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EDITED BY

S. R. BLOOM and H. S. JACOBS

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Therapeutic applications of LHRH

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Preface

This volume in the Royal Society of Medicine International Congress and Symposium Series records the proceedings of a conference on *'Therapeutic Applications of LHRH'* held at the Royal College of Physicians in London on 25 January 1985. The meeting was designed to review and discuss the physiology of luteinizing hormone-releasing hormone (LHRH) and the therapeutic potential of its pulsatile administration in the management of delayed puberty, cryptorchidism, and male and female hypothalamic infertility. Related practical aspects, such as developments in infusion pump technology, the systematic evaluation of LHRH absorption depending on site and route of administration, and the criteria to be applied in selecting patients for treatment were also considered in detail.

We believe that these proceedings will provide an objective and authoritative synopsis of the information currently available on LHRH and its therapeutic applications.

We would like to acknowledge the valuable and meticulous assistance of Dr P. J. Magill and Mr D. Beattie (Medical Department, Hoechst UK Limited) in the preparation of these proceedings for publication.

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Contents

Physiology of luteinizing hormone-releasing hormone (LHRH)

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Summary

Luteinizing hormone-releasing hormone (LHRH) is a single-chain peptide of 10 amino acids and is produced via the enzymic cleavage of a precursor of much larger molecular weight.

The brain contains several LHRH neuronal systems. Only some of these terminate on the pituitary portal blood vessels; LHRH-like peptides may be produced by the ovary, testis and mammary gland. LHRH is released into the pituitary portal blood vessels in brief pulses (perhaps of 1 min duration or less) as a result of synchronized high-frequency activation of the LHRH-containing nerve terminals. A continuous low level of LHRH secretion, possibly containing mini-LHRH pulses, is also thought to occur.

The interval between LHRH pulses is determined primarily by events within the brain and is modulated by gonadal steroids, environmental cues and sensory inputs, such as suckling.

Acute exposure to opioid peptides inhibits the release of LHRH, possibly by an action on those LHRH terminals in the median eminence that lie outside the blood-brain barrier.

Prolonged exposure to opioid peptides leads to tolerance (loss of inhibition) and hypersensitivity to opiate withdrawal (dependence). The negative feedback actions of gonadal steroids may activate this system; gonadal steroids increase the level of β-endorphin in pituitary portal blood and under conditions of high steroid feedback the opiate antagonist, naloxone, increases secretion of luteinizing hormone (LH).

LHRH stimulates pituitary gonadotrophs to synthesize LH and its own receptor; thereby one pulse of LHRH primes the pituitary to respond more effectively to a subsequent pulse of LHRH if this is appropriately timed. Mini-LHRH pulses which are too small in their own right to stimulate LH release or continuous exposure to low levels of LHRH secretion may also prime the pituitary gland.

Each major pulse of LHRH stimulates the immediate secretion of stored LH by a mechanism that may involve the polyphosphoinositide cascade of second messengers and the influx or mobilization of calcium ions.

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The secretion of follicle-stimulating hormone (FSH), while stimulated under certain circumstances by LHRH, is primarily regulated by the negative feedback actions of gonadal steroids and inhibin.

Prolonged exposure to *high levels* of LHRH agonists, modified in positions 6, 9 and 10, leads to LHRH desensitization (down-regulation). This is associated with a very marked reduction in the synthesis of LHRH receptors and LH.

LHRH antagonists, modified in positions 1, 2 and 3, bind avidly to LHRH receptors but the hormone-receptor complexes thus formed do not activate the postreceptor events involved in secretion, though LH biosynthesis may continue.

The ovulatory LH surge, even in primates, appears to involve an oestrogen-induced increase in (pulsatile) LHRH secretion and an increase in pituitary responsiveness to LHRH. The mechanisms of LHRH down-regulation may be suppressed at this time.

Introduction

LHRH is a single-chain peptide of 10 amino acids (Fig. 1) (1). Like other neuropeptides, LHRH is synthesized via a precursor of large molecular weight, and is cleaved enzymically from this precursor before secretion into the pituitary portal blood vessels (2). Secretion of LHRH is pulsatile, and the main action of this hormone on reaching the pituitary gland is to stimulate the synthesis and secretion of LH from the gonadotrophs (Fig. 2). Peptides resembling LHRH appear to be synthesized or act within a number of other structures, including the ovary (3), the testis (4) and the mammary gland (5). The action of LHRH in the context of these other systems will not be discussed in this review.

