1993

Effects of gonadotrophin releasing hormone on growth hormone release in the rat

Stephanie Robin Goodman
Yale University

EFFECTS OF GONADOTROPHIN RELEASING HORMONE ON GROWTH HORMONE RELEASE IN THE RAT

Stephanie Robin Goodman

Yale University

1993

EFFECTS OF GONADOTROPHIN RELEASING HORMONE ON GROWTH HORMONE RELEASE IN THE RAT

A Thesis Submitted to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Medicine

by

Stephanie Robin Goodman

1993

ABSTRACT

EFFECTS OF GONADOTROPHIN RELEASING HORMONE ON GROWTH HORMONE RELEASE IN THE RAT Stephanie R. Goodman, Alan S. Penzias, and Ervin E. Jones. Section of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.

While abundant research has elucidated the role of growth hormone (GH) in the adult female reproductive system, the regulation of GH secretion has not been sufficiently explained. Previous studies have shown that gonadotrophin releasing hormone (GnRH) induces GH secretion independent of gonadotrophin secretion in a non-mammalian species, *Carassius auratus* (goldfish). The purpose of this study was to determine whether GnRH acts as a GH releasing factor in a mammal. Intrajugular silastic catheters were surgically placed in forty sexually mature female Sprague-Dawley rats. Approximately five days later, rats were randomly assigned to one of four experimental groups: I) normal saline, II) human GnRH, III) apomorphine plus GnRH, or IV) somatostatin plus GnRH. Blood (0.1 mL) was withdrawn through the catheter prior to treatment and then at 30, 60, 90, and 120 minutes. Samples were immediately centrifuged, and serum was frozen at -80°C. Red cells were suspended in a volume of heparinized saline equal to the removed serum and returned to the rat to maintain constant intravascular volume. Luteinizing hormone (LH) and GH were measured by radioimmunoassay, and results were analyzed using repeated measure ANOVA or student's t-test where appropriate. Over time, GnRH caused significant

elevations in both GH and LH levels ($p < 0.0005$). There was no statistically significant rise in the GH or LH levels for the saline control group ($p > 0.05$). In addition, apomorphine caused a significant decrease in GnRH-induced LH secretion ($p < 0.05$) but did not affect GH ($p < 0.0005$). In contrast, somatostatin diminished the GnRH-induced GH rise but did not affect LH ($p < 0.0005$). Thus, this study provides evidence that in the rat GnRH causes secretion of GH in addition to LH. Because GH can be independently blocked without affecting LH and because LH can be separately diminished without changing GH, GnRH appears to act as an independent releasing hormone for both GH and LH.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the support of many people. I would like to thank Dr. Ervin Jones, my advisor, for all his help and guidance over the last two years since this project began. Dr. Alan Penzias, a dedicated physician, scientist, and role model, provided friendship and unfailing good humor.

I am grateful to the people who assisted me in the laboratory, too numerous to mention individually. In particular, I would like to thank Dr. Gabriele Rossi for sharing his microsurgical skills and love of animals with me. I also appreciate Dr. Raymond Aten, who taught me respect for radioimmunoassays and was always available when I needed a hand.

I would also like to thank the American Heart Association for providing me with funding for this project. Special thanks to the Office of Student Research for their assistance and expertise in obtaining this grant.

Finally, I would like to thank Beau Everett for his contributions to this thesis and my parents, Frederick and Susan Goodman, for their unfailing support of all that I undertake.

TABLE OF CONTENTS

INTRODUCTION 1

BACKGROUND 7

Cellular Effects of Insulin Like Growth Factors 7

GH Secretion during Puberty 9

GH Secretion in Central Precocious Puberty 11

GH and Leiomyomata Uteri 14

GH and Ovulation Induction 15

MATERIALS AND METHODS 19

Experimental Animals 19

Surgical Procedure 19

Study Design 20

Hormone Measurements 22

Statistical Analysis 24

Participants 25

RESULTS 26

DISCUSSION 29

TABLES AND FIGURES 39

REFERENCES 43

INTRODUCTION

The classical concept of neuroendocrine control of the anterior pituitary involves hypothalamic releasing factors and release inhibiting factors. These hormones control diverse events including reproduction, growth and development, internal homeostasis, and energy use and production. However, these factors were once considered specific such that one releasing factor and one inhibitor affect only one pituitary hormone. Thus, growth hormone (GH) secretion was thought to be under specific dual control--enhanced by growth hormone releasing factor (GRF) and inhibited by somatostatin.(1)

More recently, hypothalamic releasing hormones have been shown to act on more than one pituitary hormone. For example, in normal healthy humans, thyrotrophin releasing hormone (TRH) causes release of both thyroid stimulating hormone (TSH) and prolactin (PRL) and has even been shown to release GH.(2) Although variable, TRH has also been shown to increase GH in healthy animals such as birds, reptiles, fish, sheep, and rats.(3)

Other recent research has also demonstrated that TRH stimulates GH in certain diseases. One study of acromegalics showed that 500 μg of intravenous (IV) TRH stimulated GH in eight of 11 subjects.(4) This effect has been found in other syndromes including alcoholism, anorexia, cancer, cirrhosis, depression, diabetes, beta-thalassemia, and renal failure. Various explanations such as abnormal somatotroph receptors, hypothalamic dysfunction, and central aminergic dysfunction

have been forwarded to account for these findings.(3) None of these interpretations however considers TRH-induced GH a normal, biological response. Moreover, other hypothalamic peptides such as vasopressin, glucagon, neurotensin, endorphins, and metenkephalin have been reported to stimulate GH release.(5) The growing evidence that TRH and other mediators can stimulate GH secretion suggests that the control of hypothalamic-pituitary function is less specific than once thought.

Similar to TRH, gonadotrophin releasing hormone (GnRH) has been reported to directly stimulate GH secretion in numerous clinical conditions such as acromegaly, anorexia nervosa, Klinefelter's, diabetes mellitus, and major affective disorder. These responses have also been dismissed as abnormal or paradoxical due to deranged hypothalamic-pituitary control. Although the many studies documenting this effect were not randomized or placebo-controlled and often used different measures of a positive response, together they provide evidence for GnRH-induced GH secretion in pathologic conditions.

Rubin *et al* showed that three of six acromegalic subjects given 100 μg of synthetic GnRH had increased levels of GH one hour after injection.(6) Another study of 16 patients with active acromegaly had similar results. As soon as 10 minutes after a 25 μg IV injection of GnRH, six of 16 subjects had a significant rise in plasma GH defined as 50% greater than baseline level and a net rise of more than 10 ng/mL. No normal volunteers or patients with nonfunctioning adenomas exhibited this response.(7) Although these authors did not use a placebo-control or statistical analysis, they concluded that acromegalic patients had abnormal responses to GnRH

since GH secretion increased.

A study of GH response to 0.1 mg IV GnRH in 11 females with anorexia nervosa showed that the increase in GH is not limited to patients with GH secreting pituitary tumors. Although the patients had elevated mean basal GH levels secondary to starvation, they were chosen for study since they all had exhibited TRH-induced GH secretion. Sixty minutes after receiving a bolus of GnRH, their mean GH concentration was significantly higher than baseline. Interestingly, the patients had impaired luteinizing hormone (LH) and follicle stimulating hormone (FSH) responses to the GnRH. Unfortunately, this study was neither placebo-controlled, patient-controlled nor blinded.(8)

In patients with Klinefelter's syndrome, GnRH similarly seems to stimulate GH. Nine of 16 patients given 50 $\mu g/m^2$ IV GnRH had increased GH concentrations (defined as a rise from <5 ng/mL to >8 ng/mL) while no age-matched controls showed this response. These patients, unlike the anorexics, did not show a significant GH increase to TRH stimulation, but they had a normal GH response to insulin induced hypoglycemia.(9)

Giampietro *et al* examined GH regulation in 16 diabetic males (nine Type I and seven Type II) and nine healthy male controls.(10) After a 25 μg IV bolus injection of GnRH, seven diabetic patients (five Type I and two Type II) had a GH response within one hour (GH rise greater than the mean plus two standard deviations of basal levels and peak at least 5 ng/mL). This response was significantly higher than the response to TRH or saline, and no change in GH was observed in the

controls. Although GH regulation is considered abnormal in diabetes mellitus, there was no difference in baseline GH levels between subjects and controls.

Amsterdam *et al* studied the effects of GnRH on 56 males and females with major affective disorder and 38 normal males and females.(11) GH, PRL, and TSH were measured after 250 μg of a synthetic GnRH was administered as an IV bolus. This GnRH stimulation test produced statistically significant increases in GH levels when the data for all males (patients and controls) was combined. Separately, the levels did not achieve significance. In women, neither group had increased GH compared to baseline, but the GH levels in the bipolar group were higher than those in the unipolar group and controls. No significant differences were detected for PRL or TSH.

Although this study was not placebo-controlled to remove the effect of the experimental stress, the rise in GH in male controls following GnRH administration led to the conclusion that this type of response can be seen in normal healthy individuals as well as in disease states. It remains unclear why GH did not increase in the women who were all in the follicular phase of their menstrual cycle and were not taking oral contraceptives.

Further studies have involved both diabetics and depressed patients and have investigated the mechanism of GnRH-induced GH secretion. In a group of 16 male insulin-dependent diabetics, 18 unipolar depressed men, and nine healthy controls, 12 diabetics and eight depressed subjects had stimulated GH levels significantly higher than baseline 40 minutes after 25 μg IV GnRH.(12) No controls had this response,

unlike the findings of Amsterdam.(11) Nor did saline injection increase GH. Furthermore, 40 mg IV pirenzepine, a muscarinic antagonist, given 10 minutes prior to GnRH blocked the GH response in both groups but did not alter basal GH concentrations. Pirenzepine did not affect GnRH-induced LH or FSH.(12)

Scant research on GnRH-induced GH secretion has been conducted in healthy humans. In eight healthy male volunteers, 25 μg GnRH increased LH and FSH without changing GH.(13) Also, in four normal male subjects doses ranging from 25 to 200 μg GnRH significantly increased serum LH and FSH without affecting GH.(14) Despite these observations in small samples, the question of whether there is a normal direct connection between GnRH and GH release remains unanswered.

