in 50000 of LH-RH activity of the parent decapeptide. Which amino acids in the LH-RH molecule are involved in binding to the

receptors and which exert a functional effect still remains to be confirmed but we conclude that the N-terminal sequence pyroGlu-His-Trp is probably the functional part of the LH-RH molecule and that the remaining amino acids are most likely involved only in binding to receptor sites and/or in transport in blood.

One of the analogues, Des-His-2-LH-RH, was reported competitively to antagonize LH-RH in a system *in vitro*, when present in dosages 10000 times greater than LH-RH[59]. However, this analogue does not inhibit the LH-RH induced stimulation of LH-release *in vivo* in rats or ovulation in rabbits[3]. It was decided, nevertheless, to synthesize the corresponding peptide containing the modified C-terminus[57]. *In vivo* assays for inhibitory activity of Des-His-2-Des-Gly-10-LH-RH ethylamide revealed that the significant release of LH in rats induced by a standard dose of LH-RH was abolished by prior treatment with 100 μg of this peptide antagonist. Later experiments confirmed repeatedly that the peptide inhibits LH release *in vivo* in doses of 10, 1 and 0.1 μg per rat. This is the first peptide which significantly reduced LH secretion in response to LH-RH *in vivo*. Des-His-2-Des-Gly-10-LH-RH ethylamide has also proved capable, under some conditions, of inhibiting ovulation in both rats and rabbits. This substance represents only an initial member of the first generation of antagonistic LH-RH analogues to have been synthesized, and the chances that more potent inhibitors of the hormone can be developed now appear more promising. The search for still more effective inhibitors of LH-RH must continue. Irrespective of any possible success in the development of new methods of birth control based on antisera and competitive analogues of LH-RH/FSH-RH, the available results indicate that LH-RH/FSH-RH should find diagnostic and therapeutic application in clinical medicine and that it may be useful also for stimulation of fertility in domestic animals.

CONCLUSION

A family of polypeptides exist in the hypothalami of animals and human beings which specifically stimulate or inhibit the release of pituitary hormones. Several of these have been isolated from hypothalamic tissue. Their structures have been elucidated and their syntheses performed. These substances have already been used in the diagnosis and treatment of various clinical conditions. Such compounds, as well as their analogues and antisera, should open new approaches to the veterinarian and physician.

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