MINIREVIEW

Neuroendocrine and Reproductive Consequences of Overexpression of Growth Hormone in Transgenic Mice (43771)

ANDRZEJ BARTKE,*,[1] MARCELO CECIM,* KECHUN TANG,* RICHARD W. STEGER,* VARADARAJ CHANDRASHEKAR,* AND DANIEL TURYN†

Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, Illinois 62901–6512 and Instituto de Quimica y Fisicoquimica Biologicas (UBA-CONICET),† Junin 956, 1113 Buenos Aires, Argentina*

Abstract. **Availability of recombinant growth hormone (GH) and development of long-acting formulations of this material will undoubtedly lead to widespread use of GH in animal industry and in medicine. GH can act, directly or indirectly, on multiple targets, but its influence on the reproductive system and on the hormonal control of reproduction is poorly understood. Overexpression of GH genes in transgenic animals provides a unique opportunity to study the effects of long-term GH excess. Transgenic mice overexpressing bovine, ovine, or rat GH (hormones with actions closely resembling, if not identical to, those of endogenous [mouse] GH), exhibit enhancement of growth, increased adult body size, and reduced life-span as well as a number of endocrine and reproductive abnormalities. Ectopic overexpression of bovine GH (bGH) driven by metallothionein or phosphoenolpyruvate carboxykinase promoters is associated with altered activity of hypothalamic neurons which produce somatostatin, loss of adenohypophyseal GH releasing hormone (GHRH) receptors, and suppression of endogenous (mouse) GH release. Elevation of plasma levels of GH (primarily bGH) and insulin-like growth factor (IGF-I) in these transgenic mice leads to increases in the number of hepatic GH and prolactin (PRL) receptors, in the serum levels of GH-binding protein (GHBP), in the percent of GHBP complexed with GH, and in the circulating insulin levels. In addition, plasma adrenocorticotropic hormone (ACTH) and corticosterone levels are elevated. Plasma levels of luteinizing hormone (LH), as well as its synthesis and release, are not consistently affected, but follicle-stimulating hormone (FSH) levels are suppressed, apparently due to pre- and post-translational effects. Pituitary lactotrophs exhibit characteristics of chronic enhancement of secretory activity, and plasma PRL levels are elevated. Prolactin responses to mating or to pharmacological blockade of dopamine synthesis are abnormal. Reproductive life span and efficiency are reduced in both sexes, with the severity and frequency of reproductive deficits being related to plasma bGH levels. Most transgenic females expressing high levels of bGH are sterile due to luteal failure.**

Overexpression of human GH which, in the mouse, interacts with both GH and PRL receptors leads to additional endocrine and reproductive abnormalities including stimulation of LHβ mRNA levels and LH secretion, loss of responsiveness to testosterone feedback, overstimulation of mammary glands, enhanced mammary tumorigenesis, and hypertrophy of accessory reproductive glands in males.

Results of these studies indicate that GH can exert a variety of direct and indirect actions at the hypothalamic, pituitary, gonadal, and reproductive tract levels, and that the consequences of prolonged exposure to supraphysiological levels of GH cannot always be predicted from the known or the presumed physiological actions of this hormone. [P.S.E.B.M. 1994, Vol 206]

[1] To whom requests for reprints should be addressed at Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901–6512.

0037-9727/94/2064–0345$10.50/0

Indirect evidence for the ability of growth hormone (GH) to influence reproductive development and function has been available for over sixty years. GH-deficient mutant mice were described by Snell in 1929 (1) and found to be sterile in spite of the presence of apparently normal gonadotrophs in the pituitary (2, 3). Common occurrence of amenorrhea in acromegalic women with abnormally elevated plasma GH levels has been well documented for many years (4, 5). More recent data provided evidence for a role of GH in pubertal development in children (6, 7) and in experimental animals, including nonhuman primates (8–10). Growth hormone is also believed to be involved in compensatory testicular growth after hemicastration (11). However, mechanism(s) of GH action on sexual development and adult reproductive functions are poorly understood. Moreover, the influence of GH on the synthesis and release of pituitary hormones related to reproduction remained unexplored until very recently.

Technological advances of the last decade greatly increased interest in the regulation and actions of GH and its practical application. Availability of recombinant human GH (hGH) provided, for the first time, ample supply of this material for treatment of hypopituitary children and produced considerable interest in other medical uses of this hormone. This includes treatment of endocrinologically normal children with short stature, as well as obese and convalescent adults and elderly subjects in which hGH may have a considerable potential for retarding or even reversing age-related changes in bone mineralization, muscle strength, and body composition (12–14). Recombinant bovine GH (bGH, or BST for bovine somatotropin) has been formulated for prolonged action which allows relatively infrequent "depot" injections and was recently approved in this country by the Food and Drug Administration for enhancing milk yield in dairy cows. Recombinant GH is also believed to have tremendous potential in the meat industry by promoting growth, feed efficiency, and production of lean body mass (15, 16).

In addition to the recent as well as the contemplated applications of recombinant GH, a number of laboratories are exploring the possibility of manipulating growth and other economically important traits of livestock by altering the expression of GH genes using transgenic technology (17, 18). The availability of recombinant GH and long-acting somatostatin analogs suitable for suppression of normal or pathologically elevated GH levels led to an enormous increase in the interest in GH and an explosive expansion of information on GH actions, regulation of GH release, GH receptors, GH-binding proteins, mediators of GH effects, and structure-function relationships of the GH molecules.

It was against this background that we decided to utilize transgenic mice overexpressing GH to study the effects of prolonged elevation of peripheral GH levels on reproductive functions and on neuroendocrine regulation of the reproductive system. In this review, we will briefly characterize this animal model, summarize and discuss our findings as well as those reported by other workers, and attempt to relate data obtained in transgenic animals to the actions of physiologically and pathologically elevated amounts of endogenous GH.

Transgenic Animals Overexpressing GH

Reports by Palmiter, Brinster, and their collaborators (19, 20) that introduction of heterologous GH genes fused with metallothionein promoter into mouse embyros can lead to a striking increase in adult body size of the resulting animals should be considered as a milestone in biomedical research. These findings provided dramatic demonstration of the power of transgenic technology and the potential for producing novel animal models by means of gene transfer. Pioneering studies of this group (20–22) and of the group led by Wagner (23, 24) provided evidence that chronic expression of rat (r), bovine (b), or human (h) GH, or of the human placental GH variant (hGH · V) in transgenic mice can be driven by mouse metallothionein-1 (MT) or by rat phosphoenolpyruvate carboxykinase (PEPCK) promoter. It was also shown that mice resulting from injection of the corresponding gene constructs into male pronuclei of one cell embryos can transmit the transfected gene to their progeny and therefore can be used to establish stable lines of transgenic animals. Transgenic MT-GH mice express foreign GH in multiple organs and cell types including liver, kidney, intestines, skin, gonads, and adenohypophyseal gonadotropes (19, 20, 25) with expression starting during fetal development and continuing throughout the entire lifespan (19–22, 24). In PEPCK-GH animals, the expression of the introduced GH gene starts around the time of birth and appears to be limited to the kidney, liver, and adipose tissue (23). With both types of constructs, the expression of heterologous GH is driven by the promoter sequences used to produce the hybrid gene for intranuclear injection (MT or PEPCK) and therefore is not subjected to the control mechanisms which normally regulate synthesis and release of GH; i.e., GH-releasing hormone (GHRH), somatostatin (somatotropin release-inhibiting factor, SRIF) and other products of hypothalamic catecholaminergic and peptidergic neurons. Instead, the expression of transfected GH genes is regulated by factors normally influencing the corresponding promoters; i.e., heavy metal ions in case of MT and diet composition in case of PEPCK. This can be utilized to enhance the expression of MT-GH genes by

providing a 25 m*M* solution of $ZnSO_4$ in drinking water (26), while the expression of PEPCK-GH genes can be reduced by high-carbohydrate diet and stimulated by high-protein, carbohydrate-deficient diet (23). It was also documented that expression of the MT-hGH gene can be stimulated by glucocorticoid administration (27).

The use of mutated ovine MT (oMT) promoter resulted in the production of transgenic mice in which the heterologous (in this case, ovine) GH is not expressed in detectable amounts in animals maintained on a standard diet of laboratory mouse formula and tap water, but can be stimulated by providing $ZnSO_4$ (26, 28). This allows the experimenter to "turn on" and "turn off" transgene expression at any stage of postweaning life by simply providing or withholding $ZnSO_4$ solution as a source of liquid.

The incorporation of microinjected hybrid genes into the genome of developing embryos appears to be random, generally with either one or multiple gene copies being incorporated at one chromosomal site. The levels of transgene expression (as measured by plasma levels of heterologous GH) can vary enormously between different transgenic animals resulting from injection of the same gene construct into a batch of genetically uniform (usually F_1) embryos (19, 23, 26) but appears to be unrelated to the number of gene copies incorporated (29). Interestingly, comparison of hemizygous MT-hGH mice with homozygotes produced by ovarian transplantation revealed significantly greater hGH expression in homozygous animals in which the transgene is present in both maternally and paternally-derived chromosomes (30).

In lines derived from crossing a hemizygous transgenic "founder" animal with normal animals and subsequent crosses of the transgenic progeny to normal mice, the level of heterologous GH expression is incomparably less variable than among different founders and is apparently stable from generation to generation. This allows for the production of an essentially unlimited supply of hemizygous transgenic animals for studies of the consequences of transgene expression, while their nontransgenic siblings can serve as normal controls matched with respect to maternal influences, husbandry conditions, and genetic background.