Recent experimental work by Marchant *et al* in a non-mammalian species, *Carassius auratus* (goldfish), provides evidence that GnRH can release GH in normal physiologic states.(15) They found that intraperitoneal injections of salmon GnRH (0.1 μg/g BW) elevated circulating levels of both GH and gonadotrophins in female goldfish after three and nine hours when compared to saline-injected controls. Five 0.1 μg/g BW injections of a mammalian GnRH analogue significantly increased goldfish body length compared to controls, suggesting increased GH secretion.

The results of corresponding *in vitro* experiments were consistent with *in vivo* work. Using perifusion of pituitary fragments, Marchant *et al* showed that pulsed salmon GnRH stimulated GH secretion in a dose-dependent fashion and over a similar time course as gonadotrophins.(15) Their data suggest that GH and gonadotrophins are released by a common releasing hormone which acts at the pituitary level. *In*

vitro experiments also demonstrated that apomorphine (a dopamine receptor agonist) when combined with salmon GnRH decreased the gonadotrophin response with no effect on GH secretion. Somatostatin, a hypothalamic GH inhibitor, combined with salmon GnRH decreased the GH response without affecting gonadotrophin secretion. This research confirms GnRH-induced GH release and illustrates independent inhibition of GH and gonadotrophins.

The present study provides a mammalian model similar to the goldfish. The purpose was to determine the *in vivo* effect of GnRH on LH and GH release in the rat and to further elucidate its regulation. Apomorphine was co-administered with GnRH to evaluate whether GnRH can specifically stimulate GH during LH inhibition. Similarly, somatostatin was given with GnRH to investigate the specific stimulation of LH while GH was suppressed. These experiments were designed to indicate whether GnRH can selectively induce GH secretion in a normal, healthy mammal, as it does in goldfish.

BACKGROUND

Traditionally, hormones have been thought to act within one functional domain. For instance, FSH and LH function within the reproductive system and are not thought to affect energy production. GH, on the other hand, promotes linear skeletal growth through increased cartilage synthesis and functions metabolically by increasing insulin secretion, fatty acid release, and protein synthesis.(2) Contrary to previous thought, however, GH is now believed to play an active role in the reproductive system as well. These findings are substantiated by laboratory studies of granulosa cells and clinical studies of puberty, central precocious puberty, leiomyomata uteri, and ovulation induction.

Cellular Effects of Insulin Like Growth Factors

Insulin like growth factor-I (IGF-I or somatomedin-C) is a polypeptide which both mediates the effects of and is stimulated by GH. Most circulating IGF-I is synthesized in the liver, and, perhaps not surprisingly, a study of rat IGF-I messenger-RNA (mRNA) distribution in various tissues found the highest levels in the liver. Significant for the present study, the next highest concentrations were found in the uterus and ovary. Blood, lung, kidney, heart, and skeletal muscle all had significantly lower levels.(16) These findings indicate tissue specificity of growth factor expression and may even reflect its functional importance.

IGF-I receptors have been detected in porcine and murine granulosa cells, and

IGF-I has been found to stimulate bovine and porcine granulosa cell replication. During each menstrual cycle, granulosa cells within follicles must replicate and differentiate. Although gonadotrophins and sex steroids are known to function in this process, they alone cannot explain why follicles have different responses and ultimate fates. Since IGF-I can promote cellular replication and differentiation in various tissues and since IGF-I has been found in measurable amounts in follicular fluid, it may be an important factor for granulosa cell development.(17)

Lending further credence to the argument, IGF-I has been shown to have several functions in granulosa cell culture. Granulosa cells from immature, hypophysectomized, estrogen-treated female rats cultured with 50 ng/mL somatomedin-C and 20 ng/mL FSH had significantly higher progesterone accumulation compared to treatment with FSH alone.(18) Somatomedin-C augmented FSH-stimulated progesterone in a time- and dose-dependent fashion which was independent of FSH dose, cellular growth, and cell number.

In another study by the same investigators, granulosa cells obtained from similarly treated female rats were cultured and showed dose- and time-dependent increased LH binding with somatomedin-C and FSH compared to somatomedin-C alone. The increased binding was due to an increased binding capacity of functional receptors.(19) Furthermore, IGF-I and FSH synergistically increase granulosa cell aromatase activity, estrogen production, and proteoglycan synthesis which may function in antrum formation and follicular atresia.(17)

Hammond *et al* retrieved ovaries from four to six month old pigs, aspirated

follicular fluid, and obtained granulosa cell samples.(20) They found higher IGF-I concentrations in preovulatory follicles than in immature follicles and serum, suggesting that at least some *in vivo* follicular IGF-I originated in the ovary. Granulosa cells *in vitro* also produced IGF-I and IGF-I binding protein. Rat Sertoli cells, the male homology to granulosa cells, produce increased IGF-I in the presence of GH indicating that extrahepatic synthesis of IGF-I can occur by direct GH stimulation. In contrast, rat granulosa cells cultured with FSH did not have increased IGF-I.(17)

Davoren and Hsueh studied hypophysectomized female rats with diethylstilbestrol capsule implants.(21) After intraperitoneal injection of 200 μg of ovine GH, ovarian, kidney, and serum levels of IGF-I rose. The observers concluded that GH appears to directly enhance *in vivo* ovarian growth factor concentration as well as *in vitro*. Perhaps IGF-I is made by the granulosa cell for its multiple purposes and is regulated not only by GRF and GH but also by GnRH.

GH Secretion during Puberty

Puberty is a crucial period of human development that illustrates the interdependence of growth and reproduction. During this time, secondary sexual characteristics develop, and growth rapidly accelerates. Although the precise mechanisms regulating the onset of puberty remain unknown, the beginning of puberty is associated with both pulsatile GnRH secretion and increased GH pulse amplitude.(22)

If delayed, puberty can be induced with pulsatile GnRH that mimics physiologic secretion. During pulsatile GnRH treatment, an increase in GH occurs, and GH levels correlate with pubertal stage. The predominate explanation is that GnRH stimulates LH and FSH which in turn increase estrogen, a known GH stimulant. Thus, it is assumed, GH and sex steroids synergistically facilitate the growth spurt during puberty.(22) However, the hypothesis that GnRH directly stimulates GH secretion independent of estrogen has not been well tested.

Stanhope *et al* reported a case of a 16 year old girl with idiopathic hypogonadotrophic hypogonadism who was given 2 μg of GnRH subcutaneously every 90 minutes overnight (total dose 12 μg every 24 hours).(23) After six weeks, LH and FSH levels increased, and ultrasound showed an increase in follicular number and size. Increased growth velocity in the first three months was associated with an increased peak GH level. During that time, GH secretion became largely pulsatile with increased amplitude. Induction of puberty by GnRH increased physiologic GH secretion. Since the changes occurred over a short time interval, the authors did not attribute this effect to sex steroids, but rather to neurotransmitter interactions at the hypothalamic level.

The same researchers later conducted a more complete study of 26 delayed puberty patients. They gave pulsatile GnRH intended to mimic physiologic secretion by beginning only at night and later increasing the dosages throughout the day. With treatment, these patients grew as if they had undergone normal puberty. In addition, the timing of the onset of secondary sexual characteristics with the growth spurt

approximated normal development. Again, these investigators concluded that the increased GH secretion was due not only to sex steroid influence but also to GnRH and that GnRH may interact directly with somatotrophs.(24)

An older study by Sheikholislam and Stempfel involved a family of five with dwarfism due to isolated GH deficiency.(25) Each member had characteristic short stature and delayed sexual maturation. GH was administered in a dose of 2 mg intramuscularly (IM) three times each week. After three to five months, they had increased linear growth rates which regressed when treatment ended. Concomitantly, each developed secondary sexual characteristics with changes in body hair and libido that were not related to gonadotrophin release. These authors concluded that GH may alter end organ responses to sex steroids. This study suggests that GH is a necessary agent in reproductive maturation and development, in addition to GnRH and gonadotrophins.

GH Secretion in Central Precocious Puberty

Central precocious puberty (CPP) is a pathologic condition of puberty in which the onset of puberty occurs in children less than eight years of age. These children have both premature gonadal maturation and a growth spurt characteristic of puberty due to early activation of hypothalamic GnRH activity. In girls with CPP, 24 hour GH levels are higher than controls and correlate with serum somatomedin-C levels and height age compared to chronologic age.(26) For treatment, clinicians use GnRH agonists to suppress sexual maturation (27) and slow the exaggerated growth rate.(28)

Although GnRH initially stimulates LH and FSH secretion, continuous GnRH down regulates its own receptors in the anterior pituitary, eventually causing gonadotrophin secretion to diminish.(29)

Several studies of GnRH treatment for CPP indicate the effects of GnRH on growth and GH. Stanhope *et al* treated eight girls and one boy with CPP with an intranasal GnRH analogue (Buserelin).(30) Doses ranged between 17 and 40 μg/kg/day, and treatment lasted an average of 1.2 years. These patients had diminished gonadotrophin pulsatility which reversed gonadal maturation, and the researchers predicted an improved height prognosis.

In a later study, 14 girls with CPP were treated with the same intranasal GnRH analogue. The average dose was 28 μg/kg/day, and treatment lasted for an average of 2.3 years. As expected, these subjects achieved diminished secondary sexual characteristics, decreased LH pulsatility, and decreased estrogen secretion. Initially, during the stimulatory phase of treatment, GH increased. Later, GH secretion decreased, due to a change in pulse amplitude not frequency.(31) Although the authors did not examine the regulation of GH, they summarily attributed the decline to estrogen effects.

