Endocrine, *vol. 11, no. 2, 171–179, October 1999* 0969–711X/99/11:171–179/$12.25

Effects of Overexpression of Growth Hormone–Releasing Hormone on the Hypothalamo-Pituitary-Gonadal Function in the Mouse

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In this investigation, the neuroendocrine alterations induced by high, chronic circulating levels of endogenous growth hormone (GH) were studied in transgenic mice with ectopic overexpression of the human growth hormone–releasing hormone (h-GH-RH) gene. In comparison with their normal littermates, transgenic h-GH-RH mice had elevated plasma levels of GH, prolactin (PRL), and corticosterone. In addition, they had elevated body, liver, kidney, spleen, and pituitary weights compared with normal mice. Testis and seminal vesicle weights were also increased in transgenic mice. Although basal plasma luteinizing hormone (LH) levels, plasma estradiol levels in females, and plasma testosterone levels in males did not differ significantly between normal and transgenic animals, the LH response to castration was severely impaired in transgenic mice of both sexes. Among the biogenic amines studied in the hypothalamus, only dopamine concentrations were significantly lower in transgenic animals compared with their normal littermates. This decrease in hypothalamic dopamine may be related to the hyperprolactinemia in transgenic animals. In vitro, pituitaries from transgenic mice released significantly higher amounts of GH, and although the basal release of LH was not different in both normal and transgenic mice, the response to gonadotropin-releasing hormone was significantly smaller in transgenic mice. Cultured anterior pituitary cells from transgenic mice secreted high quantities of GH and PRL in vitro, but these quantities significantly decreased from 1 to 8 wk in culture.These results show that high, persistent levels of circulating endogenous GH induce alterations in neuroendocrine functions related to the hypothalamo-pituitary-gonadal and the hypothalamo-pituitary-adrenal axes.

Key Words: Growth hormone; growth hormone–releasing hormone; gonadotropins; PRL; corticosterone; transgenic mice.

Received June 10, 1999; Revised July 29, 1999; Accepted July 29, 1999.

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Introduction

There is considerable evidence that growth hormone (GH) can influence development and adult function of the hypothalamo-pituitary-gonadal axis *(1–3)*. An excess of GH as well as a deficiency or resistance of GH have been associated with disturbances of sexual development, neuroendocrine control of gonadal function, and fertility *(4–6)*. In the mouse, ectopic expression of heterologous GH can lead to alterations in hypothalamic neurotransmitter metabolism, plasma corticosterone, gonadotropin and prolactin (PRL) levels and regulation of their release, sexual behavior, and fertility *(7–10)*. The severity and the exact nature of these alterations depend on the species of origin of the GH and on the level of its expression *(7)*.

Inherited deficiency of GH with concomitant deficiency of PRL and thyroid-stimulating hormone in Snell and Ames dwarf mice is associated with profound disturbances in sexual maturation, gonadotropin release, and gonadal function, with infertility of all females and most of the males *(4,11)*. Isolated incomplete deficiency of GH in the little mouse and in the dwarf rat is associated with deficits in fertility *(12–14)*. Targeted disruption (knockout) of the GH receptor gene in the mouse causes an increase in plasma PRL levels and altered responses to gonadotropin-releasing hormone (GnRH) in males, delayed puberty in females, and reduced fertility in both sexes *(15)*.

The objective of the present study was to characterize neuroendocrine functions related to reproduction in transgenic mice with chronic elevation of endogenous GH of pituitary origin. From previous studies with transgenic mice expressing heterologous GH genes, it became evident that elevated circulating levels of bovine (b) or human (h), GH can affect the function of the hypothalamo-pituitary axis *(5,7,8)*. We therefore hypothesized that increased levels of endogenous GH could also affect gonadotropin secretion in mice. For these studies, we have utilized transgenic mice that overexpress human growth hormone–releasing hormone (GH-RH) and develop pituitary enlargement and a massive increase in plasma levels of endogenous GH *(16–19)*.

Results

In Vivo Experiments

Transgenic male mice had markedly higher levels of plasma GH than their normal littermates ($p < 0.01$) (Fig. 1).

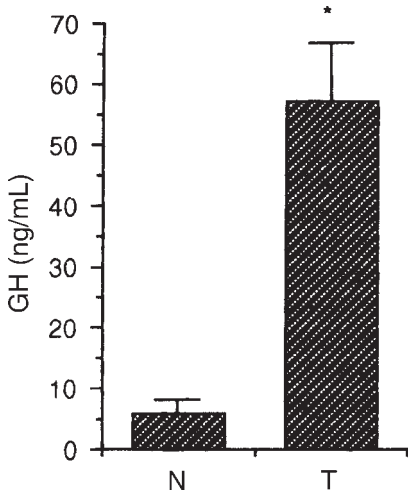


Fig. 1. Plasma GH levels in normal (N) and transgenic (T) male mice. $^{*}p < 0.05$.

In both male and female transgenic mice, plasma corticosterone levels were significantly higher than in their respective normal littermates ($p < 0.05$) (Fig. 2). In both sexes plasma PRL levels were significantly elevated in transgenic mice compared with their normal littermates ($p < 0.05$) (Fig. 2). Although plasma testosterone levels in male transgenic mice were numerically lower than in normal mice, the difference was not statistically significant. In female mice, plasma estradiol (E_2) levels were not significantly different in transgenic compared with normal animals.