Ectopic expression of heterologous GH driven by either MT or PEPCK promoters leads to enhancement of postweaning growth with adult weight exceeding body weight of normal siblings of the same sex by as much as 50%–100%. Analysis of ontogeny of alterations in body weight and in the weight of various organs in transgenic MT-rGH mice revealed existence of differential patterns of growth (31). Thus, some organs (e.g., heart and kidney) increase in weight allometrically (i.e., in proportion to increase in body weight), while others exhibit either a much greater increase (e.g., liver and spleen) or little if any change (e.g., brain and testes). Subsequent studies in a line of MT-bGH transgenic mice (32) produced very similar observations. Although splanchnomegaly may contribute to the increase in adult body weight, overexpression of GH clearly stimulates true growth, including bone growth, as evidenced by increases in the length of the tail, the extremities, and in the total body length. On the basis of studies of IGF-I expression in transgenic mice overexpressing GH (33) and known relationships between GH, hepatic production of IGF-I, and growth, it was proposed that stimulation of growth in MT-GH transgenic mice is related to GH-induced increase in peripheral IGF-I levels. However, some of the phenotypic characteristics of MT-GH transgenic mice, including increased skeletal growth, hepatomegaly, and histopathologic changes in the liver and the kidneys were not reproduced in transgenic animals expressing hIGF-I with the same promoter (34, 35) and thus are presumably due to direct effect of GH at various targets or to GH-mediated changes in local production of IGF-I or other growth factors.

Overexpression of hGHRH in transgenic mice led to pituitary enlargement, chronic overstimulation of endogenous production of mouse GH (mGH), and changes in growth, adult body size, and histopathology of the liver, which were essentially identical to previously documented phenotypic effects of overexpression of bGH in MT-bGH transgenic mice (35, 36). This would suggest that, at least with respect to the examined characteristics, ectopic expression of heterologous GH produces effects which are biologically equivalent to the consequences of excessive production of the animal's own GH by the pituitary. These observations, together with the documented specificity of bGH binding to mouse growth hormone receptor (GH · R) (37, 38), lead us to believe that effects of bGH expression in transgenic mice provide meaningful information on the physiological consequences of excessive GH production.

As expected from the well-documented feedback control of GH release, persistent ectopic production of GH and elevation of peripheral GH levels in transgenic animals leads to suppression of endogenous GH release (39). Palmiter *et al.* (20) reported reduction in the number of acidophils in the pituitaries of MT-hGH transgenic mice. Stefaneanu, Kovacs and their colleagues (25) provided morphological evidence for reduced secretory activity of somatotrophs in the anterior pituitaries of MT-hGH transgenic mice from another pedigree. In comparison to GH-producing cells of their normal siblings, the somatotrophs of transgenic mice were characterized by reductions in cytoplasmic and nuclear volume as well as in the size and number of secretory granules. In addition, the total

number of somatotrophs was severely reduced as indicated by decreases in their relative frequency and in the size of the anterior lobe of the pituitary. However, low amount of GH message could be detected in the somatotrophs, indicating continued expression of the endogenous GH gene (40). Similar changes were detected in the pituitaries of transgenic MT-bGH mice (41). Aguilar *et al.* (39) provided evidence that overexpression of bGH in transgenic mice is associated with reductions in the content of mGH and in the number of GHRH receptors in the pituitary. Comparison of transgenic animals from lines with different levels of bGH expression suggested that the severity of these changes is related to the concentration of heterologous GH in the circulation.

We suspect that reduced binding of GHRH in the pituitaries of transgenic mice is not only a reflection of reductions in the number and size of somatotrophs but also a consequence of reduced release of GHRH into the portal blood supply of the pituitary. In support of this interpretation, Hurley and Phelps (42) demonstrated reduction in hypothalamic GHRH levels in transgenic MT-hGHRH mice in which the levels of endogenous GH are markedly increased. In contrast, SRIF expression was increased in these animals and in transgenic bGH or hGH mice (43 and unpublished observations).

Chronic elevation of GH concentration in the circulation of transgenic mice is associated with marked increases in the number of hepatic GH and PRL receptors, the concentration of GHBP in serum, the amount of bound GH, and the proportion of bound GHBP (44, 45, and unpublished data). The latter changes lead to a massive increase in the levels of GHBP-GH complexes in the circulation. The half-life of GH in transgenic mice is prolonged as indicated by a significant increase in the mean residence time of injected labeled GH (45 and unpublished data).

The success of several laboratories in establishing lines of transgenic mice was mentioned earlier in this article and obviously implies that ectopic expression of heterologous GH genes can be compatible with fertility in at least one gender. However, Hammer *et al.* (21) reported that transgenic MT-hGH females do not reproduce. Moreover, the introduction of the MT-hGH gene into GH-deficient little (lit/lit) mice produced normal growth but rendered females sterile (46). Yun and Wagner described female sterility in a line of transgenic MT-hGH mice they produced (47) and provided elegant evidence that this was not due to inability to produce ova or to defects in fertilizability of oocytes. When ovaries were transplanted from transgenic to normal females, the transplant recipients were fertile and could transmit the transgene to their progeny (47). Our studies of reproductive and neuroendocrine functions in transgenic animals expressing various GH genes stem from these observations, and were made possible by the kindness and cooperation of Dr. Thomas E. Wagner and Jeung S. Yun who provided us with transgenic mice from their various lines for the establishment of a breeding colony at our institution.

Reproductive Functions in Transgenic Mice Expressing Human or Bovine GH Genes

Female Fertility. In an attempt to elucidate the cause(s) of sterility in MT-hGH females, we examined estrous cycle, incidence of mating, and effects of hormone replacement therapy in transgenic females from a line studied by Yun and Wagner (47). The results indicated that these animals exhibit slightly but significantly prolonged vaginal cycle, mate but fail to become pregnant or pseudopregnant. However, pregnancy can be established and maintained to term if the animals are treated with daily injections of progesterone or with PRL-secreting ectopic transplants of pituitaries from normal mice (48). These results were interpreted as evidence that sterility of MT-hGH transgenic mice is due to luteal failure which is caused by insufficient PRL secretion from the anterior pituitary. From the studies of plasma PRL levels and dopamine turnover in different regions of the hypothalamus in ovariectomized females and in intact males from the same line, we concluded that hGH stimulates the function of tuberoinfundibular dopaminergic neurons and thus exerts inhibitory influence on pituitary PRL release. Because this effect and the resulting sterility of transgenic MT-hGH females appears to be due to the well-documented lactogenic (PRL-like) action of hGH in the mouse (49, 50), we felt that it may represent an effect of functional hyperprolactinemia and thus provide few if any clues about the effects of GH excess on reproductive function. Therefore, we turned our attention to transgenic animals expressing bGH which is purely somatotropic in rodents (37, 38) and appears to mimic the actions of endogenous GH.

Most transgenic MT-bGH females from a line with relatively low transgene expression and plasma bGH levels of 5–20 ng/ml (39, 51, and unpublished data) are fertile, but their reproductive performance is inferior to that of their normal female siblings. Thus, transgenic MT-bGH females housed with a normal male require a longer interval to become pregnant, rarely mate in postpartum estrus, and never become pregnant from postpartum mating. This leads to a significant increase of intervals between birth of successive litters and reduced production of pups during a given time period (51 and unpublished data). Interestingly, the ability of female mice to conceive at postpartum mating was reported to be particularly sensitive to food restriction (52). Guthrie *et al.* (17) suggested that

GH overexposure in transgenic animals may mimic the effects of food restriction by antagonizing the action of insulin and interfering with glucose utilization. Although litter size in MT-bGH transgenic females is not significantly different from that in normal females, fetal mortality appears to be increased and fetal size on Day 14 of pregnancy is slightly but significantly reduced (51). In addition, preliminary data indicate that the age-related decline in fertility occurs earlier in transgenic females from this line than in their normal female siblings (unpublished observations).

In contrast to these findings, transgenic PEPCK-bGH females from lines with high levels of transgene expression and plasma bGH levels ranging from 200 to over 2,000 ng/ml (29, 51) exhibit severe reproductive impairments in spite of slight but significant advancement of sexual maturation. Although young PEPCK-bGH females have apparently normal vaginal cycle and can mate, pregnancy follows in only 20%–30% of these animals (51 and unpublished data). Since most of the sterile PEPCK-bGH females mate repeatedly without becoming pseudopregnant, we suspected that their infertility may be due to luteal failure. In support of this hypothesis, plasma progesterone levels on Day 2 and 7 after mating were significantly reduced in PEPCK-bGH transgenic as compared with normal females, and daily injections of progesterone to infertile transgenic females led to implantation of the embryos and pregnancy (Cecim and Bartke, unpublished data). Results of reciprocal transplantation of the ovaries between normal and transgenic females suggest that deficient luteal progesterone production by transgenic ovaries is due to systemic, presumably blood-borne factors, rather than to intrinsic defects in the ovary (Cecim, unpublished data). Studies of hypothalamic neurotransmitters and plasma levels of PRL at various times after mating, as well as results of twice daily PRL injections provided evidence that luteal failure in these animals is due to absence or alteration of PRL surges that normally follow mating, and that this endocrine defect is due to abnormal diurnal pattern of changes in the activity of tuberoinfundibular dopaminergic neurons (Cecim, unpublished data). Hypothalamic and adenophypophyseal function in transgenic PEPCK-bGH mice will be discussed in more detail in another section of this review.

In those transgenic PEPCK-bGH females that are fertile, the number of ova shed is significantly greater than in normal females from the same line, as evidenced by increased number of corpora lutea and implanted embryos on Day 7 and 14 of pregnancy (51 and unpublished data). We ascribe the increase in ovulation rate in transgenic females from these and other lines to GH-induced stimulation of IGF-I production and to the ability of IGF-I to augment the effects of gonadotropins on development and function of ovarian follicles (53, 54). Significant reduction in the proportion of atretic follicles in the ovaries of transgenic MT-bGH mice (55) suggests that GH, directly or indirectly, may interfere with mechanisms responsible for atresia and thus increase the number of ova shed during each ovulation.

Litter size in transgenic PEPCK-bGH females is normal at birth and normal or slightly reduced at weaning, suggesting increased fetal mortality during late gestation and/or perinatal period, and possibly also increased loss of pups during lactation.

