

# Effects of growth hormone on neuroendocrine function

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**Abstract.** Although the role of growth hormone (GH) in the control of reproductive functions is not well understood, there is considerable evidence that the states of both GH deficiency and GH excess are typically associated with reproductive deficits. To identify the possible involvement of functional alterations in the hypothalamic-pituitary system in producing these deficits, we are studying neuroendocrine function related to reproduction in transgenic animals overexpressing GH, in animals with congenital GH deficiency, and in animals with selective immunoneutralization of GH. The results indicate that GH acts on the hypothalamus to alter dopaminergic and noradrenergic control of prolactin and gonadotropin release. Life-long elevation of GH levels outside the physiological range disrupts feedback control of luteinizing hormone (LH) release by gonadal steroids. Plasma LH and follicle-stimulating hormone (FSH) levels and feedback control of LH release are also abnormal in GH-deficient animals indicating that physiological levels of endogenous GH are normally involved in the control of gonadotropin release. Differences between the effects of bovine vs. human GH in transgenic mice and differential effects of GH deficiency in mice and rats should facilitate identification of the mechanisms involved in the actions of GH on the hypothalamic-pituitary system.

Mini-review

**Key words:** growth hormone, transgenic mice, reproduction, hypothalamus

## INTRODUCTION

The role of growth hormone (GH) in the physiological control of reproductive functions and its ability to produce reproductive disorders remain to be clearly defined. In the human, congenital GH deficiency and GH resistance due to a genetic defect in GH receptors are associated with delay of sexual maturation and poor development of male external genitalia (Laron and Sarel 1970, Laron 1974, Strobl and Thomas 1994). Deficiencies in reproductive development and function exist also in mutant mice with absence of GH, prolactin, and thyrotropin-producing cells, isolated GH deficiency, or GH resistance (Bartke 1979, Chubb and Nolan 1985). In hypophysectomized rats and in hamsters with short photoperiod -induced gonadal atrophy, GH treatment can increase the number of testicular LH receptors (Bex et al. 1978, Zipf et al. 1978). This action of GH provides a very plausible explanation for the previously documented ability of GH to potentiate the action of LH on spermatogenesis, testicular growth, and testosterone secretion (Woods and Simpson 1961, Swerdloff and Odell 1977). Subsequent detailed studies in immature hypophysectomized rats provided evidence that treatment with bovine or rat GH can increase testicular weight and progesterone production *in vitro*, as well as proliferation of the Leydig cells (Closset et al. 1991). Recent studies of the effects of GH administration to prepubertal boars provided evidence for a specific role of GH in the control of functional maturation of Sertoli cells in this species (Swanbund et al. 1995). The action of GH on Sertoli cell maturation in these studies was manifested by increase in the lumen of the seminiferous tubules, which is an index of fluid secretion by the Sertoli cells, and by onset of spermatogenesis and was clearly different from the effects of FSH which promoted an increase in the length of the seminiferous tubules (Swanbund et al. 1995). In one study, administration of GH to oligospermic men was reported to augment the effects of exogenous gonadotropins on sperm count (Shoham et al. 1992).

In the female rat, GH was reported to be involved in controlling the age of sexual maturation (Ramaley

and Phares 1980, Advis et al. 1981), but this conclusion was recently questioned (Gruaz et al. 1994). Administration of GH can increase the number of ovulations in gilts (Kirkwood et al. 1988) and in adult mice (Cecim et al. 1995b). In women with anovulatory infertility, injections of recombinant human GH were reported to act as a useful adjuvant to gonadotropin therapy by allowing induction of ovulation with fewer gonadotropin injections (Homburg et al. 1990).

Prolonged elevation of peripheral GH levels above the normal range can have major impacts on reproductive function. Hypersecretion of GH by pituitary adenomas in acromegalic patients is often associated with amenorrhea or menstrual irregularity in women and reduced libido and/or impotence in men (Jadresic et al. 1982). Retarded puberty, reproductive behavior deficits, and infertility were noted in early studies of transgenic pigs and sheep overexpressing GH (Rexroad et al. 1989, Pursel et al. 1990). Reproductive deficits in transgenic mice expressing heterologous GH will be described in the subsequent sections of this article.