The modification of LHRH in positions 6, 9 and 10, especially the substitution of D-amino acids which are resistant to degradation, results in a range of powerful agonists with potencies at least 1000 times greater than that of the natural decapeptide (6–8). Conversely, modifications to the molecule in positions 1, 2 and 3 result in a range of antagonists which competitively prevent the endogenous hormone from

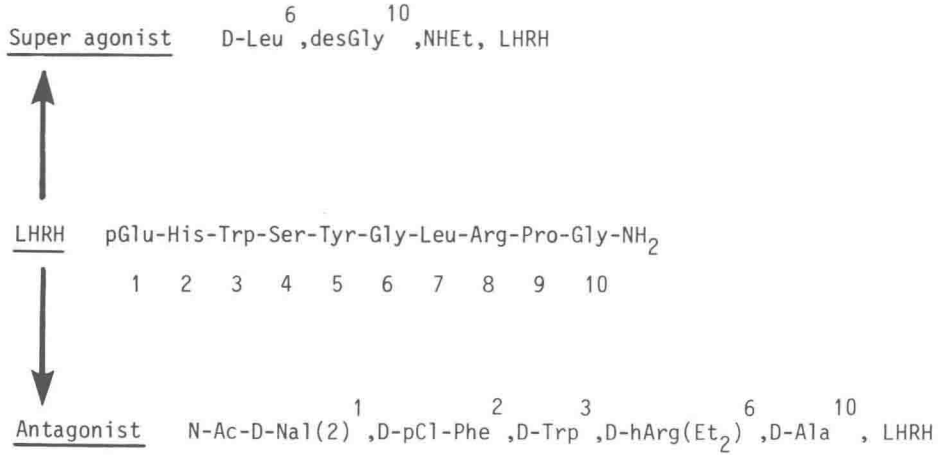

Figure 1. Structure of LHRH, and substitutions for the generation of powerful agonists and antagonists.

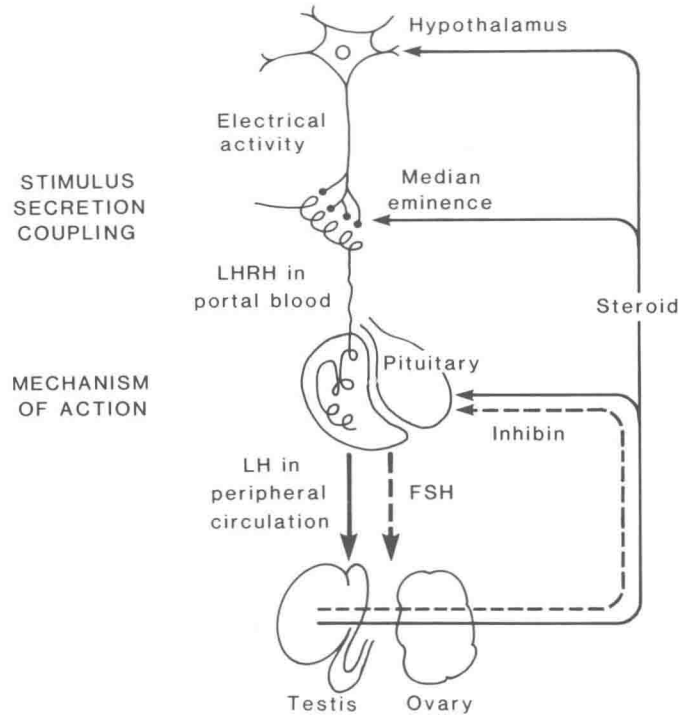

Figure 2. Diagrammatic illustration of the principal pathways involved in the physiological regulation of LHRH secretion from the brain and its action on the anterior pituitary gland to release luteinizing hormone (LH).

gaining access to its receptor (9,10). These analogues have been used extensively to explore the physiology of LHRH secretion and action.

Most of the knowledge concerning the secretion of LHRH and its action on pituitary gonadotrophs has been derived from the analysis of circulating LH levels under different reproductive conditions and/or in response to the administration of LHRH and the above-mentioned agonist and antagonist analogues. Following a brief introductory evaluation of the patterns of LH observed in peripheral blood, this review will proceed to analyse the pituitary and hypothalamic events which determine these secretory profiles, working upwards to the brain.