In terms of reproductive efficiency and productivity during their life span, fertile transgenic PEPCK-bGH females are severely impaired. Compared with normal animals, they require a longer period of cohabitation with a male to conceive, never become pregnant from postpartum estrus, and have an extremely short reproductive life span with almost all animals being acyclic and sterile by the time they reach 6–8 months of age (51 and unpublished data).

Similarity of reproductive deficits in transgenic PEPCK-bGH females from three different lines derived from different founders with different number of gene copies (29) provides circumstantial but nevertheless persuasive evidence that the observed abnormalities are due to the actions of bGH rather than to insertional mutagenesis or other "nonspecific" consequences of transgene insertion and expression.

It can be concluded that life-long excess of purely somatogenic GH in transgenic mice leads to drastic suppression of female fertility. Most females with high plasma bGH levels are sterile, while some can produce one or more litters early in life but become sterile at the age at which their normal siblings are fertile and near the peak of their reproductive performance. Comparison of results obtained in MT-bGH and PEPCK-bGH females from the lines we have examined suggests that the effects of GH excess on female reproductive functions in transgenic mice are dose-related.

It is of interest that suppression of reproductive functions by overexpression of GH in transgenic females does not seem to require GH actions during embryonic or early postnatal development. PEPCK-bGH hybrid genes begin to be expressed perinatally. Pomp *et al.* (personal communication) examined reproductive functions in oMT-1a-oGH transgenic mice in which transgene expression was induced starting at the time of weaning and found deficits which were essentially identical to those described above for PEPCK-bGH transgenic females. Moreover, the endocrine mechanisms underlying suppression of fertility appear to be the same in PEPCK-bGH and oMT-1a-oGH transgenic mice (D. Pomp, personal communication). In addition, the effects of daily injections of

large doses of bGH into adult normal mice closely resembled some of the reproductive features of transgenic PEPCK-bGH females, including suppression of estrous cycle and increased number of ovulations in those animals that do ovulate and mate (Cecim and Bartke, unpublished data).

Male Fertility. Most transgenic male mice expressing heterologous GH genes are fertile. However, this cannot be taken as evidence that GH excess does not or cannot interfere with male reproductive functions. First of all, transgenic lines are usually established and maintained by mating transgenic males with normal females. Thus, only those transgenic animals that are fertile can become founders of a stable line. Therefore, our studies as well as data reported by other workers refer only to males from lines in which males with high peripheral GH levels can breed. Moreover, quantitative analysis of reproductive performance reveals significant differences between normal and transgenic males. In the course of propagating various lines of transgenic mice, we have noticed that the breeding performance of transgenic males was inferior to that of normal male mice in terms of the interval between pairing a male with a female and conception as well as in the frequency of males which fail to sire a litter after being housed with one or more females for a period ranging from one to three months. Moreover, the reproductive life span of transgenic males is reduced, particularly in lines characterized by high level of expression of heterologous GH. Since animals from these lines age prematurely and have a drastically reduced life span (details in other sections of this review), it is important to indicate that reproductive performance of these animals deteriorates long before any obvious signs of age-related decline in body condition. In a pilot study, we have shown that four transgenic MT-hGH breeders that were no longer fertile at the age of approximately 1 year were unable to intromit or ejaculate but were very willing to mount the females and had fertile sperm in the cauda epididymidis and the vas deferens (56).

So the validity of these mostly subjective impressions could be tested, a group of young adult transgenic males from each of five different lines along with normal (control) males were each housed for one week with three females and for a successive week with three different females. The females were subsequently examined for pregnancy and for the number of live and dead implantations versus the number of corpora lutea. The results provided evidence for reduced reproductive performance of males expressing hGH or hGH · V with MT promoter in terms of the proportion of males that sired any litters during this test and the number of litters sired per fertile male (57). Unexpectedly, no significant reproductive deficits were detected in PEPCK-hGH males in which plasma hGH levels are much higher than in the MT-hGH males (491 ± 55 vs 4.8 ± 0.4 ng/ml; 58 and Milton and Bartke, unpublished data). In transgenic males expressing high levels of bGH with the PEPCK promoter, fertility was reduced as evidenced by a modest but statistically significant decrease in the proportion of females that became pregnant after one week of cohabitation with a male (57).

Reproductive deficits in transgenic mice overexpressing GH do not appear to be due to suppression of spermatogenesis or androgen production by the testis. Quantitative assessment of daily sperm production and epididymal sperm reserves revealed no deficits in transgenic males from five different lines and several instances of a small but statistically significant increase in daily sperm production per testis and/or in epididymal sperm numbers (57 and unpublished data). Plasma testosterone levels and testicular testosterone production *in vitro* were normal in males from each of the lines we have examined (59, 60, and unpublished data), except PEPCK-hGH in which release of testosterone from incubated testes was significantly increased (Milton and Bartke, unpublished observations). Testosterone responses to LH or hCG stimulation *in vivo* and *in vitro* in PEPCK-bGH and MT-hGH transgenic males did not differ from those in their nontransgenic siblings (59, 60, and unpublished data).

We have therefore examined copulatory behavior of transgenic males and their normal siblings by recording the occurrence, latency and frequency of mounts, intromissions, and ejaculations during a 1-hr test with a sexually receptive, ovariectomized, steroid-primed female. The results revealed that transgenic MT-hGH · V and PEPCK-bGH males were less likely than their normal male siblings to mount, intromit, or ejaculate during the 1-hr period of observation and that their reproductive performance generally failed to show the expected improvement with repeated testing.

Transgenic MT-hGH and MT-hGH · V exhibit striking, age-related enlargement of the seminal vesicles and the coagulating glands which, unexpectedly, is associated with depletion of androgen receptors in the seminal vesicle (56, 61). In subsequent studies we have found that male accessory reproductive glands are disproportionately enlarged also in transgenic PEPCK-hGH males and that the weight of the ventral prostate is greatly increased in transgenic males overexpressing hGH or hGH · V (unpublished data). However, these changes are absent in animals overexpressing bGH and thus presumably are mediated via PRL rather than GH receptors, or in other words, should be considered as consequences of functional hyperprolactinemia rather than GH excess.

Lactation and Mammary Tumors. There is increasing evidence that the highly complex, multi-

hormonal control of mammary gland development and function involves actions of GH. Growth hormone receptors have been localized in the mammary gland in several species (62). Growth hormone can stimulate development and differentiation of the mammary gland in mice and rats (63, 64). Administration of recombinant GH results in marked stimulation of milk production in dairy cattle and other domestic ruminants (65, 66). Increased incidence of mammary tumors in transgenic mice overexpressing hGH (67) was interpreted as evidence that GH can promote mammary tumorigenesis in this species. Overexpression of bGH in transgenic female mice from the lines we had an opportunity to examine had no obvious effects on the gross morphology of the mammary glands or on lactation (unpublished data). Mortality of pubs between birth and weaning in MT-bGH and PEPCK-bGH transgenic females is not affected (51). In contrast, mammary glands of transgenic PEPCK-hGH females are obviously stimulated. Milk visibly accumulates in the distal portion of mammary ducts in virgin animals, and growth rate of both normal and transgenic pups is significantly increased throughout lactation (68). Moreover, adult virgin PEPCK-hGH transgenic females presented with foster pups undergo apparently normal lactation as evidenced by excellent survival and normal growth rate of the pups (68). We believe that stimulation of mammary development and function in these animals is due to the well known lactogenic (PRL-like) activity of hGH in mice (49, 50) and to the very high levels (approximately 450 ng/ml) of hGH in their circulation (68). While occurrence of apparently quantitatively normal lactation in virgin animals was not expected, we suspect that it may be related to luteotrophic effects of hGH leading to pseudopregnancy after each ovulation of transgenic females from this line (unpublished observations). Progesterone derived from the corpora lutea of pseudopregnancy may have provided adequate priming of the mammary gland to allow lactogenesis in spite of absence of hormonal changes associated with pregnancy (69).

Mammary tumors were present in 80%–90% of 10- to 12-month-old transgenic females expressing hGH or hGH · V with the MT promoter, and, sporadically, also in transgenic MT-hGH males (unpublished data). In transgenic PEPCK-hGH females, the incidence of mammary tumors was much lower, which may have been due to the fact that these animals have a very short life span with most of the females dying between the ages of 7 and 10 months (unpublished data). In contrast, very few MT-bGH transgenic females from the line we have examined developed mammary tumors, and they were detected only at the age of 20–24 months; i.e., at the time when mammary tumors begin to develop in the normal siblings (Cecim, unpublished data). Development of mammary tumors in normal (nontransgenic) females from these lines was not unexpected because approximately half of their genome is derived from highly tumor susceptible C3H strain. No significant increase in the incidence of mammary tumors was detected in transgenic PEPCK-bGH females from three different lines as compared with normal females, even though these animals have extremely high levels of plasma bGH (from approximately 200 to over 2,000 ng/ml; 29, 51) and live longer than PEPCK-hGH females (70 and unpublished data). From these findings we conclude that, while the highly lactogenic hGH exerts the expected stimulatory effects on mammary tumor development in susceptible mice, the purely somatogenic bGH fails to influence the incidence or the age of onset of mammary tumors even in animals exposed to very high levels of this hormone throughout the entire postnatal life.

Effects of GH on Neuroendocrine Function in Transgenic Animals

Life-long increase in plasma GH levels in transgenic animals overexpressing various GH genes and the consequent elevation of peripheral IGF-I levels are associated with numerous changes in neuroendocrine function. Plasma levels of corticosterone, the principal glucocorticoid in this species are significantly elevated under both basal and stress conditions (71). This effect is consistently observed in both sexes in each of the lines we have examined (71) and appears to be due to increased adrenocorticotropic hormone (ACTH) secretion rather than direct stimulatory effects of GH or IGF-I on the adrenal cortex (unpublished data). We suspect that the stimulation of ACTH release reflects GH-induced changes in the release of norepinephrine or other hypothalamic neurotransmitters, and the consequent elevation of corticotrophin-releasing factor (CRF) drive to the pituitary. However, reduced corticosterone feedback, due to its increased binding to plasma proteins, may also be involved.

