TITLE:
Extrapituitary action of LHRH and LHRH-like substance in the testes

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Luteinizing hormone releasing hormone (LHRH) was first isolated from porcine hypothalamic tissue and subsequently characterized and synthesized. Synthetic LHRH and its superpotent analogs have been used in attempts to treat gonadal dysfunctions such as secondary amenorrhea, hypogonadotropic hypogonadism, cryptorchism, and delayed puberty, but these attempts have been met with only limited success. In animal experiments, chronic administrations of relatively large doses of LHRH agonists were found to have a variety of antigonadal effects including decreases in the weights of the testis, seminal vesicles and prostate, decreased secretion of testosterone, inhibition or suppression of spermatogenesis, decreased secretion of estrogen and progesterone, inhibition of implantation of fertilized ova and cessation of gestation. These paradoxical effects were first believed to be due to overstimulation of gonadotropin secretion which in turn resulted in desensitization of gonadotropin receptors on the ovaries and testes. Indeed, administration of large doses of LH or hCG are known to induce cessation of gestation and to cause decreases in testicular and ovarian LH receptor concentration.

In order to examine whether the interruption of gestation by LHRH agonists and by large doses of hCG are mediated by the same mechanisms, pregnant rats were treated with a potent LHRH agonist (D-Trp^6^-LHRH) or hCG during pregnancy. Subcutaneous administration of 6 μg D-Trp^6^-LHRH per rat per day from days 1 to 5 of pregnancy prevented the implantation of fertilized ova. This was associated with a dramatic reduction in plasma progesterone on days 4 and 8 and with a slight reduction in estradiol on day 4. Also, no ballooning of the uterus was observed upon exploratory laparatomy on days 8 and 14. Administration of 1,000U of hCG daily from days 1 to 5 partially prevented implantation and completely terminated gestation. However, plasma progesterone and estradiol levels did not differ from those in control animals. Further, the ovaries in the D-Trp^6^-LHRH treated rats appeared to be the same in size as those of control pregnant rats, whereas ovaries in the hCG-treated rats were hypertrophied. These suggest that D-Trp^6^-LHRH prevents nidation through a mechanism different from that of excess hCG. They also suggest that cessation of gestation results from decreased secretion of progesterone during the critical period and that desensitization of the ovaries by elevated circulating gonadotropin levels could not completely account for these effects. This leads us to believe that LHRH agonists could have action on the gonads that are not mediated through the pituitary gland.

My first test of this hypothesis examined whether chronic LHRH agonist treatment of hypophysectomized rats maintained on exogenous gonadotropin (PMS) could induce a reduction of ovarian LH receptors. A significant reduction was observed following administration of D-Trp^6^-LHRH in doses as low as 0.2 μg per day. A significant decrease was observed in the ovarian weight of rats treated with 2 μg of the agonist per day. This dramatic loss of ovarian LH/hCG receptors in hypophysectomized rats could indicate...
that the analog has direct effects on the ovaries. A similar experiment was also performed using male rats. Adult and immature male rats were hypophysectomized and injected daily with saline or 0.2 or 2 μg of D-Trp⁶-LHRH for seven days, with or without concomitant treatment of 1 IU hCG or 50 IU PMS. The administration of the agonist reduced LH/hCG receptors in all cases and the magnitude of this reduction was dose related. A dose as small as 0.2 μg of the peptide resulted in approximately 72% reduction in receptors. This suggests a direct action of D-Trp⁶-LHRH on the testes. It also implies that agonist-induced reduction of testicular LH/hCG receptors are not necessarily due to overstimulation of LH release from the pituitary through "down regulation".

Gonadotropins regulate their own receptors on the gonads: FSH increases, and LH decreases LH receptor content. Since LHRH agonists directly affect testicular LH receptor content, the interplay between LHRH and gonadotropins in regulating testicular LH receptors may become an important problem when the agonist is used chronically in an effort to suppress gonadal activity. My experimental data show that treatment with LHRH agonist tends to decrease testicular LH receptors in hypophysectomized rats, but the decrease is not always statistically significant. Testicular LH receptors are reduced by hypophysectomy alone and further receptor reductions were often not induced by LHRH agonist treatment. However, it should be noted that the levels of LH receptors in the testes of hypophysectomized rats were still well within the range of quantitative measurement. This may suggest that some population of testicular LH receptors persists in the absence of circulating gonadotropins. On the other hand, PMS treatment of hypophysectomized rats increases testicular LH receptors but concomitant administration of LHRH agonist prevents this LH receptor increase. This suggests that gonadotropin-dependent LH receptors are also responsive to LHRH agonists.

An interaction between gonadotropins and LHRH agonist was also observed on the testicular prolactin (PRL) receptors. In intact rats, PRL receptor levels are about 400 fmoles/testis. Administration of the LHRH agonist, D-Leu⁶-LHRH-ethylamide decreased PRL receptors to 12% of that observed in saline-injected control rats at day 1, and to 20% at day 2. PRL receptor levels were partially restored to 55% at day 7. In hypophysectomized rats given daily injections of saline for 7 days PRL receptor levels were only 20% of those in saline-injected intact rats. Injections of the agonist in hypophysectomized animals did not further decrease PRL receptor number at this time. Administration of PMS to hypophysectomized rats for 7 days partially reversed the reduction of PRL receptor that occurs after hypophysectomy, to 46% of those in intact controls. Injections of LHRH agonist into hypophysectomized, PMS-treated rats did not significantly alter PRL receptors on day 1 or day 2, but decreased PRL receptors on day 7 to 55% of same day controls (102 fmoles/testis). This latter concentration is nearly the same as that in saline-injected 7-day hypophysectomized rats not treated with PMS. These findings suggest that:

(1) the effects of LHRH agonist on testicular PRL receptors differ depending on whether or not gonadotropins are present, (2) gonadotropins, primarily FSH, maintain some population of testicular PRL receptors, and that these gonadotropin-dependent PRL receptors are suppressed by direct action of LHRH agonist upon the testes, and (3) there is a population of PRL receptors which is not affected by LHRH agonist or gonadotropin. Thus, the interaction between LHRH agonists and PMS is similar for LH receptors and PRL receptors in the testes. The dramatic fall in testicular PRL receptors levels after 1 and 2 days of LHRH agonist injections and the partial restoration at day 7 in intact rats resemble findings obtained after a single LHRH or LH injection. Therefore, it is possible that LHRH agonists may act on testicular PRL receptors at least partly through stimulating LH release.
In any case, the above findings suggest that LHRH agonists can reduce testicular LH and PRL receptors by direct testicular action. These effects are probably mediated through the specific gonadotropin-releasing hormone receptors in the gonads. The existence of such gonadal LHRH receptors has been demonstrated. However, the Kd of testicular receptors for LHRH or LHRH agonists implies that the concentration of LHRH in the general circulation is too low to activate them. Therefore, it is difficult to assume that the ligand for the gonadal LHRH receptors originates from the central nervous system as does the ligand for the pituitary LHRH receptors. Therefore, I and some others sought to determine if LHRH or an LHRH-like substance was present in the gonads. I first attempted to show the presence of LHRH-like substance in the rat testes using radioimmunoassays for LHRH and immunocytochemical methods.

Gel filtration of rat testes acid extracts on Sephadex G-50 revealed two peaks of LHRH immunoreactivity, a high molecular weight peak eluting just after the void volume and a low molecular weight form eluting at the salt volume. The immunological properties and the mobility of the low molecular weight form on reverse phase HPLC were indistinguishable from those of the synthetic LHRH, suggesting that the testicular LHRH immunoreactivity in this peak is identical to the hypothalamic hormone. However, the high molecular weight form accounted for more than 99% of the total testicular LHRH immunoreactivity and could be dissociated into a lower molecular weight form by treatment with guanidine HCL and dithiothreitol. The dissociated form eluted in an area overlapping the salt volume upon gel filtration and bound to three anti-LHRH sera whose recognition sites are directed towards the C- or th C- and N-terminal residues of the LHRH but not to an antiserum directed towards the central portion of the molecule. However, after HPLC, all four antisera recognized this form, suggesting the presence of a labile moiety in the middle portion of the compound which was altered under the HPLC conditions employed. Finally, the dissociated form was lipophilic than the synthetic decapetide as indexed by their mobilities on reverse phase HPLC in acetonitrile/phosphate buffer systems. It was also immunologically similar, but not identical to synthetic LHRH. The tissue concentration, 7.8 ng/testis is consistent with those expected of a physiologically relevant peptide.

In summary, the direct suppressive actions of LHRH or LHRH agonists on testicular functions may reflect some physiological action of endogenous ligands that possibly originate from the testes. Clarification of the physiological significance of these endogenous LHRH-like substances as well as the mechanisms underlying their actions will provide valuable information and lead to clinical applications of LHRH agonists in suppressing gonadal function as part of the effort to treat such diseases as benign prostate hypertrophy and prostatic cancer.

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和文抄録

精巣での LHRH および LHRH 様物質の下垂体外作用

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LHRH は松タ視床下部より分離され、構造などが決定され合成されるようになった。10個のペプチドから成る合成 LHRH は動物やヒトの下垂体より LH および FSH の放出を促す。合成 LHRH、強力な LHRH 同族体である D-Trp⁶-LHRH、D-Leu⁶-LHRH-ethylamide などが利用できるようになり、生理学的および臨床研究が活発となった。

性機能不全、特に神経内分泌障害に基づく性機能不全で臨床に用いられ、好成績が報告されているが、長期治療患者では性機能低下を含め、むしろ悲観的な結果になっている。性機能に対する LHRH のあきらかに矛盾した作用は始め LH 分泌の過剰刺激による LH 受容体の調節力低下によるものと考えられていたが、われわれは動物実験で一定量のゴナドトロピンにより維持された下垂体摘除動物で、LHRH 作動薬投与により、精巣の LH 受容体減少が惹起されることを見出した。この所見は LHRH および LHRH 作動薬が直接精巣組織に作用できることを示している。

近年の研究では LHRH 作動薬投与により、精巣のプロラクチン受容体数が減少し、程度は少ないが精巣 FSH 受容体も減少が見られる。これらの作用はライディヒ細胞に存在し、精巣内 LHRH 受容体を介して発現しているように思われる。これら精巣内の受容体は高い親和性を有するように思われるが、ラジオイノムノアッセイ法により測定される血中 LHRH 濃度と LHRH に対する受容体の解離定数は、血中 LHRH が精巣組織に直接作用をおよぼすとの仮定を困難にする。

近年、われわれはライディヒイノムノアッセイ法と免疫細胞化学法により精巣内に LHRH 様物質の存在を認める。この物質の精巣内濃度は視床下部内の総量に匹敵すると思われるが、その生理的特徴は不明である。しかしこの物質が精巣の LH、PRL、FSH 各受容体の調節に重要な役割を果しており、それにより精巣機能を調節し、前立腺機能に影響をおよぼすと思われる。