Another study involved five children with CPP. They were given leuprolide acetate (Lupron) in a starting dose of 20-30 μg/kg/day which was adjusted according to gonadotrophin response. GH and somatomedin-C levels were measured before and six months after treatment.(32) A decline in average GH levels consistent with the findings of Stanhope *et al* was observed in these children.(31) While the number of

GH secretory episodes did not significantly change in these children, total GH secretion diminished, again indicating a decrease in pulse amplitude not frequency. Although these patients had a decreased mean growth velocity, there was no significant difference in somatomedin-C levels before and after treatment.(32)

Mansfield *et al* studied the effects of 4-8 μg/kg/day of a GnRH analogue in 10 girls with CPP.(33) They found LH and FSH suppressed with regression of secondary sexual characteristics. During the first year, mean height velocity decreased significantly as did the rate of skeletal maturation and the mean peak nocturnal GH levels. In addition, mean somatomedin-C levels decreased, contrary to the findings of DiMartino-Nardi *et al.*(32) These effects were reversed upon discontinuation of treatment.

Although the majority of research in this area supports the above findings, not all studies concur. When 11 children with CPP were treated with a median dose of 40 μg/kg/day of leuprolide acetate for a median time of six months, decreases in LH, FSH, estradiol, and growth rate were observed. Decreases in mean basal GH level and mean peak GH response to GRF were also found, but there was no significant difference in mean IGF-I levels or nocturnal GH concentration. Possible explanations for this discrepancy in GH concentration include patient selection, degree of gonadal suppression, duration of treatment, potency of agonist, and nocturnal secretory variability.(34)

GH and Leiomyomata Uteri

Leiomyomata are benign uterine tumors and are often treated with GnRH analogues to reduce their size. Friedman *et al* studied 18 premenopausal women with leiomyomata in a randomized, placebo-controlled, double blind trial to assess the effects of leuprolide acetate-induced hypoestrogenism on serum GH and insulin like growth factors.(35) Leuprolide acetate (3.75 mg/month IM depot for four months) treated patients had significantly decreased estradiol, GH, IGF-I concentrations, and mean uterine volume compared to placebo-treated controls. There was no difference in IGF-II concentrations between the two groups. Their study suggests that GH and IGF-I, like estrogen, are potential mediators of myometrial growth and further supports a connection between growth and reproduction.

Additional *in vitro* evidence exists from essentially the same study. Fibroids from 17 and myometrial tissue from 10 of the above 18 women who received either leuprolide acetate or placebo were cultured, and culture media was assayed for hormonal content. The fibroids from women treated with the GnRH agonist secreted significantly less IGF-I and IGF-II. However, the IGF-I levels continuously decreased over time, while the IGF-II levels were only diminished at 24 hours. As a control, total protein content was assayed, and no significant difference was measured between the two groups.(36) Thus, long term GnRH administration acts *in vitro* as well as *in vivo* to diminish the secretion of GH mediators. Whether this result was due to the action of GnRH on estrogen or GH was not determined.

Word *et al* evaluated the effects of GnRH on estradiol, IGF-I, and GRF-

induced GH secretion in adult premenopausal females.(37) Seven women received 1 mg/day of leuprolide acetate for four weeks during treatment for either uterine leiomyomata or ovulation induction, while eight healthy women served as controls. They found the mean peak GH response to a GRF bolus was significantly lower in GnRH-treated women. Also, GnRH decreased baseline estradiol levels and had no significant effect on baseline GH or IGF-I. Because the GH response to GRF was positively correlated with estradiol levels, the authors concluded that GnRH-induced hypoestrogenism caused diminished GH release. Because of the potential correlation with unobserved variables, however, a direct effect of GnRH on GH independent of estrogen cannot be dismissed.

GH and Ovulation Induction

Normal ovulation requires coordination of the hypothalamic-pituitary axis, its feedback signals, and local ovarian responses.(38) Problems can occur at any functional level resulting in anovulation and infertility. A promising role for GnRH analogues and GH is in the field of ovulation induction for in vitro fertilization (IVF). One standard therapy involves treatment with human menopausal gonadotrophins (hMG) to stimulate follicle development and human chorionic gonadotrophin (hCG) when appropriate to induce ovulation.(29)

Some women, however, respond with low doses of hMG, while others require higher doses. Unfortunately, high doses can be associated with side effects like multiple gestations and ovarian hyperstimulation which can be life threatening. GnRH

analogues can sometimes be used for hypothalamic-pituitary down regulation in women to increase the chance that hMG will be effective.(29) GH-dependent production of IGF-I and the recently discovered cellular effects of IGF-I on the ovary have led to some clinical success in the addition of GH to ovulation induction protocols. GH may diminish the hMG dose necessary to achieve folliculogenesis.

Menashe *et al* have shown that the GH response to clonidine administration can help predict the subsequent response to hMG needed to induce ovulation.(39) Central alpha-adrenergic receptors are involved in the regulation of GH secretion. Clonidine, an alpha-adrenergic agent, provokes GH stimulation. Those patients with a poor response to clonidine either needed extremely high doses of gonadotrophins or completely failed to respond to exogenous gonadotrophins. This study illustrates that GH reserve correlates with the ovarian response to hMG.

One study compared estradiol and GH levels in women treated with hMG to those given GnRH (1 mg/day for 14 days) in addition to hMG. Women given both GnRH and hMG had higher peak estradiol levels and basal to peak GH increases during their menstrual cycle than those given hMG alone.(40)

An initial study using GH to induce ovulation consisted of seven patients resistant to gonadotrophin therapy. They were each given biosynthetic GH (20 IU IM on alternate days for two weeks) in addition to hMG. With this treatment, each patient had a significant decrease in the required gonadotrophin dose, as well as in the length of treatment time. Although there was no change in the number of follicles obtained, three women conceived using the combined regimen. GH appeared to

increase the ovarian response to gonadotrophin stimulation in women previously refractory to gonadotrophin stimulation.(41)

Blumenfeld and Lunenfeld then reported a case of panhypopituitarism in a patient who responded poorly to ovulation induction with the traditional use of hMG and hCG.(42) When given GH in addition to hMG, the patient required less hMG and conceived during the second cycle. Another case report involved a woman with hypothalamic amenorrhea who did not respond to either clomiphene citrate or pulsatile GnRH. After treatment with GH (0.1 IU/kg BW) each day in addition to pulsatile GnRH, she showed improved follicular recruitment, higher estradiol levels, earlier ovulation, and a successful pregnancy.(43)

Later, in a randomized, placebo-controlled, double blind trial of 16 women, Homberg *et al* tested the effect of GH (24 IU IM on alternate days for two weeks) and hMG on ovulation induction.(44) They found, as they had previously, that patients who received both hormones had a significant decrease in the required dose of hMG and required less time to achieve follicular development. Also, serum IGF-I concentrations increased in women receiving GH. They concluded that GH augments the ovarian response to gonadotrophin stimulation. Since high GH doses were used, however, a question remains whether the GH effect is ultimately physiologic or pharmacologic.

Further support for the use of GH in ovulation induction comes from a study of 10 women who were poor responders to a GnRH analogue, hMG, and hCG regimen. These patients served as their own controls, because GH was added to their

treatment only after three to six months without results. After GH addition, the patients needed less hMG, and the treatment time decreased. Follicular growth rate increased, and more oocytes were collected, although there was no significant difference in estradiol levels or the number of appropriately sized follicles. Six of the 10 women conceived and delivered healthy babies after receiving GH.(45)

Despite these studies demonstrating GH augmentation of ovulation induction, a recent study by Hughes *et al* showed that GRF does not act similarly.(46) Twelve poor responders were suppressed with a GnRH analogue and then treated with hMG, hCG, and 500 μg of GRF twice daily during the treatment cycle. Although the GH and estradiol levels did increase after GRF treatment, serum IGF-I concentrations diminished after an initial rise. Follicular fluid IGF-I levels were higher after GRF but not significantly. Although GRF treatment caused more follicular development and higher oocyte retrieval, no subjects became pregnant. While the hormonal environment improved, there was no associated increase in the pregnancy rate.

Altogether, much of these data describe the interaction between the somatotrophic axis and reproductive events. GH and somatomedins are important mediators of reproductive development and ovarian function, and GH may be complementary to gonadotrophins in ovulation induction. Whether these interactions are normally controlled by GnRH remains unknown. GnRH has clearly been shown to stimulate GH in a wide variety of human pathological conditions and normal goldfish. The present study demonstrates GnRH-induced GH secretion in a normal mammal.

MATERIALS AND METHODS

Experimental Animals:

Forty sexually-mature female Sprague-Dawley rats weighing 225-250 grams were obtained from Charles River Breeding Laboratories, Wilmington, MA. Throughout the experimental period, animals were housed in the animal care unit of Yale University School of Medicine which provided temperature and photoperiod controlled rooms maintained at 22-24°C with a 12-hour light-dark cycle. The animals were maintained on rat chow (Ralston-Purina Co., St. Louis, MO) and tap water *ad libitum*. Care was given according to standard animal care and use guidelines.

Surgical Procedure:

Approximately one day after arrival, animals were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg BW) (The Butler Company, Columbus, OH). Bilateral indwelling intrajugular silastic catheters were aseptically placed to allow venous access for bolus infusion and frequent blood drawing later in an awake, unstressed animal.(47)

Through a transverse incision on the ventral side of the neck, the jugular veins were exposed. A silastic catheter filled with heparinized saline (90 U/mL) was inserted into each internal jugular vein through an incision in the vein's anterior wall. The catheters were positioned in a retrograde fashion with the fenestrated ends approximately at the level of the right atrium. When unrestricted blood flow was

established in the catheter, the cephalad portion of the vein was ligated, and the catheter secured to the vein with 4-0 silk sutures. After catheter function was again confirmed, the catheters were filled with a heparin and polyvinylpyrrolidone (1.5 g/mL PVP) solution. This viscous solution prevents blood from diffusing back into the catheter, coagulating, and blocking blood flow during later testing. Next, the catheters were sealed with a cap and subcutaneously tunneled around the side of the neck to protrude dorsally through a second skin incision. To prevent the animals from destroying the lines, the indwelling catheters were taped together and stapled on top of the animal's back. All incisions were closed with staples, and each animal was housed individually after surgery to prevent catheter removal. All animals gained five to 30 grams of body weight post-operatively and were thus assessed to have adequately recovered.