Transgenic mice from the lines we have studied are normoglycemic with the exception of PEPCK-bGH-5 animals in which we found mild elevation of plasma glucose levels (57 and unpublished data). Data obtained from MT-bGH transgenic animals suggest that the somewhat surprising coexistence of normoglycemia with gross GH excess and persistent plasma corticosterone elevation may be due to significantly increased levels of insulin (72).

In an attempt to identify the mechanisms responsible for reproductive deficits in transgenic animals overexpressing GH, we have focused our attention on the study of plasma concentration of FSH, LH, and PRL, as well as the hypothalamic mechanisms involved in the control of their release. Because some of the effects of hGH on PRL and on gonadotropin re-

lease appear to be due to its lactogenic rather than somatotropic activity (59, 73, 74), only the effects of bGH will be discussed below.

In transgenic PEPCK-bGH males, steady state levels of LHβ and FSHβ mRNA in the pituitary are reduced (Fig. 1; 75). These alterations are significant when expressed per pituitary or per μg of pituitary DNA (75). In perifusions of the pituitaries from PEPCK-bGH males, basal LH release was increased while LH response to a LHRH pulse was attenuated in comparison with the pituitaries obtained from normal mice (75). Pituitary content of LH, basal and LHRH-stimulated LH release in static pituitary incubations, and plasma LH levels were not altered in transgenic PEPCK-bGH males (29, 75). Thus, it appears that the expression of the LHβ gene (to the extent that it can be estimated from the steady state levels of the message) and the LH storage and release are differentially regulated, possibly due to divergent effects of hGH and/or IGF-I at the pituitary and the hypothalamic levels.

In comparison with results obtained in pituitaries from normal mice, the release of FSH from incubated or perifused PEPCK-bGH pituitaries was increased, and the release of FSH from incubated pituitaries in response to LHRH was also augmented (75). However, the content of FSH in the pituitary and the plasma FSH levels were significantly lower in PEPCK-bGH than in nontransgenic males, in keeping with reduced levels of FSHβ mRNA in these animals (Fig. 1; 29, 75). Thus, similarly to the situation described for LH, our data revealed differential effects of bGH overexpression on FSH release *in vivo* and *in vitro*, with indications that both pre- and post-translational effects may be involved.

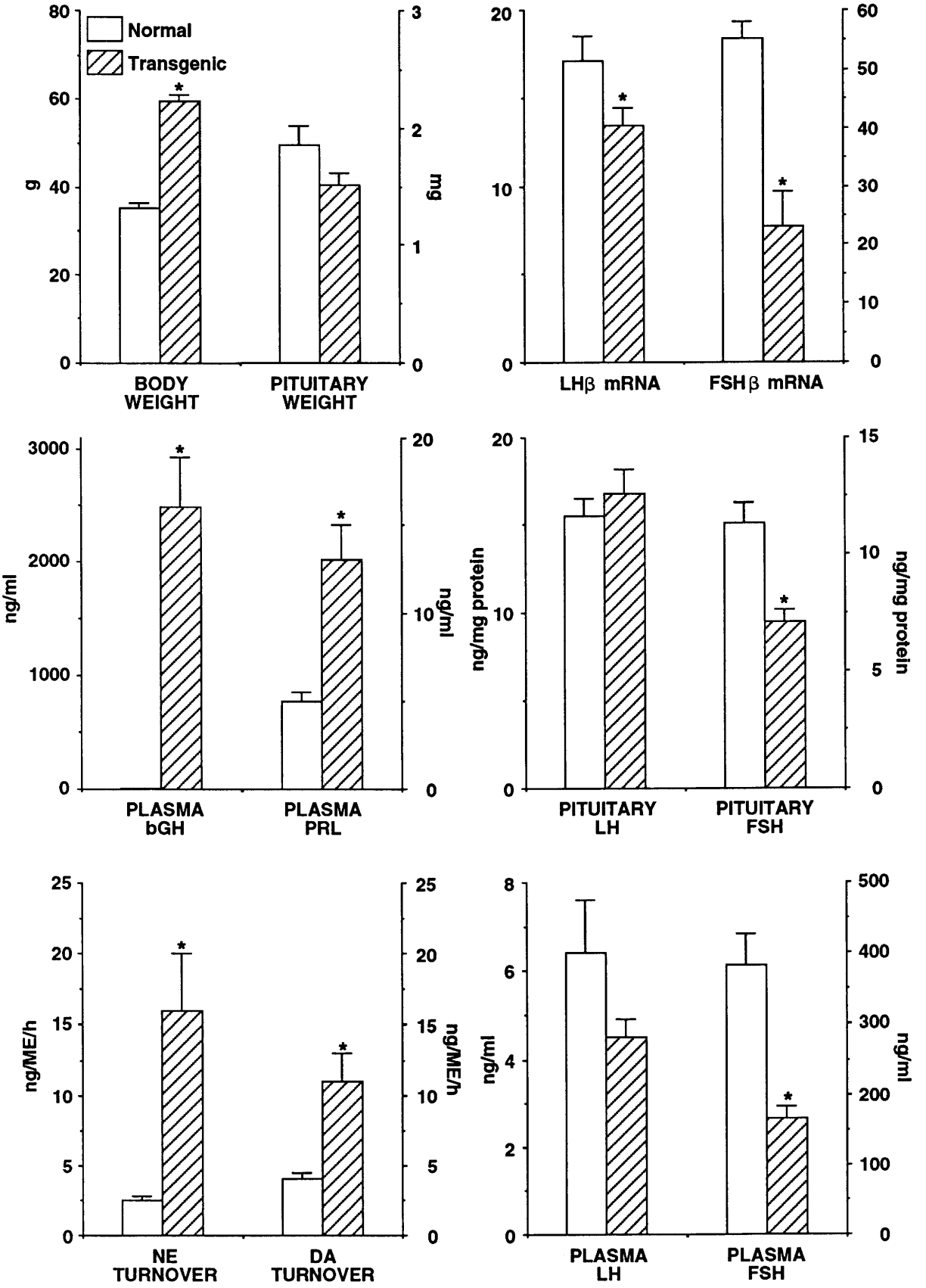
Studies of neurotransmitter function in the hypothalamus of PEPCK-bGH males revealed that in comparison with their normal siblings, transgenic males have significantly increased turnover of norepinephrine (NE) in the median eminence (Fig. 1; 29). However, the expected stimulation of NE turnover in this brain region in response to gonadectomy was severely attenuated in these transgenic animals (Fig. 2). Thus we can suspect that mechanisms responsible for altered gonadotropin synthesis, storage, and release in transgenic PEPCK-bGH males include changes in the noradrenergic input to LHRH-secreting neurons and in the regulation of noradrenergic transmission by testosterone feedback. These data also add support to our suggestion that overexpression of GH in transgenic animals influences adenohypophyseal function primarily by acting within the hypothalamus (70, 76, 77). However, effects of bGH and/or of the associated increase in plasma IGF-I at the pituitary levels may also be involved. In support of this possibility, normal pituitaries transplanted for 6 days under the renal capsules of transgenic PEPCK-bGH hosts had significantly greater ability to release LH and FSH *in vitro* than the pituitaries similarly transplanted into normal animals (75).

In a line of MT-bGH mice with low levels of bGH expression, alterations in FSHβ mRNA levels and in gonadotropin storage in transgenic males appeared to be qualitatively comparable to the changes observed in PEPCK-bGH animals but were numerically smaller and, in general, not statistically significant (75). However, in a group of middle-aged (30- to 35-week-old) MT-bGH males from the same line, plasma LH levels were significantly lower than those measured in normal males of the same age (78).

Studies of ovarian function in transgenic PEPCK-bGH females described earlier in this review suggested the presence of an essentially normal pattern of cyclic gonadotropin release in young adult animals and the premature cessation of ovulatory activity (51 and Cecim *et al.*, unpublished data). Preliminary data obtained after reciprocal transplantation of ovaries between normal and transgenic PEPCK-bGH females suggest that defects in ovarian function are due to the effects of bGH at the hypothalamic-pituitary rather than the ovarian level. The suspected suppression of cyclic gonadotropin release in middle-aged transgenic females overexpressing bGH is reminiscent of anovulatory syndromes described previously in acromegalic women (4, 5) and in transgenic pigs with bGH excess (17). In ovariectomized young adult PEPCK-bGH females from three different lines, plasma FSH levels were suppressed (29).

In 1990, we reported that plasma PRL levels were significantly increased in transgenic ovariectomized MT-bGH females as compared with their ovariectomized normal siblings (76). Reduced dopamine turnover in the median eminence of transgenic animals (76) provided a very plausible explanation for this novel effect of GH on adenohypophyseal function. Subsequent studies of Stefaneanu *et al.* (41) provided morphological evidence for marked stimulation of PRL storage and secretory activity of the lactotrophs in

Figure 1. Effects of overexpression of bovine growth hormone (bGH) in transgenic PEPCK-bGH-5 male mice on turnover rate of norepinephrine (NE) and dopamine (DA) in the median eminence (ME) of the hypothalamus, on steady state levels of mRNA for specific (β) subunits of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and on the concentration of LH, FSH, and prolactin (PRL). This figure is constructed from previously reported data (29, 75). Please note that the increase in DA turnover measured in transgenic males from this particular line was not detected in transgenic males from two different PEPCK-bGH lines (please see Fig. 2) or in transgenic females expressing the same gene construct. Mean ± SE; *values which differ from the corresponding values in normal controls; $P < 0.05$.


Normal
Transgenic
g
mg
BODY WEIGHT
PITUITARY WEIGHT
LHβ mRNA
FSHβ mRNA
ng/ml
PLASMA bGH
PLASMA PRL
ng/mg protein
PITUITARY LH
PITUITARY FSH
ng/ME/h
NE TURNOVER
DA TURNOVER
PLASMA LH
PLASMA FSH

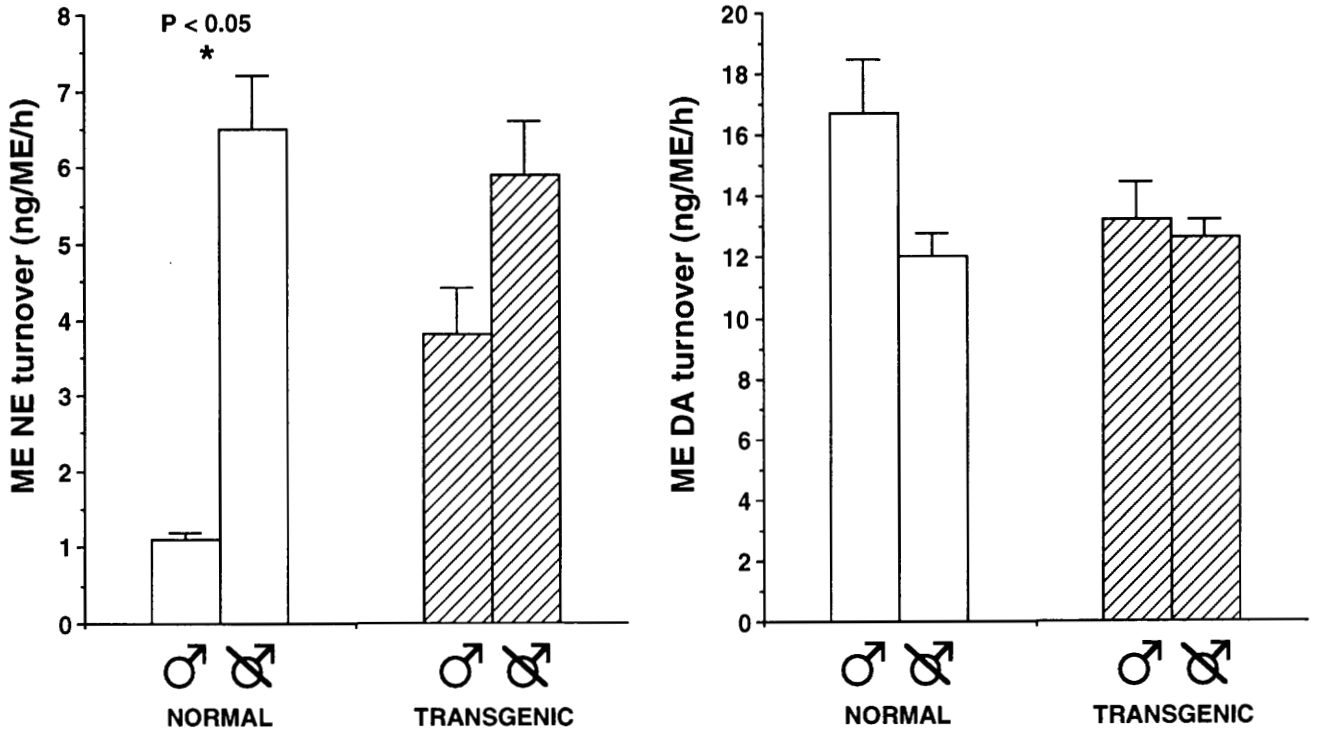


Figure 2. Effect of castration on turnover of norepinephrine (NE) and dopamine (DA) in the median eminence (ME) of normal and PEPCK-bGH-25 transgenic male mice. The expected significant increase in NE turnover in response to castration was detected in normal but not in transgenic mice. Means ± SE.

these animals. In transgenic PEPCK-bGH mice, plasma PRL levels in ovariectomized females were significantly elevated in two of three lines we have examined (29).

Surprisingly, studies of female reproductive function revealed that most transgenic PEPCK-bGH females fail to become pregnant after mating due to luteal failure which can be corrected by twice daily injections of PRL (details in an earlier section of this review). Measurements of plasma PRL levels in transgenic PEPCK-bGH females at various times after mating suggested that basal levels of PRL are increased, in keeping with the previous findings in ovariectomized animals (29), but mating-induced surges of PRL are either absent or severely attenuated (Cecim *et al.*, unpublished observations). Recent studies in our laboratory suggest that serotonergic transmission which is presumably involved in the regulation of PRL surges during the first half of pregnancy (79) may be disrupted or abnormal in transgenic PEPCK-bGH females.

Mechanisms of GH Action on Neuroendocrine and Reproductive Functions

Although effects of supraphysiological GH levels may differ substantially from the normal actions of this hormone, it seems reasonable to assume that the mechanisms involved are similar if not the same. In support of this assumption, most of the consequences of GH overexpression in transgenic animals could have either been expected from the well-established actions of GH (e.g., increase in adult body size, splanchnomegaly, suppression of endogenous GH synthesis and release) or readily ascribed to some of its known effects (e.g., increase in hepatic GH receptors, in plasma GHBP and insulin levels, and in the number of ovulations) (details and references in previous sections of this review). However, some characteristics of the transgenic animals described in this review appear to represent either novel actions of GH or effects that may be specific to chronic overstimulation of GH receptors. These include increase in plasma corticosterone levels (71), stimulation of PRL storage and release (29, 41, 76), suppression of mating-induced surges of PRL (unpublished data), and alterations of gonadotropin gene expression, storage, and release (75). Information available to date does not permit positive identification of mechanisms responsible for these effects of GH. However, some hypotheses can be proposed. For example, association of changes in the turnover rate of hypothalamic neurotransmitters, including dopamine and norepinephrine, with alterations in PRL and gonadotropin release suggests that GH acts within the hypothalamus to alter adenohypophyseal function. This suggestion is consistent with the presence of GH receptors in various brain regions including the hypothalamus (80, 81), with

behavioral effects of GH (81, 82), and particularly with the ability of intracranial administration of GH to influence behavior in birds (83). Identification of the specific neuronal groups involved will require further studies but information available to date suggests that GH can exert direct or secondary effects on the neurons secreting somatostatin, GHRH, dopamine, CRF, and NE (references in previous sections of this review). Modifications of gonadotropin gene expression and release (75) are presumably due to altered functional activity of LHRH secreting neurons. This could be due to a direct effect of GH on these neurons or to actions mediated by NE, dopamine, CRH, somatostatin, or neuropeptide Y (NPY). Each of these neurotransmitters can influence LHRH release (84–88) and each is known or suspected to be influenced by GH in transgenic animals (29, 43, 70, 71, 76, 78 and unpublished data). In support of the involvement of NPY-secreting neurons in mediating the effects of GH overexpression on adenohypophyseal hormone release, Ghosh *et al.* (78) reported that treatment with antiserum to NPY increased plasma FSH and PRL levels in normal male mice but not in transgenic MT-bGH males. However, studies of the expression and distribution of GH receptors (80, 81) and our studies in animals with ectopic pituitary transplants (75) suggest that GH may also act at the pituitary level. The latter effect is likely to be mediated by IGF-I (89). Indeed, it is well established that actions of GH on its various targets can be due to (i) direct consequences of GH binding to GH receptors, (ii) action of systemic IGF-I derived from GH-dependent hepatic synthesis, and/or (iii) effects of locally produced IGF-I. The actions of IGF-I can be modulated by alterations in the systemic or local levels of various IGF-I binding proteins and enzymes involved in their degradation. While these complex relationships and regulatory mechanisms are under active investigation in many laboratories, their presentation and discussion are outside the scope of this review.

Comparison of the effects of IGF-I and GH overexpression in transgenic mice revealed some differences in spite of considerable similarity of the resulting phenotypes (34, 35). Presumably some of the effects of GH in these animals (including increase in body weight and in the weights of various organs) are due to increase in systemic and/or local IGF-I levels, while other effects (e.g., stimulation of skeletal growth and enlargement of the liver) are due to direct actions of GH or to other mechanisms.

Abnormalities of gonadal function in transgenic animals overexpressing GH almost certainly represent a net result of GH-induced changes in the release of gonadotropins and PRL by the pituitary and direct actions of GH and/or IGF-I on various cell types within the gonads. Both ovaries and testes can bind GH (90, 91) as well as produce and bind IGF-I (54, 92–94). Considerable evidence exists for a major role of IGF-I and other growth factors in the endocrine and paracrine control of gonadal activity (53, 54, 94). Thus luteal failure in transgenic PEPCK-bGH mice is apparently due to suppression of mating-induced surges of PRL with a possible additional influence of reduced plasma FSH levels (29, 95), while the increased number of ovulations in transgenic females can be ascribed to potentiation of the action of FSH by systemic and/or locally produced IGF-I (53, 54). Alterations in testicular LH receptors and their "autoregulation" by hCG in transgenic male mice overexpressing GH (60) correspond to known actions of IGF-I on LH receptors in the testis (96).

It is important to realize that the consequences of GH overexpression in transgenic animals are reflecting not only the increase in average concentration of GH in their circulation, but also profound alterations in the pattern of GH release, particularly in males. The release of GH is normally pulsatile with large and relatively infrequent pulses in males, and much smaller and more frequent pulses in females. Sexual dimorphism in GH release was studied in considerable detail in the rat (97) but there is evidence that a similar situation exists also in the mouse (98). The release of GH from ectopic sites in transgenic MT-GH and PEPCK-GH mice is presumably nearly constant although diurnal variations were reported in plasma hGH levels in MT-hGH transgenic animals (27), and normal diurnal patterns of feeding can be expected to produce some variations in the expression of structural genes driven by the PEPCK promoter. Thus, the pattern of GH release and plasma GH levels in transgenic mice of either sex resembles the normal female pattern much more closely than the normal male pattern. The importance of these relationships was demonstrated by Norstedt and Palmiter (99) who reported that the sexually dimorphic GH-responsive expression of several genes in the liver follows the female pattern in transgenic male mice overexpressing GH. More recently, Keeney *et al.* (100) reported that expression of a male specific 42-kDa isoform of hepatic immunoreactive 3β hydroxysteroid dehydrogenase is dramatically suppressed in livers of transgenic mice expressing bGH, hGH or hGH · V genes.