Actions of LHRH on pituitary gonadotrophs

Pulsatile LH secretion

Luteinizing hormone is secreted primarily in a pulsatile pattern, but pulses are only observed with clarity when the interval between one blood sampling period and the next is substantially shorter than the interval between the LH pulses and shorter than the half-life of the hormone. In men and women, and in sheep, the half-life of LH is 30–50 min, depending on the physiological circumstances under which the

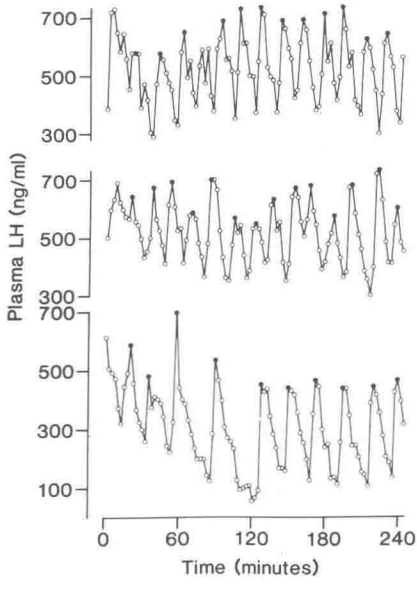

Figure 3. Plasma LH concentrations in blood samples collected every 150 s from three castrated adult male Sprague-Dawley rats. Data adapted from (16).

measurements are made (11), whilst LH pulses are commonly observed at intervals of 1–4 h or less frequently. Therefore blood samples have to be taken at intervals of 10–15 min or less if major LH secretory episodes are not to be missed. An even more frequent sampling schedule and sophisticated time series analysis (12,13) are necessary for the identification of mini-LH pulses (14) because the hormone levels may be significantly elevated above basal levels for only 5 min or less.

The major factor regulating the pulsatile secretion of LH is the negative feedback action of gonadal steroids (15). Thus, LH pulses recur at short intervals after castration and ovariectomy, and are of large amplitude (Fig. 3) (16). Conversely, LH secretion is markedly suppressed by the administration of oestrogen, progesterone and testosterone to the gonadectomized animal (Fig. 4) (17–19). Luteinizing hormone pulses usually rise to their maximal height between one sampling period and the next, before declining approximately in parallel with the half-life of the hormone. Therefore the actual period of LH secretion may be confined to a few minutes. An elevation is observed in the basal blood level of LH when LH pulses are of large amplitude and their frequency approaches or exceeds the half-life of the hormone, e.g. during the ovulatory LH surge (see Fig. 22).

The sheep and pig are excellent species in which to study LH patterns because blood samples of measurable size can be taken at intervals of 5 or 10 min over several days in succession without stress. Rats and other laboratory species present major blood sampling problems, but LH secretion is clearly pulsatile when samples are examined at short intervals (Fig. 3) (16). In sheep, changes in pulse frequency are not solely a response to steroid feedback. LH pulses fall to a rate of 1/day or less in the non-breeding season due to an augmentation in the negative feedback of gonadal steroids (20). Gonadectomy in the non-breeding season results in a massive increase in LH pulse frequency, approximating closely to that seen following gonadectomy in the breeding season (21). A similar suppression of pulsatility is seen during lactation and this could involve an enhancement in negative feedback sensitivity (22). The suckling

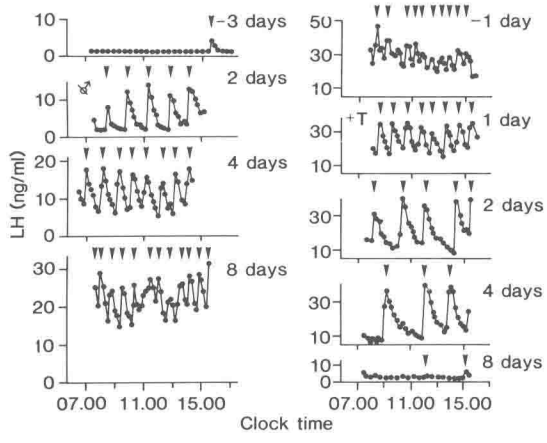

Figure 4. Plasma LH levels in two adult male rhesus monkeys sampled every 10 min for 8–9 h on various days before and after castration, and after hormone replacement therapy. The data on the left were from a monkey sampled 2, 4 and 8 days after castration. The data on the right were from a castrate monkey implanted on day 0 with a testosterone-containing Silastic capsule; this restored the circulating testosterone levels to the mid-to-upper physiological range. Data adapted from (19).

stimulus is the key to this suppression because, if the young are removed or prevented from sucking at the nipples, LH levels begin to rise within a matter of hours. It is still a matter of conjecture whether pulse frequency can exceed the castrate level under physiological circumstances, but this may occur during the ovulatory LH surge and in response to some forms of cognitive or pheromal stimulation (the 'ram effect') (23).