Survey of the reported effects of GH on reproductive development and function in both sexes suggests that GH can act at both the hypothalamic-pituitary and the gonadal level. This conclusion is compatible with what is known about the distribution of receptors for GH and for insulin-like growth factor I (IGF-I), the main mediator of its actions. Both GH and IGF-I receptors have been localized in the brain, including the hypothalamus, the anterior pituitary, the ovaries, and the testes (Prager et al. 1992, Tiong and Herington 1992, Minami et al. 1993, Edmondson et al. 1995, Jones and Clemmons 1995). Understanding of the precise mechanisms of GH actions at each of its known or potential targets is complicated by the likely existence of direct actions mediated by GH receptors, actions of systemic IGF-I primarily of hepatic origin, and actions of locally produced IGF-I. Importance of IGF-I in the local (paracrine) control of both testicular and ovarian functions is very well documented and review of this subject is outside the scope of this chapter. However, it should be noted that regulation of IGF-I production in the gonads involves factors

other than GH and therefore it is presently unclear to what extent IGF-I signaling within the ovary or the testis may be related to direct or indirect GH actions on the gonads. Most likely, both GH-dependent and GH-independent alterations in IGF-I biosynthesis and action are involved in the control of ovarian and testicular steroidogenesis, follicle growth and selection, and sperm production. It should also be indicated that GH could influence the actions of both systemic and locally produced IGF-I by altering the biosynthesis of IGF-I binding proteins as well as proteolytic enzymes involved in their degradation.

## **CURRENT AND POTENTIAL APPLICATIONS OF GH IN AGRICULTURE AND MEDICINE**

Many of the diverse actions of GH, including stimulation of linear growth, anabolic and lipolytic activity, enhancement of lactation, stimulation of immune function, and effects on bone mineralization, are potentially beneficial in both agriculture and medicine. Cloning of the GH genes and the ability to produce virtually unlimited quantities of biologically active GH by recombinant technology created opportunity for practical use of this material. Recombinant bovine GH is currently marketed in the United States for the purpose of increasing milk production in dairy cattle, and there is considerable evidence that GH treatment can markedly improve milk yield in cattle, sheep, and goats (Bauman and Vernon 1993). Detailed studies in swine provided evidence that injections of GH can improve food utilization and carcass composition by affecting nutrient partitioning to promote increase of lean body mass and to reduce fat deposition (Campbell et al. 1988). The obvious potential of these actions for reducing the cost of meat production while simultaneously improving its quality suggests that recombinant porcine GH will soon find practical use in the swine industry.

Medical usage of recombinant hGH already extends beyond replacement therapy in congenitally GH-deficient children to include GH deficient adults and some endocrinologically normal children with projected short stature. While both effec-

tiveness and ethical aspects of GH treatment in the latter group are being actively debated, other categories of patients are being considered as potential candidates for GH therapy. These include individuals with immunodeficiency, including AIDS, obese individuals, and the elderly of both genders. Results of experimental studies conducted to date indicate that GH can reduce the rate of bone demineralization in post menopausal women (Marcus et al. 1993) and improve muscle strength and general well-being while reducing adiposity in elderly men (Rudman et al. 1990). It is also believed that hGH is already included in the list of hormones available on the illicit market for use by athletes and body builders, although available evidence argues against its ability to improve muscle strength (Ohlsson and Jennische 1995). The well documented lipolytic and anabolic actions of GH and its potential to inhibit or reverse some of the correlates of normal aging suggest a possibility of wide-spread use of long term treatment with GH in essentially healthy individuals.

The increasing usage and the considerable potential for novel applications of GH in both agriculture and medicine raise an issue of possible side effects of long term exposure to elevated levels of GH in peripheral circulation. It is in this context that we decided to use transgenic mice to examine the effects of life-long exposure to very high GH levels on fertility and on parameters of neuroendocrine function related to reproduction. We were also interested in determining whether any of the effects of GH identified in the course of these studies may correspond to the physiological actions in normal animals. To address this issue, we have examined the effects of immunoneutralization of GH and of congenital GH deficiency on neuroendocrine control of gonadal function.

## **ORIGIN AND CHARACTERISTICS OF TRANSGENIC MICE**

Transgenic mice used in the present study were derived from animals kindly provided by Dr. Thomas E. Wagner and Ms. Jeung S. Yun of the Edison

Animal Biotechnology Center, Ohio University, Athens, OH. The founders of each of the lines were produced by microinjection of hybrid genes consisting of a promoter region of mouse metallothionein-I (MT) or rat phosphoenolpyruvate carboxykinase (PEPCK) gene and the coding region of the bovine GH (bGH), human pituitary GH (hGH), or human placental hGH variant (hGH-V) genes into male pronuclei of recently fertilized mouse eggs (Selden et al. 1988, Selden et al. 1989, McGrane et al. 1990). Transgenic animals in each of the resulting lines were expressing heterologous GH at multiple ectopic sites, consistent with the characteristics of the promoter, and exhibited the expected growth enhancement phenotype with an increase of adult body weight ranging from approximately 30% to approximately 100% depending on the hybrid gene employed, magnitude of expression, gender, and age. In our laboratory, hemizygous transgenic males were bred to normal C57BL/6J x C3HJ F<sub>1</sub> females. This mating system produces both hemizygous transgenic and normal (non-transgenic) animals and thus provides us with a supply of normal controls matched with transgenic animals in every respect, including the intrauterine environment. In most lines, the proportion of transgenic animals does not depart from the 1:1 ratio expected for the Mendelian transmission of a dominant mutation. In several lines, there is a significant shortage of transgenic animals for reasons which we were unable to identify but which apparently do not include differential mortality of transgenic and normal progeny (Naar et al. 1991).

In comparison to their normal siblings, transgenic animals from each of the lines exhibit enhanced and prolonged post weaning growth with a significant increase in adult body weight, along with various degrees of alterations in head shape, length of extremities, and pelage characteristics. Other characteristics of transgenic mice include splanchnomegaly with particularly striking increase in the weight of the liver (Shea et al. 1987, Bartke et al. 1992, Cecim et al. 1993), increased plasma corticosterone levels (Cecim et al. 1991), and suppression of *in situ* somatotroph function (Sotelo et al. 1993). The life-span of transgenic

mice is reduced roughly in proportion to the increase in plasma GH levels with expression of hGH or hGH-V being more detrimental to long term survival than the expression of bGH (Cecim et al. 1994). In the case of the females, differential effects of hGH vs. bGH on survival are related to early appearance of mammary tumors in almost all females expressing hGH or hGH-V but not in those expressing bGH (Cecim et al. 1994). Additional characteristics of transgenic mice were determined only in animals from one or a few of the available lines. However, on the basis of findings from other laboratories and related studies, they are assumed to represent universal characteristics of transgenic mice with ectopic overexpression of heterologous GH. These included elevation of peripheral IGF-I levels (Naar et al. 1991), increased plasma ACTH (Cecim et al. 1996), increase in plasma GH binding proteins (Sotelo et al. 1995), increased hepatic GH and PRL receptors (Orian et al. 1991, Turyn et al. 1993), increased plasma insulin levels, insulin resistance, and down-regulation of hepatic insulin receptors (Balbis et al. 1992). However, transgenic mice overexpressing GH are, in general, normoglycemic (Balbis et al. 1992, Bartke et al. 1992).

In each line of transgenic mice which we had opportunity to examine, we found evidence for reproductive deficits ranging from increased intervals between the litters and slight increases in the incidence of stillbirths to complete sterility in one of the sexes (Bartke et al. 1988, Naar et al. 1991). The reproductive deficits were generally more pronounced in females, although in the line expressing the hGH-V gene approximately 80% of females can reproduce while half or more of the males are sterile (Naar et al. 1991, Bartke et al. 1992). Female infertility is related primarily to abnormalities in the control of pituitary PRL release and the resulting luteal failure (Bartke et al. 1988, Cecim et al. 1995a,b,c) while male infertility appears to be due to suppression or alterations of sexual behavior.

Shortening of the reproductive life span appears to be a universal characteristic of these animals and is especially pronounced in animals expressing high levels of hGH or bGH.

## IMPACT OF CHRONIC GH EXCESS ON NEUROENDOCRINE FUNCTION RELATED TO FERTILITY

In an attempt to identify the sites of GH action on the hypothalamic-pituitary-gonadal axis and the GH-induced changes that may be responsible for reproductive deficits, we have examined plasma gonadotropin and PRL levels as well as indices of catecholaminergic transmission in hypothalami of transgenic and normal mice. To eliminate fluctuations related to the estrous cycle, all females were ovariectomized one week before these studies. The animals were injected with  $\alpha$ -methyl paratyrosine ( $\alpha$ -MPT), an inhibitor of tyrosine hydroxylase, and turnover of dopamine (DA) and norepinephrine (NE) in the median eminence was calculated from the rate of depletion of these neurotransmitters during a 60 min period following  $\alpha$ -MPT injection. This protocol allowed also evaluation of acute changes in plasma PRL levels in response to blockade of inhibitory DA input. Indices of serotonergic transmission, neurokinin levels, and turnover of DA and NE in brain regions other than the median eminence were evaluated in only a few of the lines and will not be discussed here.

Our findings concerning plasma LH, FSH, and PRL levels, PRL responses to  $\alpha$ -MPT, as well as DA and NE turnover in the median eminence are summarized in Table I. With respect to plasma hormone levels, the main findings included: (1) consistent and major elevation of LH in MT-hGH males (Chandrashekar et al. 1988) which resembles the effects of experimentally-induced hyperprolactinemia in mice (Klemcke and Bartke 1981) and is believed to be due to lactogenic activity of hGH, (2) suppression of FSH levels which is particularly obvious in PEPCK-bGH animals (Steger et al. 1994), (3) generally opposite changes in plasma PRL levels in females expressing bGH vs. those expressing hGH (suppression in MT-hGH, stimulation in MT-bGH and stimulation or no change in other lines expressing bGH), and instances of the corre-

sponding opposite changes in DA turnover (MT-hGH females vs. MT-bGH females, Bartke et al. 1988, Steger et al. 1991), (4) attenuated PRL responses to  $\alpha$ -MPT in transgenic animals (Bartke et al. 1988, Steger et al. 1991, 1994), which suggests an abnormality in DA control of the release of this hormone. With respect to the ability of hGH and bGH expression to lead to opposite changes in plasma PRL levels, we would like to mention a recent study in which expression of the estrogen receptor (ER) mRNA and the levels of ER protein were significantly increased in MT-bGH females and significantly reduced in MT-hGH females, while the levels of D2 DA receptor mRNA were suppressed in MT-bGH and stimulated in MT-hGH animals (Vidal et al. 1996). These findings are in excellent agreement with the results of measurements of PRL levels in the plasma and DA turnover in the median eminence (Bartke et al. 1988, Steger et al. 1991, Table I), and with morphological studies of lactotrophs in these animals (Stefaneanu et al. 1990, 1993). Thus we can conclude that hGH expression suppresses lactotroph function as expected from lactogenic activity of hGH and autofeedback effects of stimulation of PRL receptors on tuberoinfundibular dopaminergic neurons while the purely somatogenic bGH exerts a previously unknown stimulatory action on lactotrophs which appears to be due to reduced DA input. It is of interest that congenital isolated GH deficiency in two types of mutant rats is associated with reduced PRL content in the pituitary (Charlton et al. 1988, Nogami and Takeuchi 1993). In one of these mutants, plasma PRL levels, PRL mRNA and the number of lactotrophs are also reduced (Nogami and Takeuchi 1993).

## EFFECTS OF CONGENITAL OR EXPERIMENTALLY-INDUCED GH DEFICIENCY ON THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS

Data obtained in transgenic mice and discussed in the preceding sections of this article indicate that

TABLE I

Effects of expression of bovine (b) or human (h) GH in transgenic mice on plasma LH, FSH, and PRL levels, acute PRL responses to pharmacological blockade of tyrosine hydroxylase (TH), and turnover of norepinephrine (NE) and dopamine (DA) in the median eminence. Statistically significant increases and decreases are denoted by arrows pointing upward and downward, respectively

Line	Plasma levels of heterologous GH	Plasma Levels			PRL response to blocking TH <sup>xx</sup>	Median eminence turnover	
males	(ng/ml)	LH	FSH	PRL		NE	DA
MT-bGH	18 ± 4 <sup>x</sup>	-	-	-	↓	↑	-
MT-bGH-20	~400	-	NM	-	↓	-	-
PEPCK-bGH-1	810 ± 51	-	↓	-	-	↑	-
PEPCK-bGH-5	2478 ± 445	-	↓	↑	↓	↑	↑
PEPCK-bGH-25	1280 ± 103	-	-	↑	↓	↑	-
MT-hGH	<10	↑	-	↓	-	↑	-
PEPCK-hGH	491 ± 55	-	NM	↓	↓	↓	-
MT-hGH-V	NM	-	NM	-	-	↑	↓
ovariectomized females							
MT-bGH	93 ± 5 <sup>x</sup>	↓	↓	↑	↓	↓	↓
MT-bGH-20	~400	-	NM	-	↓	↓	-
PEPCK-bGH-1	744 ± 51	-	↓	↑	↓	↓	NM
PEPCK-bGH-5	2369 ± 494	-	↓	↑	↓	↑	-
PEPCK-bGH-25	1039 ± 87	↓	↓	-	-	↓	-
MT-hGH	<20	↓	↓	↓	↓	-	↑
PEPCK-hGH	430 ± 26*	↓	NM	-	↓	↓	-
MT-hGH-V	NM	↓	↓	-	-	↓	↑

NM, not measured; data derived from Bartke et al. 1988, Chandrashekar et al. 1988, Chandrashekar and Bartke 1993, Steger et al. 1991, 1993, 1994 and unpublished studies. <sup>x</sup>bGH levels in males and females from this line were measured using different RIA systems; <sup>xx</sup>a reduction is noted if  $\alpha$ -MPT failed to increase PRL level in transgenics or if % increase in transgenic was less than 50% of the increase measured in the matching normal animals; \*data from intact females.

life-long exposure to excessive levels of GH affects fertility as well as neuroendocrine functions related to reproduction in both males and females. Functional changes associated with chronic GH excess in these animals included alterations in hypothalamic neurotransmitters and in the regulation of adenohypophyseal function. These findings, along with results obtained by other workers, suggested a possibility that physiological amounts of endogenously produced GH may be normally involved in modulating the hypothalamic control of gonadotropin release. To address this issue, we examined various indices of function of the hypothalamic-pi-

uitary-gonadal axis in animals with hereditary or experimentally-induced GH deficiency.

In Ames dwarf mice, GH is not secreted due to genetically determined abnormalities in the appearance and proliferation of several cell lineages in the anterior pituitary during fetal development and the resulting absence of somatotrophs (Bartke 1979, Li et al. 1990). In these animals, plasma gonadotropin levels are significantly reduced (Chandrashekar and Bartke 1993). In male dwarfs, this is accompanied by reduced LH and testosterone responses to acute LHRH stimulation (in terms of the absolute amounts of hormone released), reduced LH respon-

ses to gonadectomy, more pronounced decline in plasma LH levels after testosterone administration, as well as reduced accumulation of androstenedione and testosterone in the incubations of testicular tissue in the presence of hCG (Chandrashekar and Bartke 1993). Treatment of male Ames dwarf mice with bGH reversed or significantly attenuated each of these abnormalities (Chandrashekar and Bartke 1993). Similar results were obtained in a subsequent study in female dwarf mice (Chandrashekar and Bartke 1996).

These observations imply that GH is normally involved in the control of hypothalamic, pituitary and testicular function. Physiological levels of GH may be necessary for the maintenance of normal levels of LH in the circulation and for full responsiveness of LH release to both stimulatory hypothalamic inputs and inhibitory influences of gonadal steroids. Because Ames dwarf mice, in addition to being GH-deficient, are also PRL deficient and hypothyroid (Bartke 1979), and various aspects of the control of gonadotropin release can be very different in different species of rodents, we decided to expand these studies to a different animal model.

Active immunization of adult male rats with repeated administration of oGH together with Freund's adjuvant (complete for the initial immunization and incomplete for the booster injections) was used to produce selective immunoneutralization of endogenous GH (Chandrashekar and Bartke 1995a,b). Presence of GH antibodies in the immunized animals was associated with a significant increase in plasma levels of both LH and FSH. When these animals were injected with a single dose of LHRH, plasma FSH and LH levels measured 15 min later were significantly higher in rats immunized with GH than in rats injected with adjuvant alone. However, the percent increase in LH levels in GH immunized rats in response to GnRH was decreased. The basal testosterone levels were not affected by GH immunization. Stimulation of LH release following GnRH treatment significantly increased plasma testosterone levels in control rats, while identical treatment of GH-immunized animals failed to alter plasma testosterone concentrations. These results indicate that neutralization of the bi-

ological activity of endogenous GH alters pituitary function and impairs the action of LH on Leydig cell steroidogenesis.

It is of interest that GH deficiency was associated with opposite effects on plasma gonadotropin levels in the two species examined, namely suppression in the mouse and stimulation in the rat. This is reminiscent of what is known about the effects of a closely related hormone, PRL, on the control of gonadotropin release in male rats and mice (Bartke et al. 1977, Klemcke and Bartke 1981).

## SUMMARY AND CONCLUSIONS

Results of our studies, including those described in this chapter, lead to several conclusions concerning effects of GH on neuroendocrine function related to reproduction. Thus (1) long term changes in peripheral GH levels are associated with alterations in the activity of hypothalamic catecholaminergic neurons which are believed to control the release of gonadotropins and PRL; (2) both GH deficiency and GH excess are associated with abnormalities in gonadotropin levels and feedback control of LH release; (3) in female mice, chronic elevation of plasma GH levels can alter the "basal" PRL release and suppress mating-induced surges of PRL which are necessary for maintenance of luteal function in this species; (4) in males, overexpression of GH can lead to reduced fertility, presumably *via* inhibitory influences on copulatory behavior; and (5) life-long GH excess dramatically shortens reproductive life span. Evidence available to date does not allow us to determine whether the effects of GH listed above are due to actions of GH itself within the hypothalamus and/or the pituitary or to GH-induced elevations in systemic or local IGF-I levels. Further studies will be necessary to distinguish between these possibilities, to identify the cellular targets of GH (or IGF-I) in the central nervous system and to elucidate the mechanism(s) of action involved.

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