The reduced life span of transgenic mice from the lines we have examined (70) and the nature of reproductive and endocrine deficits in these animals suggest that many of the neuroendocrine and reproductive alterations may represent symptoms of premature aging. Thus, changes in norepinephrine and dopamine turnover in the hypothalamus of transgenic mice resemble changes which take place in their normal siblings at a

much more advanced age (70). Suppression of ovarian cyclicity and male copulatory behavior are symptomatic of reproductive aging and are encountered in transgenic animals starting at an age of 5–8 months in different lines (70 and unpublished observations). The postcastration rise in plasma gonadotropins is compromised in transgenic mice overexpressing GH (Fig. 2; 77) as it is in normal aging rats (101). Accelerated aging of transgenic mice overexpressing GH was reported by several investigators (35, 102, 103) and is a conspicuous feature of transgenic animals from some of the lines discussed in this review (70). Groesbeck *et al.* (104) reported "toxic effects," as evidenced by increased mortality in immature and adult female rats treated with large doses of rGH. In the majority of transgenic PEPCK-bGH mice, death appears to be due to kidney failure, thus resembling the situation in normal aging mice. Evidence available to date suggests that these animals undergo normal or near-normal aging at an inappropriately early age (70 and unpublished observations). The mechanisms responsible for premature aging of transgenic GH mice remain to be elucidated.

Chronic elevation of corticosterone levels and alterations in carbohydrate metabolism (hyperinsulinemia, insulin resistance, and mild hyperglycemia in some of the lines) could also be suspected of contributing to reproductive abnormalities of transgenic mice. However, comparisons between transgenic animals expressing different GH genes with different promoters argue against any major role of hyperglucocorticoidism or diabetes in the etiology of reproductive and endocrine abnormalities in transgenic mice.

Another very interesting possibility is that alterations in neuroendocrine and reproductive functions in adult transgenic mice overexpressing GH are due to changes in hormone levels and in CNS development during fetal or early postnatal life. Expression of structural GH genes driven by either MT or PEPCK promoters starts early in development (19, 20, 23). The syndrome of "incomplete male sterility," characterized by an increase in the length of exposure to a female before conception takes place without concomitant reduction in litter size, was described in male rats treated perinatally with a progesterone antagonist (105) and closely resembles the findings in transgenic males from most of the lines we had opportunity to examine (57 and unpublished observations).

Discussion of the effects of GH overexpression in transgenic mice would not be complete without consideration of the fact that imposition of a life-long major disturbance in endocrine function is very likely to lead to activation of various alternate or abnormal regulatory mechanisms aimed at maintaining homeostasis, preserving vital functions, and perhaps also maintaining fertility. Consequently, some of the functional alterations or abnormalities detected in transgenic mice expressing various GH genes may not represent the "primary" effects of GH or IGF-I, but rather secondary adaptations which counteract the consequences of GH action. Considerable redundancy which exists in the regulation of most physiological functions provides opportunities for such compensating mechanisms to develop. For example, hyperinsulinemia and the development of islet cell adenomas in transgenic mice overexpressing GH (35, 72, 106) may occur in response to the diabetogenic effects of elevated GH and corticosterone rather than as a result of direct action of GH on pancreatic islets (107). A striking example of the ability of transgenic animals to maintain homeostasis was provided by a recent report of normal urine volume and osmolality in transgenic mice overexpressing and overselecting arginine vasopressin (108).

Future Directions

In addition to characterizing the consequences of life-long overexposure to GH and identifying the mechanisms involved, studies in transgenic mice expressing GH genes offer many challenging and exciting opportunities which only begin to be explored. For example, the developmental onset, duration, and level of expression of the structural GH genes can be controlled by the use of different promoters (19, 23, 27) and by altering the diet or other husbandry conditions (23, 26). The structural GH genes can be altered by site-directed *in vitro* mutagenesis before fusion with the desired promoter and introduction into oocytes. Elegant studies of the ability of a bGH analog generated by *in vitro* mutagenesis to antagonize the effects of endogenous GH (109) provide striking demonstration of the power of this approach. Studies of the effects of various bGH analogs in transgenic mice (110 and Chen, Kopchick, Steger and Bartke, unpublished observations) provided several examples of dissociation of different effects of GH or differential alterations of specific actions, including concomitant enhancement or preservation of some effects and suppression or elimination of others. Therefore, it should be realistic to anticipate that studies of GH expression in transgenic mice will yield new information on the actions of GH, including identification of domains and spatial characteristics of the GH molecule responsible for its specific effects. These data can be used to guide development of new applications of GH in animal industry and in medicine. For example, it may well become possible to produce selective stimulation of only some targets or functions normally responsive to GH without producing undesirable side effects and to antagonize all or selected actions of GH. It may also become feasible to produce lines of animals with improved growth, feed efficiency, and body composition

without concomitant impairments in fertility, endocrine function, carbohydrate metabolism, and viability.

Our studies of transgenic mice overexpressing GH, including previously unpublished data were supported by the National Institutes of Health via Grant HD20001 and DK42137 and by the Cooperative State Research Service, U.S. Department of Agriculture, under Agreement 89-37240-4584. These studies would not have been possible without the generosity and support of Dr. Thomas E. Wagner and Dr. Jeung S. Yun, who provided us with transgenic animals used to establish the corresponding lines in our laboratory. We thank Mrs. M. Hilt for typing.

We would also like to apologize to all those whose work pertinent to the topics discussed was not cited due to inadvertent omissions or space limitations.

1. Snell GD. Dwarf, a new mendelian recessive character of the house mouse. Proc Natl Acad Sci USA **15**:733–734, 1929.
2. Smith PE, MacDowell EC. The differential effect of hereditary mouse dwarfism on the anterior pituitary hormones. Anat Rec **50**:85–93, 1931.
3. Bartke A. Histology of the anterior hypophysis, thyroid and gonads of two types of dwarf mice. Anat Rec **149**:225–235, 1964.
4. Davidoff LM. Studies in Acromegaly III. The anamnesis and symptomatology in one hundred cases. Endocrinology **10**:461–483, 1926.
5. Jadresic A, Banks LM, Child DF, Diamant L, Doyle FH, Fraser TR, Joplin GF. The acromegaly syndrome. Q J Med **202**:189–204, 1982.
6. Laron Z. Clinical aspects of pituitary dwarfism and related conditions. In: Daughaday WH, Ed. Endocrine Control of Growth. New York: Elsevier, pp175–206, 1981.
7. Burns EC, Tanner JM, Preece MA, et al. Final height for pubertal development in 55 children with idiopathic growth hormone deficiency, treated for between 2 and 15 years with human growth hormone. Eur J Pediatr **137**:155–164, 1981.
8. Advis JP, White SS, Ojeda SR. Activation of growth hormone short loop negative feedback delays puberty in the female rat. Endocrinology **108**:1343, 1981.
9. Arsenijevic Y, Wehrenberg WB, Conz A, Eshkol A, Sizonenko PC, Aubert ML. Growth hormone (GH) deprivation induced by passive immunization against rat GH-releasing factor delays sexual maturation in the male rat. Endocrinology **124**:3050–3059, 1989.
10. Wilson ME, Tanner JM. Long-term effects of recombinant human growth hormone treatment on skeletal maturation and growth in female rhesus monkeys with normal pituitary function. J Endocrinol **130**:435–441, 1991.
11. Kosco MS, Bolt DJ, Wheaton JE, Loseth KJ, Crabo BA. Endocrine responses in relation to compensatory testicular growth after neonatal hemicastration in boars. Biol Reprod **36**:1177–1185, 1987.
12. Jorgensen JOL. Human growth hormone replacement therapy: Pharmacological and clinical aspects. Endocr Rev **12**:189, 1991.
13. Christiansen JS, Jorgensen JOL. Beneficial effects of GH replacement therapy in adults. Acta Endocrinol (Copenh) **125**:7–13, 1991.
14. Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW, Mattson DE. Effects of human growth hormone in men over 60 years old. N Engl J Med **323**:1–6, 1990.
15. Butler-Hogg BW, Johnsson ID. Bovine growth hormone in lambs: Effects on carcass composition and tissue distribution in crossbred females. Anim Prod **44**:117–124, 1987.
16. Etherton TD, Wiggins JP, Evock CM, Chung CS, Rebhun JF, Walton PE, Steele NC. Stimulation of pig growth performance by porcine growth hormone: Determination of the dose-response relationship. J Anim Sci **64**:433, 1987.
17. Guthrie HD, Pursel VG, Miller KF, Bolt DJ, Palmiter RD, Brinster RL. Effect of bovine growth hormone gene expression, sex and age on plasma gonadotropins, estrone and testosterone in prepuberal pigs. Domestic Anim Endocr **8**:423–429, 1991.
18. Rexroad CE Jr., Hammer RE, Bolt DJ, Mayo KM, Frohman LA, Palmiter RD, Brinster RL. Production of transgenic sheep with growth regulating genes. Mol Reprod Dev **1**:164–169, 1989.
19. Palmiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, Birnberg NC, Evans RM. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. Nature **300**:611–615, 1982.
20. Palmiter RD, Norstedt G, Gelinas E, Hammer RE, Brinster RL. Metallothionein human GH fusion genes stimulate growth of mice. Science **222**:809–814, 1983.
21. Hammer RE, Pursel VG, Rexroad CE Jr., Wall RJ, Bolt DJ, Palmiter RD, Brinster RL. Genetic engineering of mammalian embryos. J Anim Sci **63**:269–278, 1986.
22. Hammer RE, Pursel VG, Rexroad CE Jr., Wall RJ, Bolt DJ, Ebert KM, Palmiter RD, Brinster RL. Production of transgenic rabbits, sheep and pigs by microinjection. Nature **315**:680–683, 1985.
23. McGrane MM, deVente J, Yun J, Bloom J, Park E, Wynshaw A, Wagner T, Rottman FM, Hanson RW. Tissue-specific expression and dietary regulation of a chimeric phosphoenolpyruvate carboxykinase/bovine growth hormone gene in transgenic mice. J Biol Chem **263**:11443–11451, 1988.
24. Selden RF, Wagner TE, Blethen S, Yun JS, Rowe ME, Goodman HM. Expression of the human growth hormone variant in cultured fibroblasts and transgenic mice. Proc Natl Acad Sci USA **85**:8241–8245, 1988.
25. Stefaneanu L, Kovacs K, Horvath NE, Losinski N, Mayerhofer A, Wagner TE, Bartke A. An immunocytochemical and ultrastructural study of adenohypophyses of mice transgenic for human growth hormone. Endocrinology **126**:608–615, 1990.
26. Shanahan CM, Rigby NW, Murray JD, Marshall JT, Townrow CA, Nancarrow CD, Ward KA. Regulation of expression of a sheep metallothionein 1a-sheep growth hormone fusion gene in transgenic mice. Mol Cell Biol **9**:5473–5479, 1989.
27. Selden RF, Yun JS, Moore DD, Rowe ME, Malia MA, Wagner TE, Goodman HM. Glucocorticoid regulation of human growth hormone expression in transgenic mice and transiently transfected cells. J Endocrinol **122**:49–60, 1989.
28. Pomp D, Nancarrow CD, Ward KA, Murray JD. Growth, feed efficiency and body composition of transgenic mice expressing a sheep metallothionein 1a-sheep growth hormone fusion gene. Livestock Prod Sci **31**:335–350, 1992.
29. Steger RW, Bartke A, Parkening TA, Collins T, Cerven R, Yun JS, Wagner TE. Effects of chronic exposure to bovine growth hormone (bGH) on the hypothalamic-pituitary axis in transgenic mice: Relationship to the degree of expression of the PEPCK.bGH hybrid gene. Transgene (in press).
30. Yun JS, Li Y, Wight DC, Portanova R, Selden RF, Wagner TE. The human growth hormone transgene: Expression in hemizygous and homozygous mice (43096). Proc Soc Exp Biol Med **194**:308–313, 1990.
31. Shea BT, Hammer RE, Brinster RL. Growth allometry of the organs in giant transgenic mice. Endocrinology **121**:1924–1930, 1987.
32. Cecim M, Bartke A, Yun JG, Wagner TE. Growth allometry of transgenic mice expressing the mouse metallothionein-I/bovine growth hormone gene. Transgene **1**:125–132, 1993.
33. Matthews LS, Hammer RE, Brinster RL, Palmiter RD. Expression of insulin-like growth factor I in transgenic mice with elevated levels of growth hormone is correlated with growth. Endocrinology **123**:433, 1988.
34. Mathews LS, Hammer RE, Behringer RR, D'Ercole AJ, Bell GI, Brinster RL, Palmiter RD. Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology **123**:2827–2833, 1988.
35. Quaife CJ, Mathews LS, Pinkert CA, Hammer RE, Brinster

RL, Palmiter RD. Histopathology associated with elevated levels of growth hormone and insulin-like growth factor I in transgenic mice. Endocrinology **124**:40–48, 1989.
36. Hammer RE, Brinster RL, Rosenfeld MG, Evans RM, Mayo KE. Expression of human growth hormone releasing factor in transgenic mice results in increased somatic growth. Nature **315**:413–416, 1985.
37. Knobil E, Hotchkiss J. Growth hormone. Annu Rev Physiol **26**:47–74, 1964.
38. Aguilar RC, Fernandez HN, Dellacha JM, Calandra RS, Bartke A, Turyn D. Identification of somatogenic binding sites in liver microsomes from normal mice and transgenic mice expressing human growth hormone gene. Life Sci **50**:615–620, 1992.
39. Sotelo AI, Bartke A, Turyn D. Effects of bovine growth hormone (GH) expression in transgenic mice on serum and pituitary immunoreactive mouse GH levels and pituitary GH-releasing factor binding sites. Acta Endocrinol (in press).
40. Stefaneanu L, Kovacs K, Bartke A. *In situ* hybridization study of growth hormone, prolactin, and proopiomelanocortin mRNAs in adenohypophyses of mice transgenic for human growth hormone. Endocr Pathol **4**:73–78, 1993.
41. Stefaneanu L, Kovacs K, Bartke A, Mayerhofer A, Wagner TE. Pituitary morphology of transgenic mice expressing bovine growth hormone. Lab Invest **68**:584–591, 1993.
42. Hurley DL, Phelps CJ. Altered growth hormone-releasing hormone mRNA expression in transgenic mice with excess or deficient endogenous growth hormone. Mol Cell Neurosci **4**:237–244, 1993.
43. Hurley DL, Phelps CJ. Hypothalamic preprosomatostatin messenger ribonucleic acid expression in mice transgenic for excess or deficient endogenous growth hormone. Endocrinology **130**:1809–1815, 1992.
44. Orian JM, Snibson K, Stevenson JL, Brandon MR, Herington AC. Elevation of growth hormone (GH) and prolactin receptors in transgenic mice expressing ovine GH. Endocrinology **128**:1238–1246, 1991.
45. Bartke A, Turyn D, Aguilar RC, Sotelo AI, Steger RW, Chen XZ, Kopchick JJ. Induction of growth hormone (GH) receptors by GH in transgenic mice, and receptor binding of hGH and bGH analogs. Proceed Workshop Superfamily of Receptors for Growth Hormone, Prolactin, Erythropoietin and Cytokines, Haifa, Israel, 1993 (in press).
46. Hammer RE, Palmiter RD, Brinster RL. Partial correction of murine hereditary growth disorder by germ line incorporation of a new gene. Nature **311**:65–67, 1984.
47. Yun JS, Wagner TE. Study of human growth hormone transgenic mice: Female reproductive system. In: Program of the 10th Korea Symposium on Science and Technology. Seoul, Korea **3**:279–282. 1987.
48. Bartke A, Steger RW, Hodges SL, Parkening TA, Collins TJ, Yun JS, Wagner TE. Infertility in transgenic female mice with human growth hormone expression: Evidence for luteal failure. J Exp Zool **248**:121–124, 1988.
49. Hartree AS, Kovacic N, Thomas M. Growth-promoting and luteotrophic activities of human growth hormone. J Endocrinol **33**:249–258, 1965.
50. Posner BI, Kelly PA, Shiu RPC, Paud R, Friesen HG. Studies of insulin, growth hormone and prolactin binding: Tissue distribution, species variation and characterization. Endocrinology **95**:521–531, 1974.
51. Naar EM, Bartke A, Majumdar SS, Buonomo FC, Yun JS, Wagner TE. Fertility of transgenic female mice expressing bovine growth hormone or human growth hormone variant genes. Biol Reprod **45**:178–187, 1991.
52. Krackow S. Effect of food restriction on reproduction and lactation in house mice mated post partum. J Reprod Fert **86**:341–347, 1989.
53. Jia X, Kalmijn J, Hsueh AJW. Growth hormone enhances FSH-induced differentiation of cultured rat granulosa cell. Endocrinology **118**:1401–1409, 1986.
54. Katz E, Ricciarelli E, Adashi EY. The potential relevance of growth hormone in female reproductive physiology and pathophysiology. Fertil Steril **59**:8–34, 1993.
55. Mayerhofer A, Weis J, Bartke A, Yun JS, Wagner T. Effects of transgenes for human and bovine growth hormones on age-related changes in ovarian morphology in mice. Anat Rec **117**:175–186, 1990.
56. Bartke A, Shire JGM, Chandrashekar V, Steger RW, Mayerhofer A, Amador AG, Bain P, Tang K, Yun JS, Wagner TE. Effects of human growth hormone on reproductive and neuroendocrine functions in transgenic mice. In: First NL, Haseltine FP, Eds. Transgenic Animals. Boston: Butterworth-Heinemann, pp237–248, 1991.
57. Bartke A, Naar EM, Johnson L, May MR, Cecim M, Yun JS, Wagner TE. Effects of expression of human or bovine growth hormone genes on sperm production and male reproductive performance in four lines of transgenic mice. J Reprod Fertil **95**:109–118, 1992.
58. Steger RW, Bartke A, Yun JS, Wagner TE. Neuroendocrine function in transgenic mice with the phosphoenolpyruvate carboxykinase/human growth hormone (PEPCK/hGH) hybrid gene and very high peripheral levels of hGH. Transgene **1**:19–26, 1993.
59. Chandrashekar V, Bartke A, Wagner TE. Endogenous human growth hormone (GH) modulates the effect of gonadotropin-releasing hormone on pituitary function and the gonadotropin response to the negative feedback effect of testosterone in adult male transgenic mice bearing human GH gene. Endocrinology **123**:2717–2722, 1988.
60. Amador AG, Mayerhofer A, Bartke A. Genetics and luteinizing hormone (LH) receptors. In: Bartke A, Ed. Function of Somatic Cells in the Testis. Proceedings of the XIIth North American Testis Workshop, 1994 (in press).
61. Prins GS, Cecim M, Birch L, Wagner TE, Bartke A. Growth response and androgen receptor expression in seminal vesicles from aging transgenic mice expressing human or bovine growth hormone genes. Endocrinology **131**:2016–2023, 1992.
62. Hauser SD, McGrath MF, Collier RJ, Krivi GG. Cloning and *in vivo* expression of bovine growth hormone receptor mRNA. Mol Cell Endocrinol **72**:187–200, 1990.
63. Plaut K, Ikeda M, Vonderhaar BK. Role of growth hormone and insulin-like growth factor-I in mammary development. Endocrinology **133**:1843–1848, 1993.
64. Feldman M, Ruan W, Cunningham BC, Wells JA, Kleinberg DL. Evidence that the growth hormone receptor mediates differentiation and development of the mammary gland. Endocrinology **133**:1602–1608, 1993.
65. Grings EE, De Avila DM, Eggert RG, Reeves JJ. Conception rate, growth and lactation of dairy heifers treated with recombinant somatotropin. J Dairy Sci **73**:73–77, 1990.
66. Ludri RS, Updhyay RC, Singh M, Guneratne JRM, Basson RP. Milk production in lactating buffalo receiving recombinantly produced bovine somatotropin. J Dairy Sci **72**:2283–2287, 1989.
67. Törnell J, Rymo L, Isaksson OGP. Induction of mammary adenocarcinomas in metallothionein promoter-human growth hormone transgenic mice. Int J Cancer **49**:114–117, 1991.
68. Milton S, Cecim M, Li YS, Yun JS, Wagner TE, Bartke A. Transgenic female mice with high human growth hormone levels are fertile and capable of normal lactation without having been pregnant. Endocrinology **131**:536–538, 1992.
69. Skarda J, Fremrova V, Bezeeny I. Progesterone alone is responsible for stimulation of the growth of ducts and of mammary alveolar structures in mice. Endocrinologica Experimentalis **23**:17–27, 1989.
70. Steger RW, Bartke A, Cecim M. Premature ageing in transgenic mice expressing different growth hormone genes. J Reprod Fert (Suppl)46:61–75, 1993.
71. Cecim M, Ghosh PK, Esquifino AI, Began T, Wagner TE, Yun JS, Bartke A. Elevated corticosterone levels in transgenic mice expressing human or bovine growth hormone genes. Neuroendocrinology **53**:313–316, 1991.
72. Balbis A, Dellacha JM, Calandra RS, Bartke A, Turyn D. Down regulation of masked and unmasked insulin receptors in the liver of transgenic mice expressing bovine growth hormone gene. Life Sci **51**:771–778, 1992.
73. Chandrashekar V, Bartke A, Wagner TE. Interactions of hu-