Dramatic changes in pulsatile LH secretion are observed in man during puberty, with LH pulses being confined primarily to the hours of darkness/sleep (Fig. 5) (24,25). By adult life, these circadian changes in LH pulse frequency are far less obvious, though there is evidence that the LH surge commences most commonly during the early morning hours (26). A substantial change in pulse frequency is evident during the menstrual cycle. As the follicular phase advances, LH pulses tend to increase in frequency and decline in amplitude and pulses become relatively indistinct towards

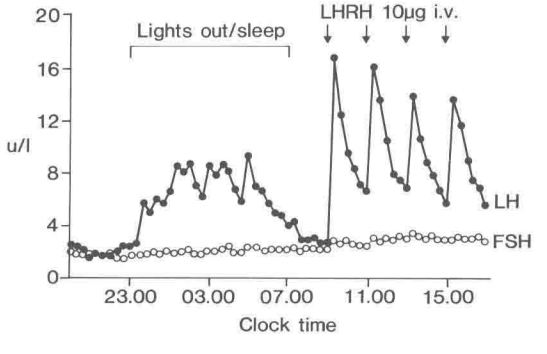

Figure 5. Plasma LH and FSH levels in a mid-pubertal boy of 15 years of age sampled every 20 min from 20.00 to 17.00 h on the following day. The pituitary responsiveness was measured by the administration of four bolus intravenous (i.v.) injections of 10 μg LHRH at 2-h intervals. Note the increase in LH secretion during the night, and the failure of FSH to increase markedly in response to even the bolus injection of LHRH. Data from (25).

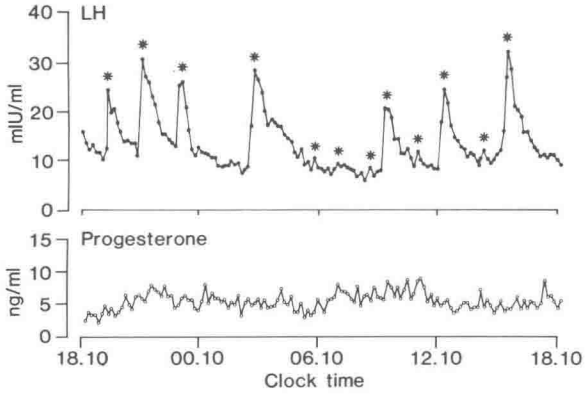

Figure 6. Plasma LH and progesterone levels in a woman sampled every 10 min for a 24-h period commencing 2 days *after the mid-cycle LH surge. The mean progesterone level was 6·2 ng/ml and that for oestradiol was 99 pg/ml. The asterisks indicate statistically significant LH pulses. Data from (14).*

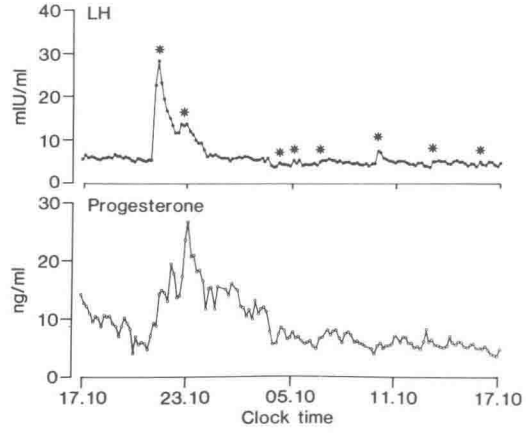

Figure 7. Plasma LH and progesterone levels in a woman sampled every 10 min for a 24-h period commencing 10 days *after the mid-cycle LH surge. The mean progesterone level was 9·2 ng/ml and that for oestradiol was 121 pg/ml. The asterisks indicate statistically significant LH pulses. Note the correlation between the major LH pulse shown and the elevation in the secretion of progesterone. Data from (14).*

ovulation. Pulses are again obvious during the luteal phase and their frequency declines towards the period of menstruation (12,27). In the mid- and late luteal phase each pulse is associated with a surge in progesterone secretion, and blood levels of progesterone may change five-fold (7–35 ng/ml) within a few hours (Figs 6 and 7) (14,28). This dynamic relationship between pulsatile LH secretion and progesterone secretion during the mid-luteal phase raises doubts concerning the value of single progesterone measurements for the assessment of luteal function. Such short-term changes in steroid secretion may also be important in steroid feedback (29), but this is an entirely unresearched area. One relatively new point of possible significance is the presence of mini-LH pulses between the major secretory episodes. Such mini-pulses are very noticeable in the late luteal phase (Fig. 7) and these could signify the

presence of two superimposed LHRH pulse generator systems. The significance of these mini-LH pulses will be discussed further in a later section with regard to the priming of the pituitary gland.