Plasma luteinizing hormone (LH) levels in intact normal and transgenic male mice were not significantly different (Fig. 3). Plasma LH levels were found significantly increased on the 2 and 4 d after orchidectomy in both normal and transgenic mice ($p < 0.05$), with no significant differences between the two groups (Fig. 3). However, in normal males, plasma LH levels continued to increase, reaching a plateau by the wk 2, whereas in transgenic mice, there was no further increase in plasma LH after d 4 postcastration. Plasma LH levels in transgenic mice at d 8, 15, 22, and 29 after castration were significantly lower than in normal mice at the same postcastration periods ($p < 0.01$) (Fig. 3). In intact female mice, plasma LH levels in normal and transgenic animals were not significantly different (Fig. 3). Immediately after ovariectomy, plasma LH rose significantly in normal mice ($p < 0.05$), reaching a plateau between the wk 2 and 3 after surgery. In transgenic mice, however, there was no increase in plasma LH levels on d 2 and 4 after ovariectomy, with a slight increase afterward, which was significant only 2 wk after ovariectomy ($p < 0.05$). Plasma LH levels in transgenic mice were significantly lower than in their normal littermates at all the postovariectomy periods studied ($p < 0.05$).

The ovariectomized GH-RH transgenics had significantly elevated body, liver, kidney, and spleen weights compared with ovariectomized controls (Table 1). Pituitary weights were also markedly elevated in the GH-RH transgenic mice. The liver, kidney, spleen, and anterior pituitary weights of the transgenic mice were greater than what may have been expected in relationship to the total body growth. A similar pattern was seen in the intact males. In addition, testes and seminal vesicle weights were significantly greater in the transgenic compared with the normal males, but this greater weight was proportional to the total body growth. The presence of the transgene did not effect adrenal weight in either sex (Table 2).

Neither the content nor the turnover of norepinephrine or dopamine in the median eminence was affected by the expression of the transgene in the female mice (Fig. 4). In the mediobasal hypothalamus, the concentrations of norepinephrine, serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) were similar in both normal and transgenic ovariectomized mice (Fig. 5). However, dopamine content in the mediobasal hypothalamus was significantly lower in the transgenic than in the normal mice. In the male mice, median eminence norepinephrine content and turnover were similar. Dopamine content and turnover were significantly lower in the transgenic male mice (Fig. 6). In the mediobasal hypothalamus, there were no significant differences in norepinephrine, dopamine, serotonin, and 5-HIAA concentrations between normal and transgenic male mice (Fig. 7).

In Vitro Experiments

Anterior pituitaries from male or female transgenic mice released significantly more GH into the medium than anterior pituitaries from normal male or female mice ($p < 0.01$) (Fig. 8). The release of PRL by anterior pituitaries was much greater in female than in male mice. The anterior pituitaries from normal female mice released significantly more PRL than anterior pituitaries from transgenic female mice ($p < 0.05$) (Fig. 8). Although the anterior pituitaries from normal male mice released somewhat higher quantities of PRL than those from transgenic mice, the difference was not statistically significant. The basal release of LH by anterior pituitaries from normal and transgenic mice was similar in both the male and female groups (Fig. 9). The response to GnRH by anterior pituitaries from female transgenic mice was significantly lower than that from their normal littermates ($p < 0.05$). No significant differences in the response to GnRH between anterior pituitaries from normal and transgenic male mice were detected.

Pituitary Cell Cultures

The release of GH into the media decreased significantly from its highest levels at d 8 of culture up to 5 wk of culture ($p < 0.05$) (Fig. 10). Between 5 and 8 wk, no further significant decrease in GH release was observed. PRL release into the media was highest at d 8 of culture, with significant decreases at 3 and 5 wk ($p < 0.05$) (Fig. 10). After 5 wk in culture, the release of PRL by pituitary cells remained stable. In spite of the marked decrease of GH and PRL release into the medium, after 8 wk of culture, the microscopic examina-