man growth hormone and prolactin on pituitary and Leydig cell function in adult transgenic mice expressing the human growth hormone gene. Biol Reprod **44**:76–82, 1991.
74. Chandrashekar V, Bartke A, Wagner TE. Neuroendocrine function in adult female transgenic mice expressing the human growth hormone gene. Endocrinology **130**:1802–1808, 1992.
75. Tang K, Bartke A, Gardiner CS, Wagner TE, Yun JS. Gonadotropin secretion, synthesis, and gene expression in two types of bovine growth hormone transgenic mice. Biol Reprod **49**:346–353, 1993.
76. Bartke A, Steger RW, Parkening TA, Collins TJ, Yun JS, Wagner TE. Influence of human and bovine growth hormones on the regulation of prolactin release in transgenic mice. In: Gupta D, Wollmann HA, Ranke MB, Eds. Neuroendocrinology: New Frontiers. Tübingen, Germany: Brain Research Promotion, pp 39–48, 1990.
77. Steger RW, Bartke A, Parkening TA, Collins T, Yun JS, Wagner TE. Neuroendocrine function in transgenic male mice with human growth hormone expression. Neuroendocrinology **52**:106–111, 1990.
78. Ghosh P, Debeljuk L, Wagner TE, Bartke A. Effect of immunoneutralization of neuropeptide Y on gonadotropin and prolactin secretion in normal mice and in transgenic mice bearing bovine growth hormone gene. Endocrinology **129**:597–602, 1991.
79. Mistry A, Voogt JL. Role of serotonin in nocturnal and diurnal surges of prolactin in the pregnant rat. Endocrinology **125**:2875–2880, 1989.
80. Matthews LS, Enberg B, Norstedt G. Regulation of rat growth hormone receptor gene expression. J Biol Chem **264**:9905–9910, 1989.
81. Harvey S, Hull KL, Fraser RA. Growth hormone: Neurocrine and neuroendocrine perspectives. Mini review. Growth Reg **3**:161–171, 1993.
82. Matte AC. Growth hormone and isolation-induced aggression in wild male mice. Pharmacol Biochem Behav **14**:85–87, 1981.
83. Buntin JD, Figge GR. Prolactin and growth hormone stimulate food intake in ring doves. Pharmacol Biochem Behav **31**:533–540, 1989.
84. Kalra SP, Kalra PS. Neural regulation of luteinizing hormone secretion in the rat. Endocrinol Rev **4**:311–351, 1988.
85. Meites J, Sonntag WE. Hypothalamic hypophysiotropic hormones and neurotransmitter regulation: Current views. Annu Rev Pharmacol Toxicol **21**:295–322, 1981.
86. Rotsztejn WH, Drovva SV, Epelbaum J, Kordon C. Somatostatin inhibits *in vitro* release of luteinizing hormone-releasing hormone from rat mediobasal hypothalamic slices. Esperientia **38**:974–975, 1982.
87. Rivest S, Plotsky PM, Rivier C. CRF alters the infundibular LHRH secretory system from the medial preoptic area of female rats: Possible involvement of opioid receptors. Neuroendocrinology **57**:236–246, 1993.
88. Reznikov AG, McCann SM. Effects of neuropeptide Y on gonadotropin and prolactin release in normal, castrated or flutamide-treated male rats. Neuroendocrinology **57**:1148–1154, 1993.
89. Kanematsu T, Irahara M, Miyake T, Shitsukawa K, Toshihiro A, Aono, T. Effect of insulin-like growth factor I on gonadotropin release from the hypothalamus-pituitary axis *in vitro*. Acta Endocrinologica **125**:227–233, 1991.
90. Lucy MC, Collier RJ, Kitchell ML, Dibner JJ, Hauser SD, Krivi GG. Immunohistochemical and nucleic acid analysis of somatotropin receptor populations in the bovine ovary. Biol Reprod **48**:1219–1227, 1993.
91. Lobie PE, Breipohl W, García J, Aragón, Waters MJ. Cellular localization of the growth hormone receptor/binding protein in the male and female reproductive system. Endocrinology **126**:2214–2221, 1990.
92. Hammond JM, Baranao JLS, Skaleris D, Knight AB, Romanus JA, Recher M. Production of insulin-like growth factors by ovarian granulosa cells. Endocrinology **117**:2553–2555, 1985.
93. Adashi EY, Resnick CE, Svoboda ME, Vanwyk J. Follicle-stimulating hormone enhances somatomedin C binding to cultured rat granulosa cells. J Biol Chem **261**:3923–3930, 1986.
94. Bellvé AR, Zheng W. Growth factors as autocrine and paracrine modulators of male gonadal functions. J Reprod Fertil **85**:771–793, 1989.
95. Choudhary JB, Greenwold GS. Luteotropic complex of the mouse. Anat Rec **163**:373–388, 1969.
96. Bernier M, Chatelain P, Mather JP, Saez JM. Regulation of gonadotropin receptors, gonadotropin responsiveness, and cell multiplication by somatomedin-C and insulin in cultured pig Leydig cells. J Cell Physiol **129**:257–263, 1966.
97. Tannenbaum GS, Martin JB. Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology **98**:562–570, 1976.
98. MacLeod JN, Pampori NA, Shapiro BH. Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. J Endocrinology **131**:395–399, 1991.
99. Norstedt G, Palmiter R. Secretory rhythm of growth hormone regulates sexual differentiation of mouse liver. Cell **36**:805–812, 1984.
100. Keeney DS, Murry BA, Bartke A, Wagner TE, Mason JI. Growth hormone transgenes regulate the expression of sex-specific isoforms of 3β-hydroxysteroid dehydrogenase/Δ^{5-4}-Isomerase in mouse liver and gonads. Endocrinology **133**:1131–1138, 1993.
101. Matt DW, Sayles TE, Jih MH, Kauma SW, Lu JKH. Alterations in the postovariectomy increases in gonadotropin secretion in middle-aged persistent-estrous rats: Correlation with pituitary gonadotropin subunit gene expression. Neuroendocrinology **57**:351–358, 1993.
102. Pendergrass WR, Li Y, Jiang D-Z, Wolf NS. Decrease in cellular replicative potential in "giant" mice transfected with the bovine growth hormone gene correlates to shortened life span. J Cell Physiol **156**:96–103, 1993.
103. Wolf E, Kahnt E, Ehrlein J, Hermanns W, Brem G, Wanke R. Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: Lessons from transgenic animal models. Mech Ageing Dev **68**:71–87, 1993.
104. Groesbeck MD, Parlow AF, Daughaday WH. Stimulation of supranormal growth in prepubertal, adult plateaued, and hypophysectomized female rats by large doses of rat growth hormone: Physiological effects and adverse consequences. Endocrinology **120**:1963–1975, 1987.
105. van der Schoot P, Baumgarten R. Effects of treatment of male and female rats in infancy with infepristone on reproductive function in adulthood. J Reprod Fert **90**:255–266, 1990.
106. Messing A, Chen HY, Palmiter RD, Brinster RL. Peripheral neuropathies, hepatocellular carcinomas and islet cell adenomas in transgenic mice. Nature **316**:461–463, 1985.
107. Brelje TC, Sorenson RL. Role of prolactin versus growth hormone on islet B-cell proliferation *in vitro:* Implications for pregnancy. Endocrinology **128**:45–57, 1991.
108. Miller M, Kawabata S, Wiltshire-Clement M, Reventos J, Gordon JW. Increased vasopressin secretion and release in mice transgenic for the rat arginine vasopressin gene. Neuroendocrinology **57**:621–625, 1993.
109. Chen WY, White ME, Wagner TE, Kopchick JJ. Functional antagonism between endogenous mouse growth hormone (GH) and a GH analog results in dwarf transgenic mice. Endocrinology **129**:1402–1408, 1991.
110. Yang C-W, Striker LJ, Pesce C, Chen WY, Peten EP, Elliot S, Doi T, Kopchick JJ, Striker GE. Glomerulosclerosis and body growth are mediated by different portions of bovine growth hormone. Lab Invest **68**:62–70, 1993.