Response to LHRH stimulation and/or inhibition

The response to administration of LHRH or its analogues is not always simple to interpret and is frequently blurred by endogenous LHRH secretion and by changes in pituitary responsiveness. This also applies when LHRH is administered during periods of absent or infrequent LH pulses, e.g. during the day in pubertal children (Fig. 5) or during seasonal infertility in sheep. Even in such circumstances, the gonadal response elicited may modify the pattern of endogenous LHRH secretion or pituitary responsiveness. An alternative approach is to use an experimental situation in which endogenous LHRH secretion has been abolished by lesions placed in the mediobasal hypothalamus (see Fig. 21) (30) or by pituitary stalk transection (31). A similar effect can be obtained using active or passive LHRH immunoneutralization (32,33). This latter approach is particularly attractive because it selectively removes just one hypothalamic hormone without entailing surgery. Furthermore, an LHRH agonist that does not cross-react with the antibodies raised against native LHRH can then be used to explore the response of the pituitary gland (Fig. 8) (34). All these procedures, whilst effectively removing pulsatile secretion, have varying and largely unquantifiable effects on the basal level of LHRH secretion, and any remaining differences in basal secretion may confound the interpretation of any subsequent studies.

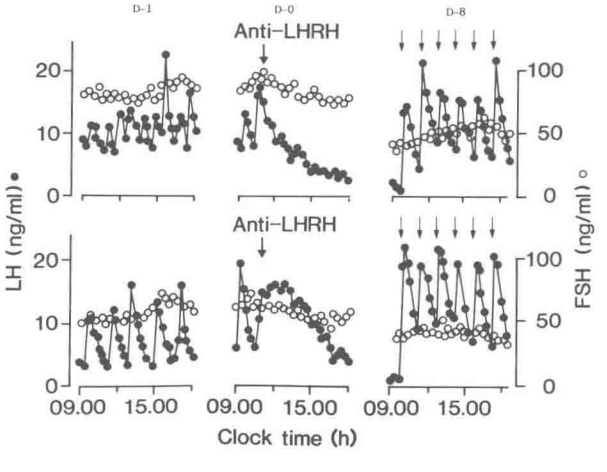

Figure 8. Passive immunoneutralization of endogenous LHRH. Plasma LH (●) and FSH levels (○) in two long-term ovariectomized Romanov × Prealpes ewes sampled every 10 min on the day before (D – 1), the day of passive immunization (D – 0) and 8 days later during replacement with an LHRH analogue (D – 8). The animals were each given 50 ml (i.v.) of an antiserum raised against an LHRH–glutaraldehyde–human serum albumin conjugate. The LHRH analogue used for replacement therapy was des-Gly^{10}(Pro^{9})NHEt-LHRH; this had a potency 2–5 times that of native LHRH, but displayed negligible cross-reactivity with antibody used for passive immunization. The analogue was administered in six 200-μg i.v. injections (arrowed). Data adapted from (34).

Major LH pulses are eliminated very effectively by any procedure that interrupts the supply of LHRH to the pituitary gland. Likewise, a bolus injection of LHRH or an LHRH agonist readily stimulates the release of LH, and the profile obtained closely parallels that associated with an LH pulse evoked by endogenous LHRH (Figs 5 and 8). Taken together, these observations provide irrefutable evidence that each LH pulse is driven by a corresponding LHRH pulse. The delay between LHRH administration and LH secretion is only a matter of seconds, a fact which indicates that LHRH primarily releases stored LH.

LHRH priming

In addition to promoting LH secretion, LHRH acts to stimulate the synthesis both of LH and of LHRH receptors. Thus a dramatic fall in pituitary LH content and pituitary LHRH receptor numbers is observed when endogenous levels of LHRH secretion (or their effects) are reduced by lesions (35) or immunization (33) (Fig. 9), and the situation is reversed by LHRH agonist administration (36). Therefore one LHRH pulse can prime the pituitary gland to respond more effectively to a subsequent LH pulse if the interval separating them is appropriately timed (37,38). This priming phenomenon can also be augmented or attenuated by gonadal steroids, but this depends on their level and time of administration (39). The considerable attention focused on the priming phenomenon elicited by repetitive LHRH pulses has perhaps caused another possible explanation to be overlooked, namely that the pituitary gland might also be primed by the continuous secretion of low levels of LHRH. Indeed, continuous low levels of LHRH administration have been used to evoke an LH surge in anoestrous ewes pretreated with progesterone (40). In the same way, mini-LHRH pulses too small in their own right to elicit LH secretion or which cause only minimal LH secretion might also prime the pituitary gland. Thus, the response to a major LHRH secretory episode might be augmented considerably by small episodes of

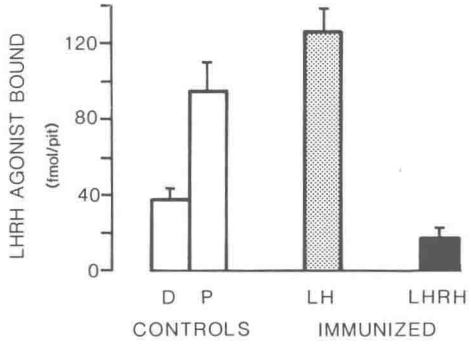

Figure 9. Active immunoneutralization of LH and LHRH on pituitary LHRH receptors in female rats. The control group (n = 10) was immunized against human serum albumin, the LH group (n = 6) against LH (NIAMDD oLH-S23, 100 μg/rat), and the LHRH group (n = 10) against LHRH conjugated to human serum albumin. Booster immunizations were given 3 months after the initial injections. All animals were killed 3 weeks later, the human serum albumin control group at 10.00 h on the day of dioestrus (D) or at 12.00 h on pro-oestrus (P). [D-Ser(tBu)^6]des-Gly^{10}-NHEt-LHRH was used as an agonist in measurement of receptor.

LHRH secretion in the period immediately preceding the major pulse, and this could correspond to the situation observed in the late luteal phase of the menstrual cycle (Fig. 7) (14).

LHRH down-regulation

After a brief period of LH secretion, prolonged exposure to high levels of LHRH agonists results in a very marked fall in pituitary responsiveness to LHRH, and this is associated with a dramatic fall in LHRH receptor numbers and LH biosynthesis (Fig. 10) (41). Such a phenomenon constitutes in effect a non-surgical form of gonadectomy because it produces a prolonged withdrawal of gonadotrophin support to the ovaries and testis, thus providing the rationale for the use of LHRH nasal spray in the treatment of androgen-dependent prostatic disease (42–44). Some LHRH receptors (or binding sites) remain during down-regulation, but these are ineffective at releasing LH when challenged by a bolus injection of LHRH (Fig. 11): this phenomenon could be attributable in part to the extremely low level of LH biosynthesis and the low LH stores present (35).

Action of LHRH antagonists

Antagonists of LHRH, whilst also eliminating LH secretion, express a mechanism of action entirely different from that discussed above. Modifications to the decapeptide in positions 1, 2 and 3 abolish the ability of the molecule to stimulate LH secretion,

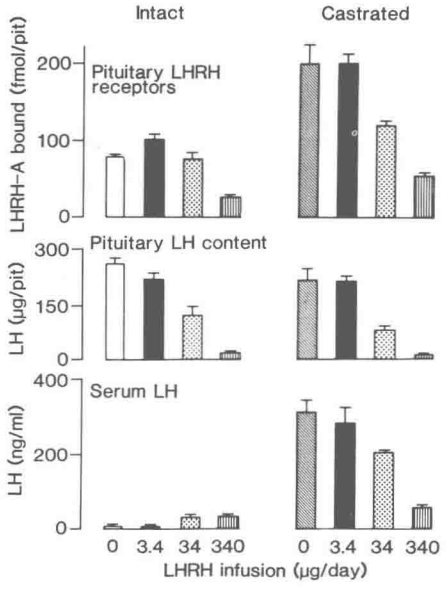

Figure 10. Down-regulation by LHRH infusion in intact and castrated male rats. LHRH was infused continuously at the doses stated above for 6 days commencing on the day of castration, using osmotic mini-pumps implanted into the peritoneal cavity. The values shown are the mean ± SEM of five animals per group. Data adapted from (36).