Study Design:

Approximately five days after surgery, the animals were moved from the animal care facility to the laboratory for testing. They were weighed and randomly assigned to one of four groups: I) 0.9% normal saline control, II) human GnRH 0.1 μg/g BW (Factrel, Wyeth-Ayerst Laboratories, Philadelphia, PA), III) Apomorphine 2 nM/g BW (A-4393, Sigma Chemical Company, St. Louis, MO) plus GnRH 0.1 μg/g BW, or IV) Somatostatin 2 nM/g BW (SRIF-14, 5-8769, Sigma Chemical Company, St. Louis, MO) plus GnRH 0.1 μg/g BW.

At the beginning of the study, the heparin/PVP solution, now hardened, was

aspirated from one venous catheter, and the external tip of the catheter was attached to a heparinized saline (50 U/mL) filled polyethylene line. To help maintain catheter patency, a syringe was attached to the other end of the line which was periodically flushed. The line extended outside the cage to allow the awake animal complete freedom of movement throughout the study period. If blood did not easily flow through the first catheter, the second catheter was used.

To avoid dilution errors and insure complete drug administration, approximately 1 mL of blood was removed prior to each injection or sample withdrawal and then returned to the animal. Blood was withdrawn (1 mL) through the catheter and was immediately centrifuged for 15 seconds (Brinkmann Centrifuge 3414). This time 0 (baseline) serum was pipetted off and stored frozen at -80°C for later assays. The remaining blood cells were resuspended to a volume of 1 mL with saline and the assigned experimental substance according to treatment group (Table 1, Compound 1). This solution was injected through the catheter. Removed red blood cells were always returned to the animal in order to keep the circulating blood volume constant and prevent anemia. Within five minutes, a second intrajugular injection of 1 mL normal saline with or without GnRH was injected, again according to study group (Table 1, Compound 2).

After the injections, every 30 minutes for a total of 120 minutes, 1 mL of blood was withdrawn and centrifuged, yielding approximately 400 μL of serum. Again, sera were frozen and cells reinfused when suspended to a volume of 1 mL in heparinized normal saline (27 U/mL). Studies generally occurred between 1100 and

1500 hours.

At the end of the study period, vaginal wet smears were obtained to determine estrus cycle day by light microscopy. A majority of cornified cells indicated estrus, a leucocytic smear indicated metestrus, and epithelial cell predominance indicated proestrus. Diestrus was signified by a relatively acellular smear with a mixture of all cell types.(48) Rats were sacrificed by an intravenous bolus of pentobarbital.

Hormone Measurements:

After completion of all *in vivo* studies, sera were divided into four aliquots of 100 μL. Each portion was placed in a separate tube and stored at -80°C. Rat LH and GH were measured using radioimmunoassay (RIA) kits provided by the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, University of Maryland School of Medicine. Samples were measured in duplicate within the same assay. Necessary solutions included PBS (10 mM sodium phosphate, 150 mM sodium chloride, and 0.1% sodium azide at pH 7.6) and RIA buffer (1% bovine serum albumin in PBS). Dilution experiments were performed for each assay to determine appropriate antibody concentrations achieving substrate excess and adequate separation.

NIDDK-rLH-I9 was iodinated with ^{125}I (New England Nuclear Research Products, DuPont Company, Boston, MA) by the lactoperoxidase method for the LH RIA.(49) Each tube contained approximately 10,000 cpm/0.1 mL RIA buffer (trace). NIDDK-anti-rLH-s-10 in 2% normal rabbit serum (Sigma Chemical Company, St.

Louis, MO) was used as the initial antibody in a dilution of 1:40,000. NIDDK-rLH-RP-3, used to generate a standard curve, was diluted in 0.1 mL RIA buffer and used in quantities ranging from 0.05 ng to 10 ng. Sample tubes contained 50 μL of serum, and the total volume in each tube was 0.4 mL. The final antibody dilution was therefore 1:160,000.

After addition of all reagents (RIA buffer, standard or sample, trace, and antibody), the tubes were incubated at room temperature overnight. To separate the bound antibody-antigen complex from the unbound, 100 μL of a 1:20 dilution of goat anti-rabbit immunoglobulin (AB21, Chemicon International Company, Temecula, CA) in PBS was added to the tubes which then incubated for two hours again at room temperature. To aid the precipitation, 1.0 mL of cold polyethylene glycol (6% in PBS) was then added. Tubes were vortexed, incubated for five minutes, and centrifuged at 2,500xg for 15 minutes at 4°C (Sorvall RT6000 Refrigerated Centrifuge, DuPont Company). At this point, the supernatant was aspirated and the pellet counted by Automatic Gamma Counter (Micromedic Systems 4/200) for one minute. The counts per minute were transformed by a computerized program using four parameter weighted logit-log regression analysis.(50)

All samples were analyzed in four separate assays with an average ED_{50} of 494.75. The average intra-assay coefficients of variation (CV) for the LH assay were 13.5%, 12.6%, and 24.3% at 20%, 50%, and 80% B/Bo respectively. An external standard was not used.

For the GH assay, NIDDK-rGH-I-7 was iodinated with ^{125}I (New England

Nuclear Research Products, DuPont Company, Boston,MA) by the chloramine-T method.(51) Again, each tube contained approximately 10,000 cpm/0.1 mL RIA buffer. NIDDK-anti-rGH-S-5 in 2% normal monkey serum (Antibodies Incorporated, Davis, CA) was used as the first antibody in a dilution of 1:3750. NIDDK-rGH-RP-2 produced a standard curve ranging from 0.1 ng to 10 ng/0.1 mL RIA buffer. Again, 50 μL serum samples were used in a total volume of 0.4 mL bringing the final antibody dilution to 1:15,000.

The assay for GH followed the same procedure as LH, however, the second antibody for separation was 100 μL of 1:20 goat anti-monkey immunoglobulin (P3, Antibodies Incorporated, Davis, CA) in PBS. Again, all the samples were measured in four assays with an average ED_{50} of 865.75. Rat GH average intra-assay CV was 10.5%, 9.7%, and 18.7% for 20%, 50%, and 80% B/Bo, respectively. The interassay coefficient of variation was 5.2%. All hormone levels are reported as the mean $\pm$ standard errors of the mean (SEM).

Statistical Analysis:

Any serum sample with an LH level below the limit of assay sensitivity (0.5 ng/mL) was assigned a value of 0.5 ng/mL. For the GH assay, the lower limit of detection was 1.2 ng/mL, and again, samples with GH levels below this limit were assigned this value. Hormone data were analyzed with Systat (SYSTAT Intelligent Software, Evanston, IL). Repeated measures analysis of variance (RM-ANOVA) and one- or two-tailed student's t-tests were used, as indicated. Because a normal

distribution of data was confirmed by the Lilliefors test of normality, parametric analysis was used. RM-ANOVA allows comparison of subjects under repeated measures over time. Probabilities <0.05 were considered significant.

Participants:

I performed surgery on all animals involved in this study and with Dr. Alan S. Penzias, undertook the necessary experimental interventions. I received instruction on specific surgical techniques from Dr. Gabriele Rossi. Dr. Raymond F. Aten iodinated rat LH and GH and defined the LH assay protocol. I developed the GH assay procedure and executed both LH and GH radioimmunoassays.

RESULTS

The forty animals were divided into four treatment groups as follows: 10 received normal saline, 16 got GnRH alone, 10 were injected with GnRH plus apomorphine, and four received GnRH with somatostatin. Due to the unavoidable financial constraints of this initial study, animals were assigned to treatment groups in unequal numbers. Specifically, the high cost of somatostatin compared to other treatment compounds limited this group to only four animals. When occasional difficulties were encountered withdrawing blood, time points were omitted from statistical analysis.

Table 2 indicates the number of animals in each estrus cycle day by treatment group. Analysis of results by estrus cycle day was not useful because the number of animals in each treatment group were not large enough for meaningful statistical comparison. Thus, the LH and GH values for each group were averaged to include all cycle days. Since estrogen levels vary with estrus cycle day and since estrogen stimulates GH, GH levels in female rats may vary with day of estrus.

Over the two-hour study period, GnRH caused a significant elevation in LH levels as a function of time (Figure 1) ($n=14$, $p<0.0005$). The mean basal LH level was 0.62 ± 0.05 ng/mL and rose to a maximum of 17.1 ± 2.7 ng/mL at 90 minutes.

When animals were pretreated with apomorphine, GnRH-induced LH levels were significantly diminished. From a mean basal LH level of 0.71 ± 0.11 ng/mL, LH peaked to only 10.6 ± 1.9 ng/mL again after 90 minutes. Although these

animals responded to GnRH with an LH elevation, at 60, 90, and 120 minutes the LH levels were all significantly lower than in animals receiving only GnRH ($n=8$, $p<0.05$).

Somatostatin pretreatment, however, did not affect the GnRH-induced LH rise. These animals had a mean basal LH level of 0.5 ng/mL, which at 90 minutes rose to a maximum of 26.7 $\pm$ 6.5 ng/mL ($n=4$, $p<0.0005$). When these animals were compared to those receiving only GnRH, there was no statistically significant difference in LH concentration at 60, 90, or 120 minutes ($p>0.05$).

The mean serum LH concentration in saline treated animals ranged from 0.95 $\pm$ 0.33 ng/mL at time 0 to 1.9 $\pm$ 1.2 ng/mL at 120 minutes. There was no statistically significant difference in LH levels as a function of time for this control group using RM-ANOVA ($n=8$, $p>0.05$).

In addition to stimulating LH levels, GnRH also caused a significant rise in serum GH levels as a function of time (Figure 2) ($n=14$, $p<0.0005$). After administration of GnRH, the mean basal GH level of 3.9 $\pm$ 1.5 ng/mL increased to a maximum of 26.6 $\pm$ 3.7 ng/mL at 90 minutes.

When apomorphine was added to GnRH treatment, GH levels were still stimulated over time in contrast to the blunting effect on LH ($n=9$, $p<0.0005$). The mean basal serum GH level in this group was 18.2 $\pm$ 6.5 ng/mL, which fell to 3.7 $\pm$ 0.8 ng/mL at 30 minutes, rose to a maximum of 37.1 $\pm$ 7.1 ng/mL at 60 minutes, and then fell again over the next hour. Comparing this group to the GnRH-only group, neither is consistently higher or lower than the other. Although there is a

difference between the groups for one time point, the other four time points are not significantly different ($p > 0.05$).

Nevertheless, somatostatin clearly diminished the GnRH-induced GH response. This treatment group had mean serum GH levels that remained almost constant over time ($n=4$, $p > 0.05$) with a minimum of 1.2 ng/mL at 30 minutes and a maximum of 5.7 ± 4.3 ng/mL at 90 minutes. When compared to animals given GnRH alone, this group had a significantly lower GH response ($p < 0.01$).

The saline treated animals had varying GH concentrations over time, from 4.5 ± 1.6 ng/mL baseline to 22.8 ± 8.9 ng/mL at time 90. Despite this wide range in values, RM-ANOVA did not detect a statistically significant difference in GH level as a function of time ($n=8$, $p > 0.05$).

DISCUSSION

GnRH has been known to stimulate gonadotrophin secretion in a wide range of species including goldfish, rats, and humans. GnRH increases synthesis and storage of gonadotrophins, moves gonadotrophins from a reserve pool to a secondary pool, and directly releases gonadotrophins from the pituitary. Normally, the arcuate nucleus secretes GnRH in a pulsatile fashion to control the reproductive cycle and receives negative feedback from gonadotrophins and sex steroids. When GnRH is given to humans as an IV bolus, serum LH rises within five minutes, peaks at 20-25, and then returns to baseline over a few hours.(52)

GnRH has also been found to stimulate human GH secretion, but most of these observations have been limited to pathologic conditions such as acromegaly (6,7), anorexia nervosa (8), Klinefelter's syndrome (9), diabetes mellitus (10,12), and depression (11). Alternatively, long-term GnRH analogue treatment down regulates gonadotrophins and has been associated with decreased GH secretion. This evidence is documented in studies performed on subjects with central precocious puberty (32,33), leiomyomata (35,36), and infertility (37).

This study of awake, chronically catheterized rats provides evidence that GnRH significantly increases both serum GH and LH as a function of time. Apomorphine, a dopamine receptor agonist, selectively decreased the LH response to GnRH without affecting GH. On the other hand, somatostatin successfully blocked GnRH-induced GH secretion but did not change LH. Because GH can be

independently blocked without affecting LH and because LH can be separately diminished without changing GH, GnRH's action appears specific to each anterior pituitary hormone. Thus, this study suggests that GnRH induces independent secretion of LH and GH.

Overall, these findings are consistent with those of Marchant *et al* in their study of GH regulation in goldfish, although differences exist between GH peaks and time course.(15) They found GH levels elevated to 100 ng/mL at three, six, nine, and 24 hours post injection. The present results were obtained over a two hour time period using identical drug dosages to those effective in goldfish. During this time, both GH and LH were stimulated to peaks of 20-30 ng/mL which then began to fall. Within two hours, however, the levels had still not returned to baseline. These effects over time are more consistent with human data in acromegalics (6), depressed patients, and healthy volunteers.(11) It is not clear whether the fall in GH and LH at the 120 minute time point represents a real trend toward baseline or a momentary decline during a longer, continuous rise. This decline may be due to GnRH clearance or simply the release of stored pools of GH and LH. A possible explanation for the different time periods between studies is the likely difference in clearance rates of mammalian GnRH in goldfish compared to rats. If the present study had observed a longer time period, perhaps a true return to baseline could have been demonstrated. Alternatively, a second peak might have been discovered which could represent new GH and LH synthesis.

While it seems clearer that GnRH may induce mammalian GH secretion, GH

secretion itself is sensitive to circulating sex steroids. In humans, estrogen alone stimulates GH. This effect is illustrated by a study of estrogen replacement (20 μg/day ethinyl estradiol for 15 days) in 17 postmenopausal healthy women. After estrogen treatment, the women's mean resting GH level and 22 hour GH secretion significantly increased. Exercise-induced and GRF-induced GH secretion increased as well.(53)

Rats also exhibit estrogen-dependent GH secretion. When male rats were gonadectomized, the amplitude of GH pulses decreased. When these rats were implanted with estradiol containing silastic capsules, their basal GH increased. Furthermore, they displayed a female-like pattern of GH secretion characterized by more frequent, lower amplitude pulses compared to the male-like higher amplitude bursts.(54) With pituitary autotransplantation to the kidney capsule, estradiol still caused a dose-dependent increase in GH levels.(55)

An initial *in vitro* study of estrogen effects on rat GH secretion used cultures of rat pituitary tumor cells that actively produced GH. Estradiol in milli- and micro-molar concentrations caused a dose-dependent and reversible decrease in GH secretion over 24 hours.(56) Other *in vitro* studies using normal pituitary cell cultures also contradict *in vivo* findings. Webb *et al* found that estradiol had no effect on basal or GRF-induced GH secretion in male rat pituitary cells.(57) Fukata and Martin later found no influence of estradiol on GRF-induced GH secretion in female rat pituitary cells.(58)

Still, many *in vitro* studies support the *in vivo* findings. In normal female

pituitary cell cultures, 0.5 μg/mL estradiol doubled the GH release after a three hour incubation.(59) Similarly, another study of normal female pituitary cell culture revealed that estradiol, as well as estrone and estriol, caused significant increases in spontaneous and GRF-stimulated GH release after 72 hours.(60) Current evidence obtained by Shirasu *et al* from immunostained hypothalami and pituitaries from ovariectomized rats exposed to increasing estrogen concentrations indicates that certain GRF neurons are estrogen targets.(61) They concluded that estrogens may function in GH regulation by modulating GRF release.

What remains unclear is whether the effect of GnRH on GH is due to these estrogen effects alone or whether GnRH can act directly at the hypothalamic or pituitary level to regulate GH secretion. When long-term GnRH administration in humans diminishes gonadotrophin and estrogen synthesis, it has also been shown to diminish GH secretion.(32,33,35,36) In demonstrating that GnRH induces GH independent of LH, the present study suggests that the effect of GnRH on GH is not secondary to LH-induced estrogen. Future investigation of the effects of GnRH on GH independent of estrogen status is needed to address this question.

Rats in different estrus stages have varied estrogen levels. In proestrus, there is a peak of estradiol to approximately 40 pg/ml which falls to a baseline of about 10 pg/ml in estrus. Throughout diestrus and metestrus estradiol levels gradually rise. Corresponding to this, LH levels peak at 35 ng/ml during proestrus and return to baseline by the beginning of estrus. LH levels remain at a baseline of about 1 ng/ml during diestrus and metestrus.(62) Normal rats exhibit pulsatile GH release which in

males has been shown to peak at greater than 200 ng/mL and decline to less than 1 ng/mL. In male rats, a pulse of GH secretion occurs approximately at 3.3 hourly intervals. Female rats have more continuous GH secretion with lower peaks and higher troughs.(63) The timing of these GH surges is associated with the light-dark cycle.(64)

For statistical analysis, within the present study, animals in different estrus stages were grouped together by treatment. Using this approach, results obtained were statistically significant. It is unlikely that the LH peaks obtained soon after GnRH administration were simply due to spontaneous secretion. If significant spontaneous secretion of LH had occurred, the saline-treated control rats should have manifested the same increases, which they did not.

Although the saline-treated control rats did not have a statistically significant GnRH-induced GH rise as a function of time by RM-ANOVA, there appears to be some increase in GH in this group, especially at 90 minutes, as illustrated in Figure 2. As a sample size increases it becomes a better representation of the population from which it is taken. Thus, more reliable statistics are obtained from larger sample sizes. The failure of the saline group to achieve significance may simply be a problem with the sample size. Working within the constraints of limited funding necessarily decreased the sample sizes in this study. Perhaps if a larger sample size had been used with equal numbers of animals in each group, statistically significant differences in saline-induced GH would have been detected.

Alternatively, spontaneous estrogen-induced GH secretion might account for

the apparent increase in GH in the saline-treated group and the statistically significant rise in the GnRH-treated group. Two of 10 (20%) saline-treated animals were in proestrus, whereas four of 16 (25%) GnRH-treated animals were in proestrus. It is possible that the slightly higher percentage of proestrus animals in the GnRH-treated group resulted in a statistically significant rise in GH. If the saline-treated group also had 25% of animals in proestrus, then perhaps a statistically significant rise in GH would have been obtained from saline alone. Again, a larger sample size might allow differences in estrus cycle day to be discerned. Future experiments may also involve stricter time of day control for better synchronization of estrus cycle phase. Furthermore, vaginal smears should be done every day to follow cytology over a longer period of time since a single smear is not sufficient to accurately determine day of estrus and since a surgical procedure can disrupt normal estrus patterns. Another method to determine estrogen status more accurately would simply be to measure estrogen levels in addition to LH and GH. To eliminate the effect of estrogen on GH altogether, future studies could use ovariectomized animals to determine the effect of GnRH on GH.

Estrogen is not unique in its ability to stimulate GH secretion. Multiple other physiologic and pathologic states and mediators have similar effects. Exercise, sleep, hypoglycemia, pain, vasopressin, glucagon, neurotensin, endorphins, metenkephalin, gastrin, catecholamines, sedatives and anesthetics can all increase circulating GH concentrations.(5) GH regulation is indeed complex, and it remains difficult to strictly control for everything that influences it.

In contrast to humans, stress causes inhibition of GH secretion in rats.(64) Stress increases somatostatin thereby decreasing GH. In the present study, stress cannot account for the increased serum GH levels in saline-treated animals. Prolactin levels in rats do increase in response to stress. An estimation of the animal's stress may be obtained in future investigations by measuring prolactin levels in addition to GH and LH.

Although little controversy exists about somatostatin's ability to inhibit GH in different species, the effect of apomorphine on GH is less certain. As a dopamine agonist, apomorphine has been shown to increase GH in men but appears less effective in women. Reserpine, which depletes norepinephrine and dopamine stores, decreases rat GH levels. Whereas clonidine overcomes this and stimulates GH, apomorphine is not effective. Thus, in the rat, the alpha-adrenergic system appears to play a more substantial role in GH regulation than does the dopamine pathway.(5) In female rat pituitary cell culture, dopamine itself had no effect on GH secretion.(59) In the present study apomorphine did not consistently change the GH response to GnRH in a statistically significant manner, but the complex response obtained suggests that other factors already discussed may be involved.

The ability of apomorphine to inhibit gonadotrophins in goldfish, humans, and rats is well documented. In normal female goldfish, intraperitoneal injection of dopamine and apomorphine significantly decrease serum gonadotrophins after one hour and six hours, respectively. After fish were given GnRH and apomorphine together, their serum gonadotrophin levels were significantly lower than with GnRH

alone.(65) Domperidone, a specific D_2 receptor antagonist, increases gonadotrophins in male and female goldfish at a dose of 10 μM/kg BW.(66) In cultured goldfish cells, apomorphine inhibited the gonadotrophin response to GnRH. Although apomorphine is a non-selective dopamine agonist, experiments using selective agonists and antagonists show that the mechanism is D_2 receptor mediated.(67)

In a study using eight normal males and females, a 4 μg/kg/min IV dopamine infusion over three hours caused a significant decrease in circulating LH and prolactin levels. The LH rose rapidly upon cessation of the infusion. No significant changes in FSH levels were found, and GH levels fluctuated randomly.(68) In ovariectomized rats, apomorphine (1.5 mg/kg) inhibited episodic LH secretion for one hour.(69) The findings of the present study in rats are consistent with these cross species results. Apomorphine significantly blunted the LH response to GnRH.

Where GnRH acts on the hypothalamic-pituitary axis to cause GH release was not addressed in this study. Evidence does exist, though, to support GnRH action at the pituitary level. Marchant *et al* found that goldfish pituitary fragments exposed to GnRH *in vitro* had dose-dependent increased GH secretion.(15) Panerai *et al* transplanted rat anterior pituitaries under the renal capsule after hypophysectomy.(70) The rats were exposed to 0.6 and 1.2 μg of GnRH and after five and 10 minutes had increased GH levels. An intact hypothalamic-pituitary portal blood system was not required for GnRH-induced GH secretion.

Future experiments to determine where GnRH affects GH are required. Using *in situ* hybridization techniques, the present study could be repeated using only

GnRH, and probes for mRNA to rat GRF and GH could be used in the hypothalamus and pituitary, respectively. If GnRH induces hypothalamic production of rat GRF mRNA, it will indicate paracrine stimulation of a hypothalamic trophic factor by another hypothalamic trophic factor. If GnRH acts in an endocrine mode, pituitary production should be detected by rat GH mRNA. Otherwise, if increased mRNA synthesis is not detected in either the hypothalamus or the pituitary, the most likely explanation would be that GnRH caused the release of accumulated pituitary GH stores.

GH appears to play an important role in various reproductive events--from granulosa cell replication and differentiation to sexual development and ovulation induction. Previous studies describe GnRH-induced GH secretion in clinical conditions as an abnormal or paradoxical response. The present study provides evidence that GH secretion may be induced by GnRH independent of its effect on LH in normal female rats similar to the findings in normal female goldfish. These results provide a functional explanation for how GH and possibly IGF-I are involved in a mammalian reproductive system.

If GnRH does indeed have a direct stimulatory action on human GH, certain mechanisms would become clearer. In puberty, for instance, the association between secondary sexual characteristics and accelerated growth could be explained by the onset of hypothalamic GnRH pulses, stimulating both GH and gonadotrophins. Similarly, normal follicular development and fertility may depend on adequate GnRH to stimulate not only gonadotrophins but also GH. And finally, granulosa cell

replication and differentiation, which appears dependent on IGF-I, may actually be regulated directly by GnRH via GH. The findings and preliminary conclusions of the present study will enable future research to elucidate these effects in normal humans and provide further understanding of GH regulation.

TABLE 1: Experimental Treatment Design

Animal Group	Compound 1*	Compound 2**
I	Normal Saline	Normal Saline
II	Human GnRH	Normal Saline
III	Apomorphine	Human GnRH
IV	Somatostatin	Human GnRH

* Compound 1 was injected into the animal at time 0 in 1 mL reconstituted blood.

** Compound 2 was injected into the animal within five minutes of Compound 1 in a total volume of 1 mL.

TABLE 2: Number of Animals in Each Estrus Day by Treatment Group

	TREATMENT GROUP			
	I saline	II GnRH	III apomorphine/ GnRH	IV somatostatin/ GnRH
ESTRUS DAY				
estrus	2	2	1	0
diestrus	1	4	3	1
metestrus	5	6	3	3
proestrus	2	4	3	0

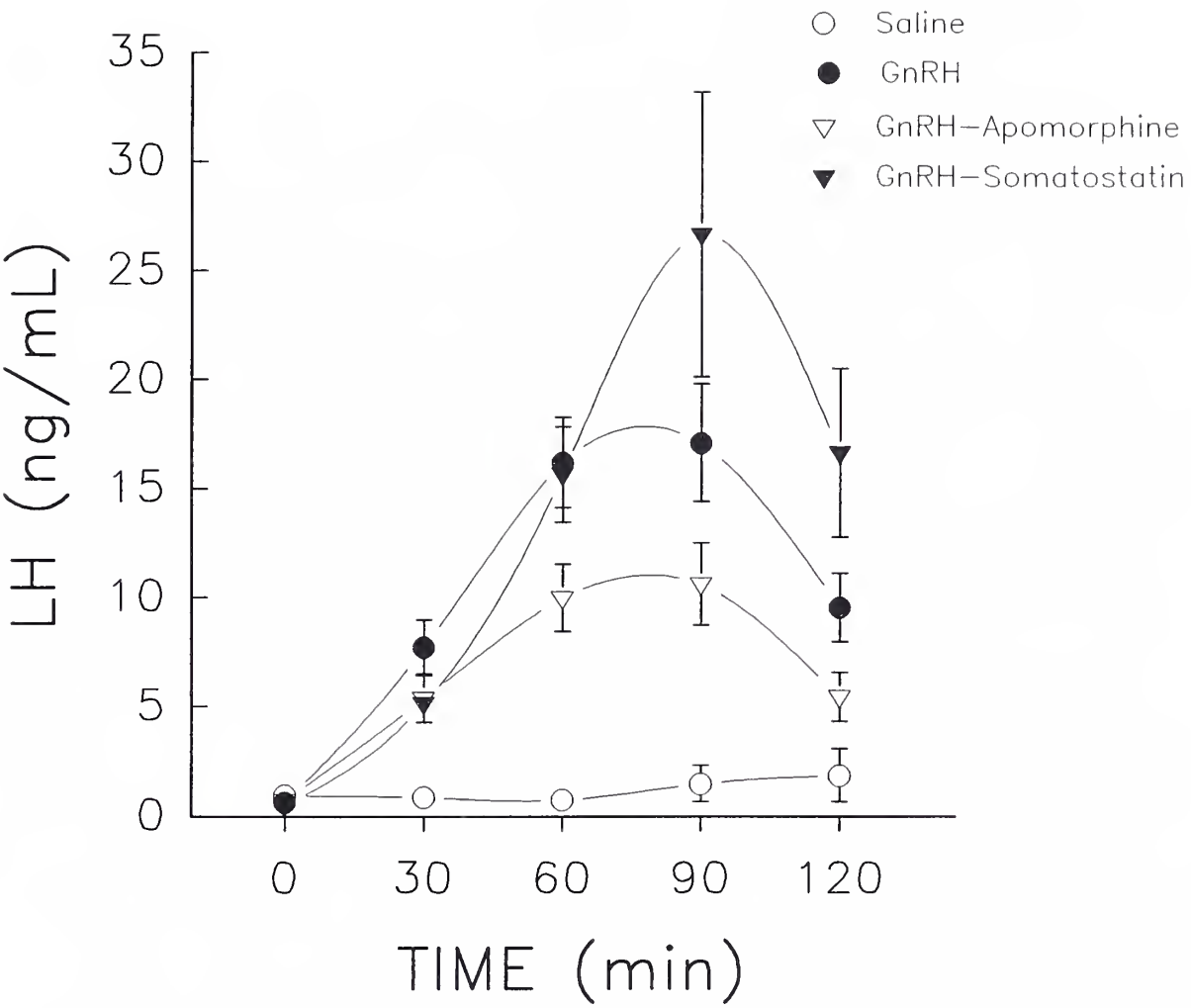

FIGURE I: LH Concentration vs. Time
(mean ± SEM)

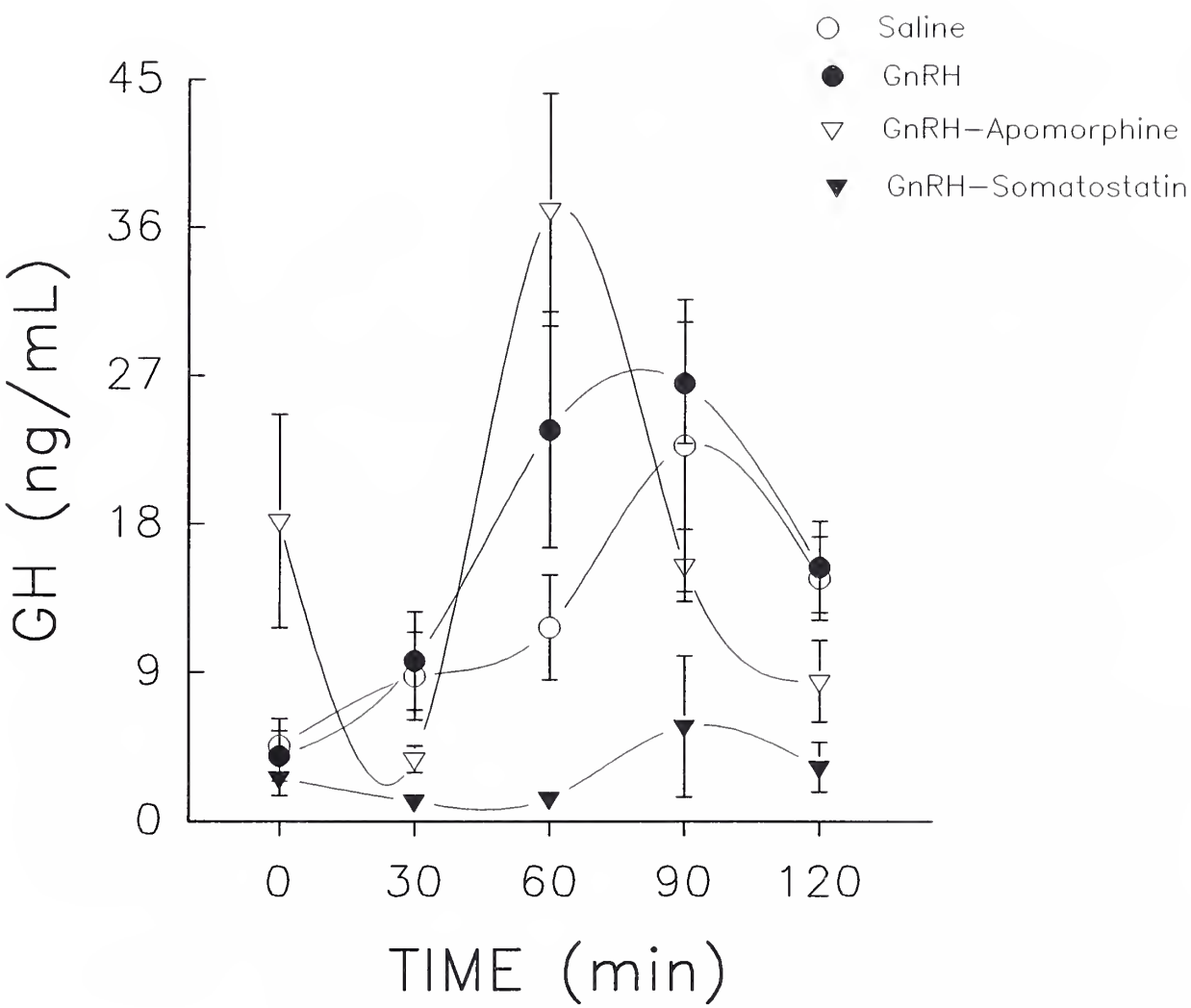

FIGURE 2 : GH Concentration vs. Time

(mean ± SEM)

REFERENCES

1. Wilson JD, Foster DW 1992 Hormones and Hormone Action In:Wilson and Foster (eds) Textbook of Endocrinology. WB Saunders Co., Philadelphia, 8th ed., 1-90

2. Reichlin S 1992 Neuroendocrinology In:Wilson and Foster (eds) Textbook of Endocrinology. WB Saunders Co., Philadelphia, 8th ed., 135-220

3. Harvey S 1990 Thyrotropin-releasing hormone: a growth hormone-releasing factor. J. Endocrinol. 125:345-358

4. Irie M, Tsushima T 1972 Increase of serum growth hormone concentration following thyrotropin-releasing hormone injection in patients with acromegaly or gigantism. J. Clin. Endocrinol. Metab. 35:97-100

5. Martin JB 1980 Functions of central nervous system neurotransmitters in regulation of growth hormone secretion. Federation Proc. 39:2902-2906

6. Rubin AL, Levin SR, Bernstein RI, Tyrrell JB, Noacco C, Forsham PH 1973 Stimulation of growth hormone by luteinizing hormone-releasing hormone in active acromegaly. J. Clin. Endocrinol. Metab. 37:160-162

7. Faglia G, Beck-Peccoz P, Travaglini P, Paracchi A, Spada A, Lewin A 1973 Elevations in plasma growth hormone concentration after luteinizing hormone-releasing hormone (LRH) in patients with active acromegaly. J. Clin. Endocrinol. Metab. 37:338-340

8. Maeda K, Tanimoto K, Chihara K 1987 Abnormal growth hormone release following luteinizing hormone releasing hormone in anorexia nervosa. Jpn. J. Psychiatr. Neurol. 41:41-45

9. Dickerman Z, Rachmel A, Gil-Ad I, Prager-Lewin R, Galatzer A, Laron Z 1981 Rise in plasma growth hormone in response to exogenous LRH in Klinefelter's syndrome. Clin. Endocrinol. 15:403-410

10. Giampietro O, Miccoli R, Ferdeghini M, Penno G, Masoni A, Navalesi R 1987 Inappropriate growth hormone response to luteinizing hormone-releasing hormone in diabetes mellitus. Metabolism 36:1149-1153

11. Amsterdam JD, Winokur A, Lucki I, Snyder P, Harris RI, Caroff S, Rickels K 1982 Growth hormone, prolactin and thyrotropin responses to gonadotropin-releasing hormone in depressed patients and healthy volunteers. Psychoneuroendocrinology 7:177-184

12. Coiro V, Volpi R, Capretti L, Speroni G, Castelli A, Mosti A, Marchesi C, Gardini E, Rossi G, Chiodera P 1989 Simultaneous inhibition by pirenzepine of the GH responses to GnRH and TRH in insulin-dependent diabetics and in patients with major depression. Acta. Endocrinol. 120:143-148

13. Wagner H, Bockel K, Hrubesch M, Grote G, Hauss WH 1972 On the effect of synthetic LH-releasing hormone on the blood levels of LH, FSH, HGH, TSH, ACTH, insulin and blood sugar in man. Horm. Metab. Res. 4:403

14. Haug E, Torjesen P 1973 Effect of synthetic luteinizing hormone- and follicle stimulating hormone- releasing hormone (LH/FSH-RH) on serum levels of LH, FSH, thyrotrophin (TSH) and growth hormone (HGH) in normal male subjects. Acta. Endocrinol. 73:465-474

15. Marchant TA, Chang JP, Nahorniak CS, Peter RE 1989 Evidence that gonadotropin-releasing hormone also functions as a growth hormone releasing factor in the goldfish. Endocrinology 124:2509-2518

16. Murphy LJ, Bell GI, Friesen HG 1987 Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinology 120:1279-1282

17. Adashi EY, Resnick CE, D'Ercole J, Svoboda ME, Van Wyk JJ 1985 Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endo. Rev. 6:400-420

18. Adashi EY, Resnick CE, Svoboda ME, VanWyck JJ 1985 Somatomedin-C synergizes with follicle-stimulating hormone in the acquisition of progestin biosynthetic capacity by cultured rat granulosa cells. Endocrinology 116:2135-2142

19. Adashi EY, Resnick CE, Svoboda ME, VanWyck JJ 1985 Somatomedin-C enhances induction of luteinizing hormone receptors by follicle-stimulating hormone in cultured rat granulosa cells. Endocrinology 116:2369-2375

20. Hammond JM, Baranao JLS, Skalens D, Knight AB, Romanus JA, Rechler HM 1985 Production of insulin-like growth factors by ovarian granulosa cells. Endocrinology 117:2553-2555

21. Davoren JB, Hsueh JW 1986 Growth hormone increases ovarian levels of immunoreactive somatomedin C/insulin-like growth factor I *in vivo.* Endocrinology 118:888-890

22. Stanhope R, Preece MA, Grant DB, Brook CGD 1988 New concepts of the growth spurt in puberty. Acta. Paediatr. Scand. 347:30-37

23. Stanhope R, Pringle PJ, Brook CGD 1985 Alteration in the nocturnal pulsatile release of GH during the induction of puberty using low dose pulsatile LHRH: a case report. Clin. Endocrinol. 22:117-120

24. Stanhope R, Pringle PJ, Brook CGD 1988 The mechanism of the adolescent growth spurt induced by low dose pulsatile GnRH treatment. Clin. Endocrinol. 28:83-91

25. Sheikholislam BM, Stempfel RS 1972 Hereditary isolated somatotrophin deficiency: effects of human growth hormone administration. Pediatrics 49:362-374

26. Ross JL, Pescowitz OH, Barnes K, Loriaux DL, Cutler GB 1987 Growth hormone secretory dynamics in children with precocious puberty. J. Pediatr. 110:369-372

27. Crowley WF, Comite F, Vale W, Rivier J, Loriaux DL, Cutler GB 1981 Therapeutic use of pituitary desensitization with a long-acting LHRH agonist: A potential new treatment for idiopathic precocious puberty. J. Clin. Endocrinol. Metab. 52:370-372

28. Mansfield MJ, Beardsworth DE, Loughlin JS, Crawford JD, Bode HH, Rivier J, Vale W, Kushner DC, Crigler JF, Crowley WF 1983 Long-term treatment of central precocious puberty with a long-acting analogue of luteinizing hormone-releasing hormone. N. Engl. J. Med. 309:1286-1290

29. Speroff L, Glass RH, Kase NG 1989 Induction of Ovulation. In: Brown CL(ed) Clinical Gynecologic Endocrinology and Infertility. Williams and Wilkins, Baltimore, 4th ed. 583-609.

30. Stanhope R, Adams J, Brook CGD 1985 The treatment of central precocious puberty using an intranasal LHRH analogue (Buserelin) Clin. Endocrinol. 22:795-806

31. Stanhope R, Pringle PJ, Brook CGD 1988 Growth, growth hormone and sex steroid secretion in girls with central precocious puberty treated with a gonadotrophin releasing hormone (GnRH) analogue. Acta. Paediatr. Scand. 77:525-530

32. DiMartino-Nardi J, Wu R, Fishman K, Saenger P 1991 The effect of long-acting analog of luteinizing hormone-releasing hormone on growth hormone secretory dynamics in children with precocious puberty. J. Clin. Endocrinol. Metab. 73:902-906

33. Mansfield MJ, Rudlin CR, Crigler JF, Karol KA, Crawford JD, Boepple PA, Crowley WF 1988 Changes in growth and serum growth hormone and plasma somatomedin-C levels during suppression of gonadal sex steroid secretion in girls with

central precocious puberty. J. Clin. Endocrinol. Metab. 66:3-9

34. Sklar CA, Rothenberg S, Blumberg D, Oberfield SE, Levine LS, David R 1991 Suppression of the pituitary-gonadal axis in children with central precocious puberty: effects on growth, growth hormone, insulin-like growth factor-I, and prolactin secretion. J. Clin. Endocrinol. Metab. 73:734-738

35. Friedman AJ, Rein MS, Pandian MR, Barbieri RL 1990 Fasting serum growth hormone and insulin-like growth factor-I and -II concentrations in women with leiomyomata uteri treated with leuprolide acetate or placebo. Fertil. Steril. 53:250-253

36. Rein MS, Friedman AJ, Pandian MR, Heffner LJ 1990 The secretion of insulin-like growth factors I and II by explant cultures of fibroids and myometrium from women treated with a gonadotropin-releasing hormone agonist. Obstet. Gynecol. 76:388-394

37. Word RA, Odom MJ, Byrd W, Carr BR 1990 The effect of gonadotropin-releasing hormone agonists on growth hormone secretion in adult premenopausal women. Fertil. Steril. 54:73-78

38. Speroff L, Glass RH, Kase NG 1989 Anovulation. In: Brown CL(ed) Clinical Gynecologic Endocrinology and Infertility. Williams and Wilkins, Baltimore, 4th ed. 213-231.

39. Menashe Y, Frenkel Y, Lunenfeld B, Mashiach S, Pariente C 1990 Can growth hormone increase, after clonidine administration, predict the dose of human menopausal hormone needed for induction of ovulation? Fertil. Steril. 53:432-435

40. Wilson EE, Word RA, Byrd W, Carr BR 1991 The effect of superovulation with human menopausal gonadotropins on growth hormone levels in women. J. Clin. Endocrinol. Metab. 73:511-515

41. Homburg R, Eshel A, Abdalla HI, Jacobs HS 1988 Growth hormone facilitates ovulation induction by gonadotrophins. Clin. Endocrinol. 29:113-117

42. Blumenfeld Z, Lunenfeld B 1989 The potentiating effect of growth hormone on follicle stimulation with human menopausal gonadotropin in a panhypopituitary patient. Fertil. Steril. 52:328-331

43. Volpe A, Coukos G, Artini PG, Silferi M, Petraglia F, Boghen M, D'Ambrogio G, Genazzani AR 1990 Pregnancy following combined growth hormone-pulsatile GnRH treatment in a patient with hypothalamic amenorrhea. Hum. Reprod. 5:345-347

44. Homburg R, West C, Torresani T, Jacobs HS 1990 Cotreatment with human

growth hormone and gonadotropins for induction of ovulation: a controlled clinical trial. Fertil. Steril. 53:254-260

45. Ibrahim ZHZ, Matson PL, Buck P, Lieberman BA 1991 The use of biosynthetic human growth hormone to augment ovulation induction with buserelin acetate/human menopausal gonadotropin in women with a poor ovarian response. Fertil. Steril. 55:202-204

46. Hughes JN, Martin-Pont B, Torresani T, Tamboise A, Herve F, Santarelli J 1991 Interest of growth hormone-releasing hormone administration for improvement of ovarian responsiveness to gonadotropins in poor responder women. Fertil. Steril. 55:945-951

47. Steffens AB 1969 A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. Physiol. Behav. 4:833-836

48. Nalbandov AV 1964 Reproduction in female mammals and birds In: Salisbury and Crampton (eds) Reproductive Physiology. WH Freeman and Co., San Francisco, 2nd ed. 119-152

49. Thorell JI, Johansson BG 1971 Enzymatic iodination of polypeptides with ^{125}I to high specific activity. Biochim. Biophys. Acta. 251:363-369

50. Rodbard D 1974 Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clin. Chem. 20:1255-1270

51. Peake GT, Morris J, Buckman MT 1978 Growth Hormone. In:Jaffe BM and Behrman HR (eds.) Methods of Hormone Radioimmunoassay. Academic Press, New York, 2nd ed:229

52. Speroff L, Glass RH, Kase NG 1989 Neuroendocrinology. In: Brown CL(ed) Clinical Gynecologic Endocrinology and Infertility. Williams and Wilkins, Baltimore, 4th ed. 51-89

53. Dawson-Hughes B, Stern D, Goldman J, Reichlin S 1986 Regulation of growth hormone and somatomedin-C secretion in postmenopausal women: effect of physiological estrogen replacement. J. Clin. Endocrinol. Metab. 63:424-432

54. Painson JC, Thorner MO, Krieg RJ, Tannenbaum GS 1992 Short term adult exposure to estradiol feminizes the male pattern of spontaneous and growth hormone-releasing factor-stimulated growth hormone secretion in the rat. Endocrinology 130:511-519

55. Carlsson L, Eriksson E, Seeman H, Jansson JO 1987 Oestradiol increases

baseline growth hormone levels in the male rat: Possible direct action on the pituitary. Acta. Physiol. Scand. 129:393-399

56. Haug E, Gautvik KM 1978 Effects of sex steroids on growth hormone production in cultured rat pituitary cells. Acta. Endocrinol. 87:40-54

57. Webb CB, Szabo M, Frohman LA 1983 Ectopic growth hormone-releasing factor and dibutyryl cyclic adenosine monophosphate stimulated growth hormone release *in vitro*: Effects of corticosterone and estradiol. Endocrinology 113:1191-1196

58. Fukata J, Martin JB 1986 Influence of sex steroid hormones on rat growth hormone-releasing factor and somatostatin in dispersed pituitary cells. Endocrinology 119:2256-2261

59. Komolov IS, Perez-Arce JA, Fedotov VP 1980 The effects of estradiol on prolactin and growth hormone secretion in cultured rat pituitary cells from intact and ovariectomized rats. Endokrinologie 75:278-284

60. Simard J, Hubert JF, Hosseinzadeh T, Labrie F 1986 Stimulation of growth hormone release and synthesis by estrogens in rat anterior pituitary cells in culture. Endocrinology 119:2004-2011

61. Shirasu K, Stumpf WE, Sar M 1990 Evidence for direct action of estradiol on growth hormone-releasing factor (GRF) in rat hypothalamus: localization of [^{3}H]estradiol in GRF neurons. Endocrinology 127:344-349

62. Freeman ME 1988 The ovarian cycle of the rat. In: Knobil E and Neill (eds) The Physiology of Reproduction. Raven Press, New York, vol 2:1893-1928

63. Tannenbaum GS, Martin JB 1976 Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 98:562-570

64. Tuomisto J and Mannisto P 1985 Neurotransmitter regulation of anterior pituitary hormones. Pharm. Rev. 37:249-332

65. Chang JP, Peter RE 1983 Effects of dopamine on gonadotropin release in female goldfish, *Carassius auratus*. Neuroendo. 36:351-357

66. Omeljaniuk RJ, Shih SH, Peter RE 1987 *In vivo* evaluation of dopamine receptor-mediated inhibition of gonadotrophin secretion from the pituitary gland of the goldfish, *Carassius auratus*. J. Endocr. 114:449-458

67. Chang JP, Yu KL, Wong OL, Peter RE 1990 Differential actions of dopamine receptor subtypes on gonadotropin and growth hormone release *in vitro* in goldfish.

Neuroendocrinology 51:664-674

68. Leblanc H, Lachelin GCL, Abu-Fadil S, Yen SSC 1976 Effects of dopamine infusion on pituitary hormone secretion in humans. J. Clin. Endocrinol. Metab. 43:668-674

69. Drouva SV, Gallo RV 1977 Further evidence for inhibition of episodic luteinizing hormone release in ovariectomized rats by stimulation of dopamine receptors. Endocrinology 100:792-798

70. Panerai AE, Cocchi D, Gil-Ad I, Locatelli V, Rossi GL, Muller EE 1976 Stimulation of growth hormone release by luteinizing hormone-releasing hormone and melanocyte-stimulating hormone-release inhibiting hormone in the hypophysectomized rat bearing an ectopic pituitary. Clin. Endocrin. 5:717-722

HARVEY CUSHING / JOHN HAY WHITNEY
MEDICAL LIBRARY

MANUSCRIPT THESES

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS DATE