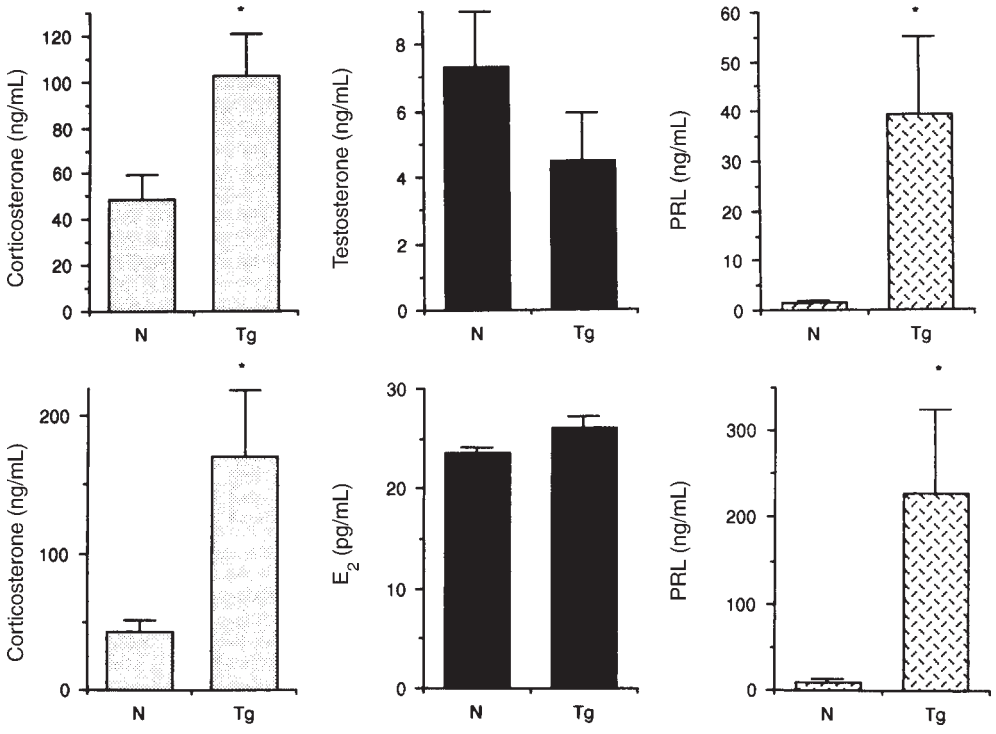
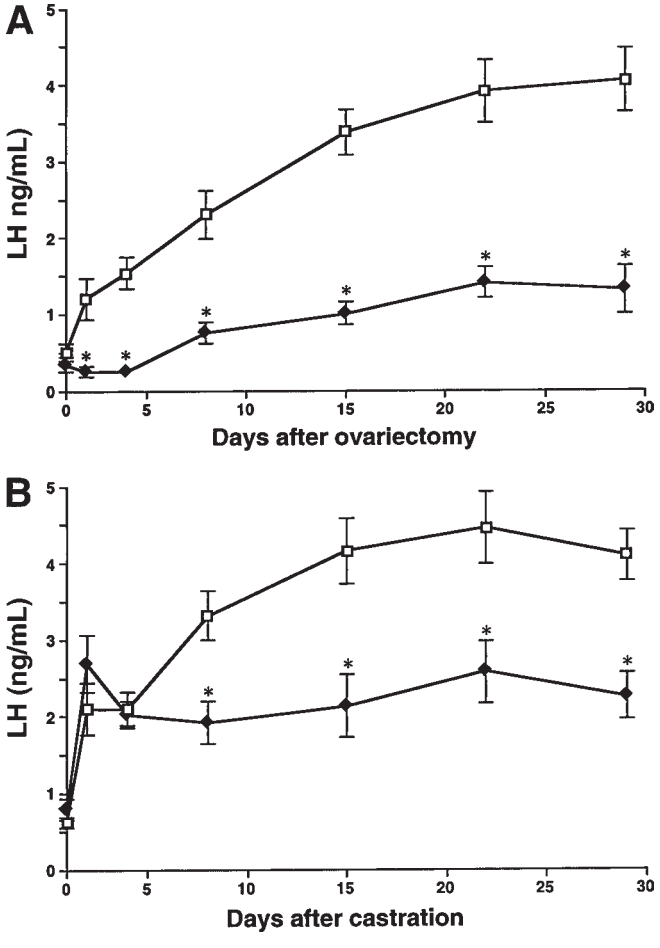


Fig. 2. (Top) Corticosterone, testosterone, and PRL levels; and (bottom) corticosterone, E_2, and, PRL levels in plasma of normal (N) and transgenic (Tg) **(A)** male and **(B)** female mice. $^{*}p < 0.05$.



tion of these cultures showed an abundance of enlarged cells with marked hyperplasia and relatively small nuclei (Fig. 11).

Discussion

Several reports from our laboratory had previously shown that the ectopic expression of bovine or human GH in transgenic mice is associated with disturbances in the hypothalamo-pituitary-gonadal axis *(7–10,15)*. In the present investigation, we have widened these studies to include the effects of endogenous ecutopically produced GH on the hypothalamo-pituitary function.

The GH-RH transgenic mouse produces large amounts of endogenous GH stimulated by the ectopic expression of h-GH-RH driven by mouse metallothionein-1 (MT-1) promoter. The continuous stimulation of the anterior pituitary function by GH-RH results in increased secretion of GH, enlarged anterior pituitaries, and marked hyperplasia of somatotrophs and mammosomatotrophs *(17,18)*. The advantage of this type of transgenic mice in comparison to previously studied transgenic mice overexpressing heterologous GHs is that in this case we were able to study the effect of the hypersecretion of endogenous homologous

Fig. 3. *(left)* **(A)** Plasma LH levels in intact male normal —□— and transgenic —◆— mice and the response to orchidectomy **(B)** and plasma LH levels in intact female normal —□— and transgenic —◆— mice and the response to ovariectomy. $^{*}p < 0.05$.

Table 1
Weights in Normal and Transgenic Ovariectomized Female Mice

	Normal	Body Weight (%)	Transgenic	Body Weight (%)
Body (g)	29.269 ± 1.417	—	38.789 ± 0.991*[b]	—
Anterior pituitary (mg)	2.258 ± 0.166	0.0077	7.727 ± 0.417*[b]	0.0199
Liver (g)	1.43 ± 0.06	4.86	2.98 ± 0.128*[b]	7.68
Kidney (g)	0.366 ± 0.019	1.25	0.646 ± 0.023*[b]	1.68
Spleen (g)	0.162 ± 0.013	0.553	0.288 ± 0.016*[b]	0.742
Adrenal (mg)	12.027 ± 0.869	0.041	13.094 ± 0.823	0.034

[a]Values are means ± SE.
[b]*$p < 0.05$ vs normal.

Table 2
Weights in Intact Normal and Transgenic Male Mice

	Normal	Body Weight (%)	Transgenic	Body Weight (%)
Body (g)	33.69 ± 1.08	—	43.10 ± 1.30b*[b]	—
Anterior pituitary (mg)	1.58 ± 0.112	0.0047	5.775 ± 0.413*[b]	0.0134
Liver (g)	1.598 ± 0.059	4.74	3.192 ± 0.162*[b]	7.4
Kidney (g)	0.504 ± 0.021	1.496	0.732 ± 0.030*[b]	1.7
Spleen (g)	0.115 ± 0.005	0.341	0.226 ± 0.012*[b]	0.524
Adrenal (mg)	8.636 ± 0721	0.025	10.300 ± 0.735	0.024
Testis (g)	0.231 ± 0.008	0.685	0.256 ± 0.009*[b]	0.594
Seminal vesicles (g)	0.278 ± 0.013	0.825	0.346 ± 0.025*[b]	0.803

[a]Values are means ± SE.
[b]*$p < 0.05$ vs normal.

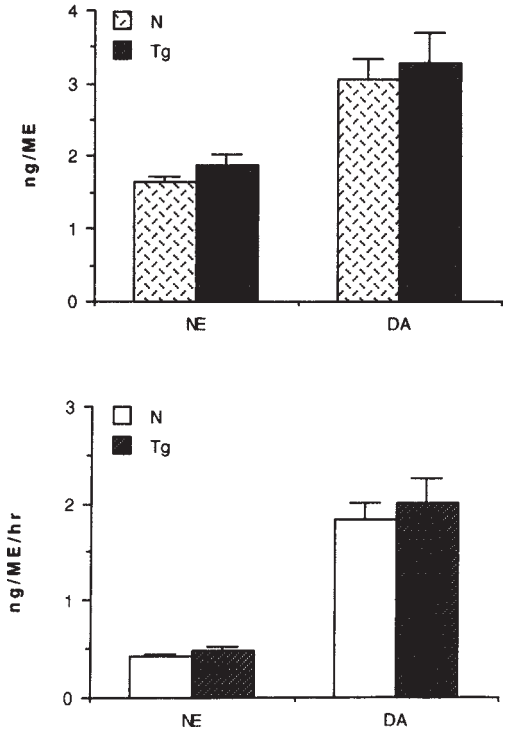


Fig. 4. Norepinephrine (NE) and dopamine (DA) content **(A)** and turnover **(B)** in the median eminence of normal (N) and transgenic (Tg) ovariectomized mice. *$p < 0.05$.

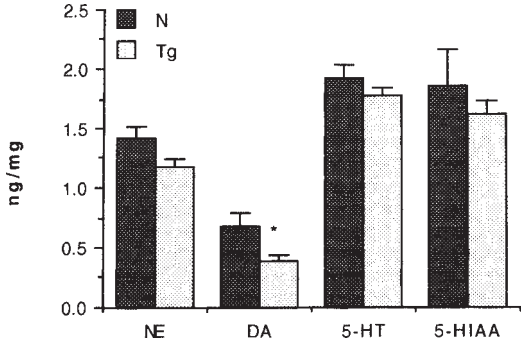


Fig. 5. Norepinephrine (NE), dopamine (DA), serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) concentrations in the mediobasal hypothalamus of normal (N) and transgenic (Tg) ovariectomized mice. *$p < 0.05$.

GH in mice. Moreover, high levels of GH in these animals originate from the anterior pituitary rather than in the liver, kidneys, intestines, and other ectopic sites.

Our present study shows that the increased levels of plasma GH seen in these transgenic mice are associated with an impaired response of LH to castration in both sexes of mice, although this difference was more marked in the female than in the male. In the male mouse, there were no differences in the early response to castration between

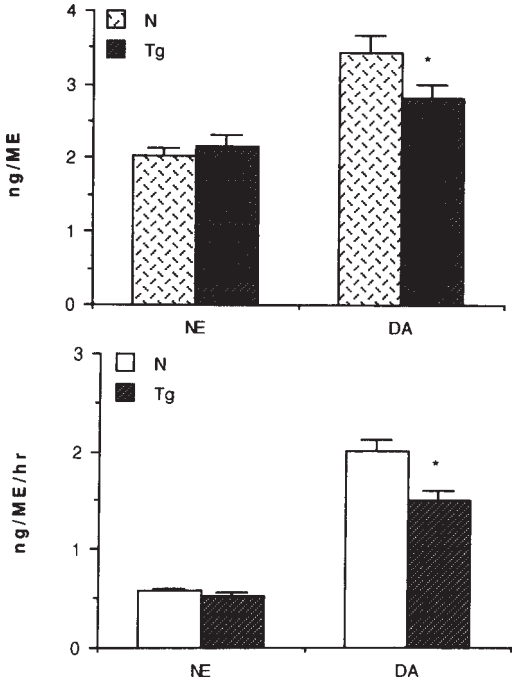


Fig. 6. Norepinephrine (NE) and dopamine (DA) content **(A)** and turnover **(B)** in the median eminence of normal (N) and transgenic (Tg) male mice. $^{*}p < 0.05$.

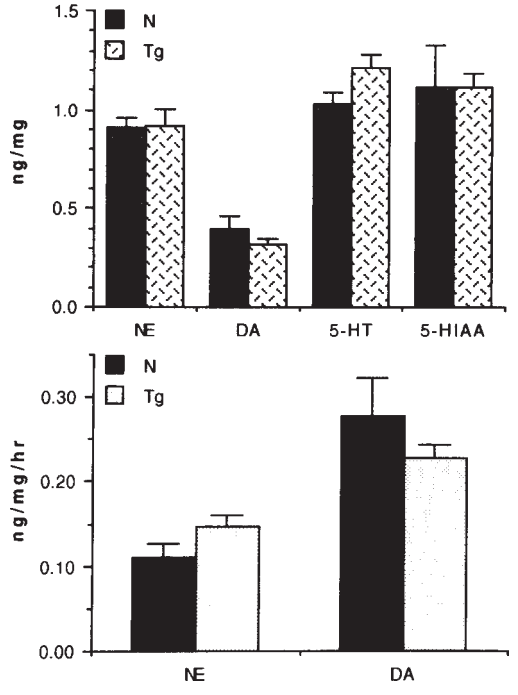


Fig. 7. Norepinephrine (NE), dopamine (DA), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) concentrations **(A)** and turnover **(B)** of norepinephrine and dopamine in the mediobasal hypothalamus of normal (N) and transgenic (Tg) male mice. $^{*}p < 0.05$.

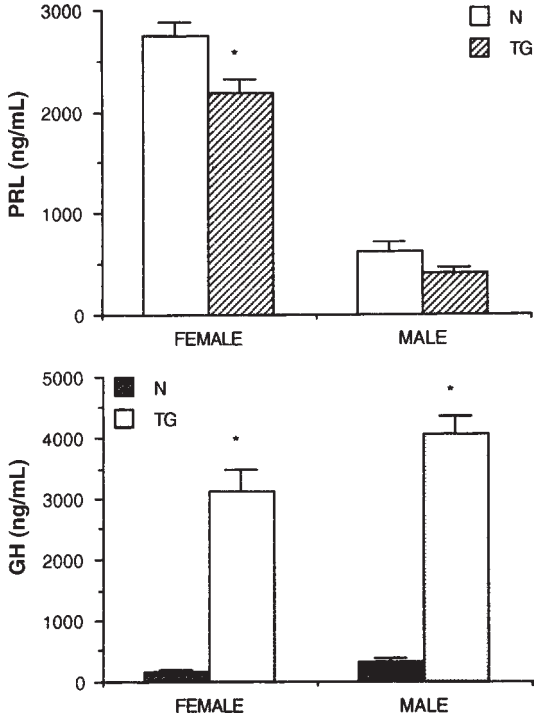


Fig. 8. Release of GH and PRL by anterior pituitaries from normal (N) and transgenic (Tg) male and female mice in vitro. $^{*}p < 0.05$.

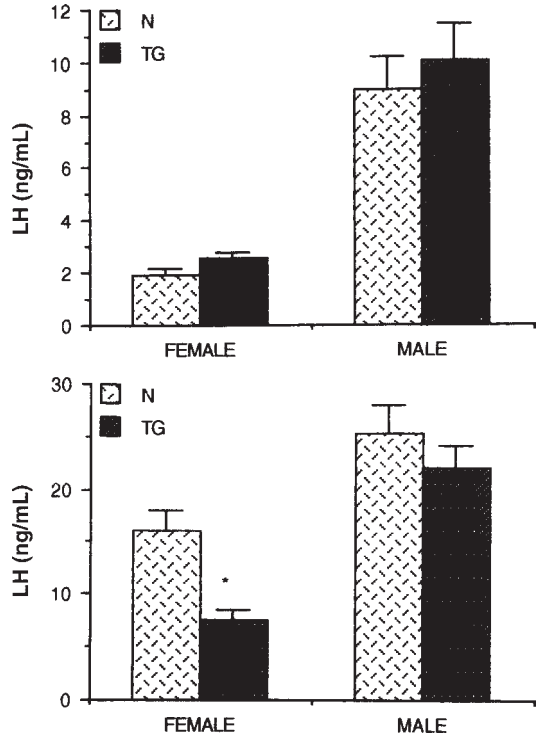


Fig. 9. Release of LH by anterior pituitaries from normal (N) and transgenic (Tg) male and female mice in vitro, in **(A)** basal conditions and **(B)** after stimulation with Gn-RH (LH-RH). $*p < 0.05$.

normal and transgenic animals, but a lower response was evident by the d 8 after surgery in transgenic mice. In the female mice, however, the response to ovariectomy in terms of LH release was markedly lower in the transgenic mice. In the incubated pituitaries, the basal release of LH was similar in both groups of animals, but the LH response to

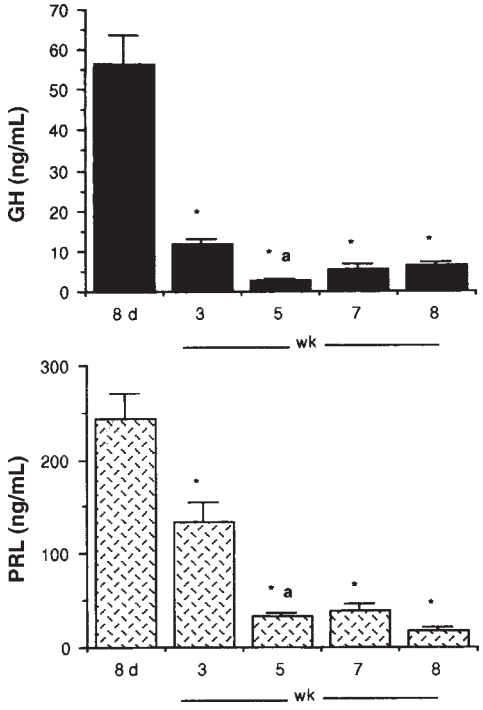


Fig. 10. Release of GH and PRL in anterior pituitary cells from transgenic male mice, from 8 d to 8 wk in culture. *$p < 0.05$ vs 8 d; *[a]$p < 0.005$ vs 3 wk.

GnRH was lower in transgenic than in normal female mice. These observations show that the chronic elevation of endogenous GH in transgenic mice resulted in alterations in the regulation of LH secretion. In spite of the differences in the secretion of LH in castrated male and female transgenic mice and in the in vitro response to GnRH, norepinephrine content and turnover in the hypothalamus were not apparently altered in these animals.

In numerous previous reports, reviewed by Barraclough and Wise *(19)*, norepineprhine, among other catecholamines (CATS) and neurotransmitters, was shown to be a major factor in the regulation of gonadotropin secretion. The lack of apparent alterations in norepinephrine content and turnover in the hypothalamus of transgenic mice could be explained by the fact that measuring these levels in the whole mediobasal hypothalamus may have missed changes at the level of discrete nuclei in the hypothalamus, which are directly related to the control of gonadotropin secretion. Alternatively, the modifications in the secretion of LH observed in the transgenic mice may have been owing to alterations in the secretion of some other neurotransmitter also related to the control of gonadotropin secretion. In addition to norepinephrine, several other neurotransmitters and neuropeptides have been shown to be involved in the control of LH secretion, among them, GABA *(20)*, *n*-methyl D-aspartic acid *(21)*, neuropeptide Y *(22)*, and tachykinins *(23)*. The mechanism through which the high expression of GH-RH affects the secretion of gonadotropins is likely to be mediated by the hypersecretion of GH. GH, in turn, by altering some of the neurotransmitters or neuropeptides just mentioned, may modify gonadotropin secretion.

The hypersecretion of endogenous GH was also accompanied by increased levels of plasma PRL in transgenic mice. Stefaneanu et al. *(18)* reported that in transgenic mice expressing the h-GH-RH gene, there is a marked hyperplasia of mammosomatotrophs, bihormonal cells that contain both GH and PRL. These investigators reported also the presence of some hyperplastic cells containing PRL in the anterior pituitary of h-GH-RH transgenic mice. This finding suggests that the chronic and sustained stimulation of pituitary cells by GH-RH could result in a stimulation not only of GH secretion, but also PRL.

In previous publications from our laboratory, we reported that a moderate hyperprolactinemia was found in transgenic mice overexpressing heterologous GH *(24)*. In ovariectomized transgenic mice, dopamine concentrations in the mediobasal hypothalamus were found to be lower than in the same tissue of normal ovariectomized mice. In male transgenic mice, the content and turnover of dopamine were also lower than in the normal littermates. Since dopamine is a very well-known physiological inhibitor of PRL secretion *(25)*, it is possible that its decreased levels in the hypothalamus may be related to the hyperprolactinemia in the transgenic mice. More difficult to explain, however, is the moderate decrease in the release of PRL from pituitaries of female transgenic mice in vitro. It is possible that the pituitaries from normal animals, when relieved from the tonic hypothalamic inhibition of dopamine, were maximally stimulated to release PRL. Since the pituitaries from transgenic mice were already stimulated in vivo (or less effectively inhibited), when incubated in vitro, they could not be further stimulated, or were relatively less stimulated.

Just as already reported in several lines of transgenic mice overexpressing b or hGH *(9)*, plasma corticosterone was significantly elevated in the GH-RH transgenic mice in the present investigation. It is evident that elevated circulating levels of GH are able to stimulate corticosterone secretion by the adrenal gland. On the basis of results obtained in bGH transgenic mice *(9)*, we suspect that the elevation of plasma corticosterone in GH-RH transgenic mice was possibly owing to stimulation of adrenotorticotropic hormone release, through a metabolic stress induced by the overexpression of GH-RH.

Interestingly, in spite of the fact that the hyperplastic somatotrophs can continue secreting high amounts of GH when cultured in vitro, this secretory activity greatly decreased after 1 wk in culture. This is also the case with the secretion of PRL, although the release of this hormone markedly decreased after 3 wk in culture. In spite of the decrease in the secretion of GH and PRL, after 8 wk in culture, the anterior pituitary cells of transgenic mice were still hyperplastic and showed marked enlargement. This suggests that, although these pituitary cells secrete less

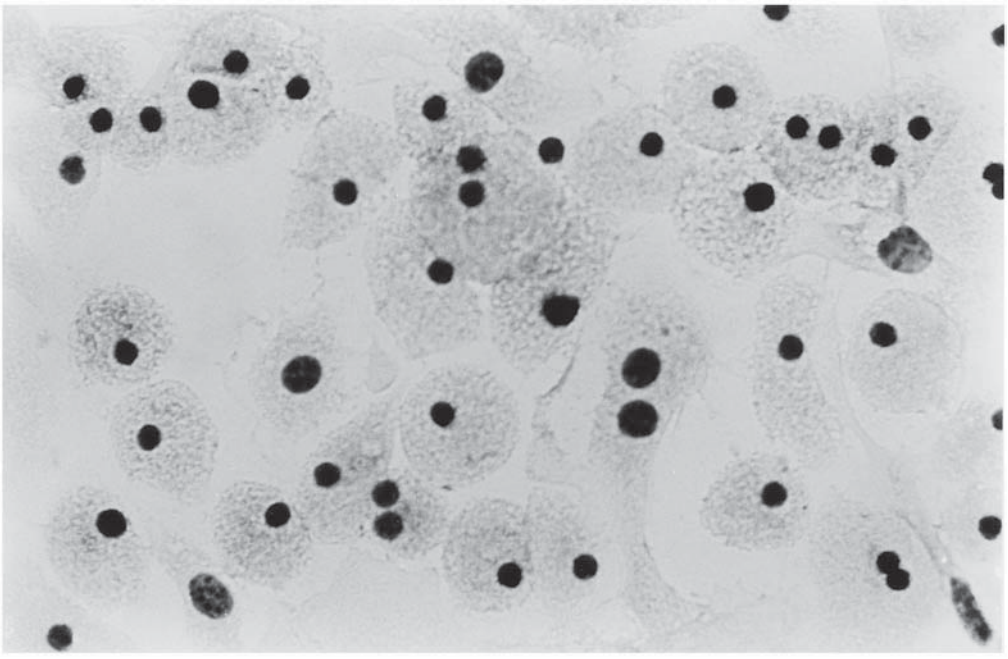


Fig. 11. Anterior pituitary cells after 8 wk in culture. Staining, toluidine blue; magnification, ×1840.

GH and PRL after 8 wk in culture, they still seem to be hyperstimulated at that time. It is therefore possible that GH-RH was secreted by the pituitary cells in vitro. Adenohypophyseal expression of hGH under control of the same promoter (MT) was previously detected in transgenic mice *(26)*. In future studies, it may be interesting to determine GH-RH activity in the pituitary cell extracts. From our results, it is evident that morphologically the pituitary cells in culture show marked signs of hyperstimulation as long as after 8 wk in culture. These cells, however, seem to have diminished their secretory potential in terms of GH and PRL release into the medium.

It has been demonstrated that the anterior pituitary gland of GH-RH transgenic mice contains markedly increased amounts of different peptides, such as galanin *(27)* and tachykinins *(28)*. Galanin has been shown to be able to modify the secretion of anterior pituitary hormones *(29)*. Tachykinins have also been demonstrated to modulate the function of the hypothalamo-pituitary-gonadal axis *(23)*. It is possible that some of the neuroendocrine alterations observed in these transgenic mice may be owing, directly or indirectly, to the increased levels of these peptides. Alternatively, the increment of the bioactive peptide concentrations in the pituitary gland may be merely a side effect of the hyperstimulation of the somatotrophs.

In summary, it is evident that the hypersecretion of endogenous GH in h-GH-RH transgenic mice results in a number of alterations in the neuroendocrine functions. Among these, the present investigation shows that there is an impaired LH response to castration and to GnRH, as well as elevated plasma PRL and corticosterone levels.

Materials and Methods

Animals

Hemizygous transgenic (expressing h-GH-RH with mouse MM-1 promoter; MT-GH-RH) and normal littermate mice were raised in our vivarium by mating transgenic males with C57BL/6 × C3H F1 hybrid females, purchased from the Jackson Laboratory (Bar Harbor, ME). The animals were fed laboratory chow with free access to tap water. They were kept in quarters with controlled temperature (21–23°C) and light cycle (12 h light/12 h dark). These transgenic mice were derived from animals kindly provided by Dr. J. Hyde (University of Kentucky) and originated by Dr. K. Mayo (Northwestern University). The treatments of the animals were carried out following protocols previously approved by the Animal Care Committee, Southern Illinois University.

Experiments

In Vivo

Adult normal (n = 9) and transgenic (n = 12) male mice (approx 3 mo old) were anesthetized with ether, and blood was drawn by orbital sinus puncture using a disposable glass capillary pipet. Blood was collected in tubes containing 20 μL of 6% EDTA solution in water, to avoid clotting, and centrifuged, and plasma was aspirated and kept frozen until assayed for mouse GH. These experiments were carried out between 9:00 and 10:00 AM. In this as well as in the following experiments, no attempt was made to check the stage of the estrous cycle in female mice. Since the animals were always killed in the morning, the stage of the cycle should not have major effects on LH levels. To study corticosterone and PRL levels in plasma, groups of normal and

transgenic male and female mice (mean age 2 mo) were kept in a quiet room with no personnel access allowed overnight in order to minimize stress. On the following morning, the animals were killed by decapitation in an adjacent room, within 30 s of the initial disturbance of their cage. Blood was taken from the trunk and collected in tubes containing 50 μL of 6% EDTA in order to prevent clotting. Plasma was aspirated and kept frozen until assayed for corticosterone and PRL. Additionally, the levels of testosterone in the plasma from male mice, and E_2 in the plasma from female mice, were determined. In the male group, 8 normal and 8 transgenic mice were used; in the female group, 13 normal and 7 transgenic mice were used.

In another experiment, the LH response to castration was investigated in male and female transgenic animals, and compared with the same response in normal mice of the same age and sex. Normal and transgenic male mice (mean age 3 mo) were lightly anesthetized with ether, and blood was drawn from the orbital sinus, as already described.

The following day, the animals were orchidectomized under ether anesthesia using the abdominal route. Blood was drawn by orbital sinus puncture on d 2, 4, and 8 after surgery and then once a week up to the wk 4 after castration. In each plasma sample, LH levels were determined. Eighteen normal mice and 10 transgenic mice were used in this experiment.

Normal and transgenic female mice (approx 3 mo old) were bled, and the following day they were ovariectomized using a dorsal approach, under light ether anesthesia. Blood was again drawn on d 2, 4, and 8 after surgery and then once a week until the wk 4 after ovariectomy. In each plasma sample, LH levels were determined. Seventeen normal and 8 transgenic mice were used in this experiment.

Additional male and female mice were used to determine hypothalamic neurotransmitter content and turnover. Intact male and ovariectomized female (ovariectomized 8 d prior to sacrifice) were studied. Ovariectomized mice, instead of intact females, were used to avoid potential problems owing to possible variations in CAT levels at different stages of the estrous cycle. Sixty minutes before sacrifice, one-half of the mice were injected with tyrosine hydroxylase inhibitor, alpha-methyl-*p*-tyrosine ([aMPT], 250 mg/kg intraperitoneally) for determination of CAT turnover rates in defined regions of the brain. All mice were sacrificed between 8:00 and 10:00AM. Body, liver, kidney, spleen, anterior pituitary, adrenal, testes, and seminal vesicle weights were recorded. At the time of autopsy, the brain was quickly removed, and the mediobasal hypothalamus and median eminence were dissected free and frozen.

Prior to assay, median eminence and mediobasal hypothalami were sonicated in 0.1 *M* $HClO_4$ containing the internal standards for the CAT assay (dihydroxybenzylamine) and 1 m*M* sodium bisulfite. The median eminence and mediobasal hypothalami were subjected to alumina extraction and norepinephrine, dopamine, 5-HT, and 5-HIAA in the extracts were separated by high-performance liquid chromatography.

In Vitro

Normal and transgenic mice of both sexes were killed by decapitation, and the anterior pituitary was removed and placed in a test tube containing 1 mL of medium 199 plus antibiotics (1 mL/100 mL of medium) (Sigma, St. Louis, MO). One anterior pituitary was put in each tube, and 10 pituitaries per group were used. The glands were preincubated for 30 min in a Dubnoff metabolic shaker at 37°C under constant gassing with 95% O_2 and 5% CO_2. At the end of the preincubation, the medium was discarded and replaced with new medium. The incubation was carried out for 90 min. Media were then aspirated and kept frozen until assayed for mouse GH, PRL and LH. New medium containing synthetic GnRH (Calbiochem, LaJolla, CA) (10 ng/mL per tube) was dispensed in each tube and the incubation proceeded for an additional 90 min. Media were aspirated and kept frozen until assayed for LH.

Pituitary Cell Cultures

Long-term cultures of anterior pituitary cells from transgenic animals were used to determine whether overproduction of GH and PRL in these cells becomes autonomous and whether overproduction of GH depends on GH-RH produced at extrapituitary sites. Anterior pituitary cells from transgenic male mice were enzymatically dispersed with collagenase followed by pancreatin, as previously described *(30)*. After the dispersion, the cells were suspended and cultured in F-12 medium containing fetal calf and horse serum and antibiotics (penicillin, streptomycin, and fungizone) (Sigma), for 8 wk. Media were changed every 3 d. At 8 d, and 3, 5, 7, and 8 wk during culture, media were aspirated, the cultures were rinsed with F-12 medium, and 1 mL of F-12 medium and antibiotics per well was dispensed. The cells were put back in the incubator for 7 h, after which the media were aspirated and kept frozen until assayed for GH and PRL. F-12 medium containing fetal calf and horse serum and antibiotics was dispensed in each well and put back in the incubator. After the last incubation, on the wk 8, the cells were fixed with Bouin's fluid, processed for histology, and stained with toluidine blue.

Assays

Mouse GH and PRL were determined using homologous radioimmunosasay (RIA) kits and plasma LH was determined using a heterologous rat LH kit; both kits were distributed through the National Hormone and Pituitary Program and Dr. A. F. Parlow. Corticosterone, testosterone, and E_2 were determined using commercial kits (DPC, Los Angeles, CA). Biogenic amines were quantitated by electrochemical detection as previously described *(31)*. CAT turnover rates were estimated using the formula $K = k[CA]_0$ in which $[CA]_0$ = the mean CAT concentration at zero time (noninjected controls); and the rate constant, k, = the –log of the slope of the line describing the decline of norepinephrine or dopamine concentration during the 1 h following the blockade of tyrosine hydroxylase with aMPT *(32)*.

Statistics

The significance of the differences among groups was determined using analysis of variance followed by post-hoc tests (Fisher's PLSD and Dunnett) or the student's *t*-test whenever pertinent.

Acknowledgments

We are grateful to Clare Fadden for her excellent technical assistance, the National Hormone and Pituitary Program, Dr. A. F. