

Gonadotropin-Releasing Hormone Mediated Luteinizing Hormone and Follicle Stimulating Hormone Release from Human Pituitary Tissue in vitro

With the availability of synthetic and highly purified natural gonadotropin releasing hormone, or gonadotropin-releasing hormone (GRH), many investigations in vitro and in vivo in several animal species have demonstrated the effects of this hormone on luteinizing hormone (LH) and follicle stimulating hormone (FSH) release¹⁻³. Studies in man have been limited to in vivo observations⁴⁻⁶. This prompted us to investigate the effects of synthetic GRH on human pituitary glands in vitro.

Materials and methods. Four female patients, 53-64 years of age, underwent hypophysectomy for advanced, metastatic adenocarcinoma of the breast. All patients were either post-menopausal or post-castration. Patients No. 1, 2, and 3 were on exogenous androgens (Halotestin) as well as other chemotherapeutic drugs at the time of operation. Patient No. 4 was on prednisone pre-operatively. All were felt to be free of pituitary dysfunction at the time of operation.

Pituitary fragments obtained at surgery were immediately placed in 10 ml of freshly oxygenated (95% O₂, 5% CO₂) TC 199 medium (Difco). The fragments were then divided into approximately equal portions and allowed to pre-incubate in more freshly oxygenated medium at 37°C for 1½ h. The fragments were finally

placed in 1 ml of medium containing no additive (control) or varying amounts of synthetic GRH (a gift of the National Institutes of Health, Bethesda, Maryland). This was designated time 0', and the pituitary fragments allowed to incubate at 37°C with frequent gentle shaking. Aliquots in duplicate were removed at various time intervals for the radioimmunoassay of LH and FSH. The reagents for assay of LH and FSH were obtained courtesy of the NIH. Radioimmunoassays of LH and FSH were performed by a double antibody technique similar to that describ-

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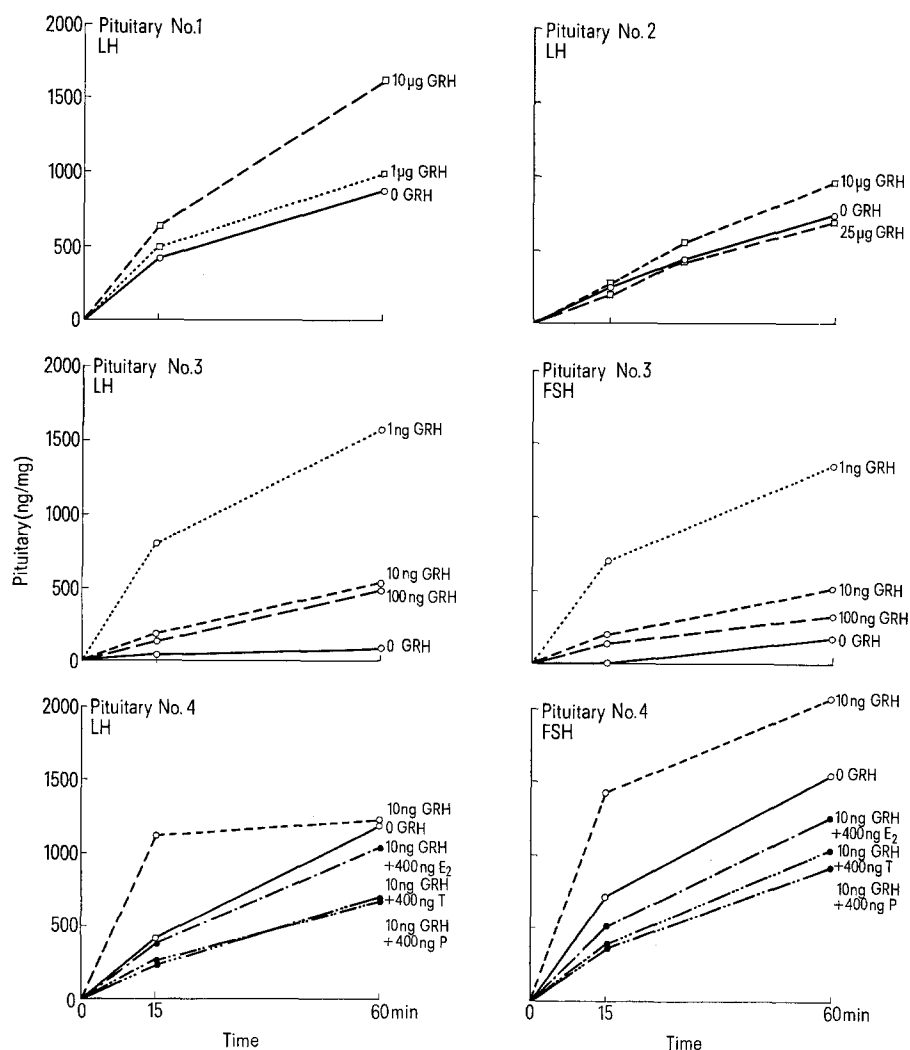
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The effect of various concentrations of GRH on LH and FSH release alone or in combination with E₂, T, and P from anterior human pituitary tissue in vitro. In each case the quantities of GRH, E₂, T, and P are concentrations per ml.

ed by MIDGLEY⁷. The protocol for pituitary No. 4 was varied slightly to include an extra $\frac{1}{2}$ h of pre-incubation during which time the pituitary fragments were exposed to no additives (controls) or 400 ng/ml of estradiol (E_2), testosterone (T), or progesterone (P), respectively. These fragments were then removed to the 1 ml incubation media containing no additives (controls) or 10 ng/ml of GRH. After incubation, the pituitary fragments were weighed to the nearest 0.01 mg on a micro balance.

Results. Results as presented in the Figure are expressed as ng of LER907 present in the medium per mg of pituitary tissue at the various time intervals. Control values, or baseline release, were somewhat variable. However, all the pituitaries showed some response to the varying doses of GRH either at 15 or 60 min. Only 1 pituitary fragment (no. 2 with 25 μ g of GRH) was less than or equal to the control. The release of LH and FSH (as measured in no. 3 and 4) were of the same order of magnitude with release of both hormones significantly above the control at 15 min. Pre-incubation for $\frac{1}{2}$ h with 400 ng of E_2 , P or T appeared to decrease the response to 10 ng of GRH in pituitary No. 4. Despite the responses seen with most fragments, a dose-response effect was not apparent in these experiments. 1 and 10 ng doses of GRH appeared to be as effective as the larger doses.

Discussion. The relative unavailability of normal human pituitary for in vitro study is a limiting factor in this study. Nevertheless, the data presented indicate that synthetic GRH is capable of releasing both LH and FSH from human pituitaries in vitro. As little as 1.0 ng of GRH appears capable of causing this release. This is in agreement with in vitro studies in rats where nanogram amounts of GRH released significant amounts of LH and FSH⁸. In vivo human studies have indicated that i.v. injections of GRH result in maximum elevation of serum LH and FSH within 15 min to 1 h⁹. Our studies indicate that isolated pituitaries are also capable of such release within 1 h, though the response over baseline was not always as marked. In the 2 pituitaries where concomitant

LH and FSH release was studied, FSH-RH activity of GRH was equal to, or greater than, the LH-RH activity of this molecule. This is in contrast to most human in vivo studies where quick i.v. injection of GRH results in proportionally greater serum LH levels than FSH levels⁹⁻¹¹.

Large doses of estradiol, testosterone, and progesterone had significant suppressive effects on both LH and FSH release in the one pituitary in which this was studied. This in vitro phenomenon suggests a direct effect of steroids on human pituitary tissue.

Zusammenfassung. Menschliche Hypophysen wurden mit und ohne LH-RH inkubiert und eine Freisetzung von LH und FSH in vitro gefunden, die sich durch Sexualsteroid hemmen lässt.

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Analysis of Environmental Factors Regulating the Gonadal Cycle in a Tropical Pond Turtle, *Lissemys p. granosa* (Schoepff.)

The role of external factors in regulating the sexual cycle of mammals and birds has been extensively studied, but the present literature regarding similar studies in reptiles is meagre¹. Further, the studies on the gonadal changes in the species of order chelonians appear not to have attracted much attention² and hence remain the most unexplored in the whole reptilian group. Some data which are available have been exclusively devoted to temperate species of turtle³⁻¹² and apparently no literature is at hand on sexual cycle of tropical forms. In this report an attempt has been made to study the seasonal gonadal cycle in a tropical pond turtle *Lissemys p. granosa* in relation to natural environmental changes, particularly photoperiod, temperature, rainfall and humidity.

The gonosomatic index (GSI, weight of gonads divided by body weight and quotient multiplied by 100)¹³, volume of gonads and histological studies were the parameters used for the evaluation of gonadal cycle. The results obtained from various parameters for the assessment of sexual cycle of *Lissemys p. granosa* were uniform. 6-8 specimens of both sexes were collected in every month for 12 months from the ponds in and around Varanasi and were sacrificed after recording their body weight indi-

dually at a preset date similar for each month. The data for environmental changes were collected from the local newspaper office. *Lissemys p. granosa* exhibited marked seasonal cyclical changes in its gonadal activity. In natural habitat, this turtle breeds once a year during July-August and fertilized eggs are laid in September. Its sexual cycle has been divided into six phases (Figure). The cycle in both sexes began from the later half of January

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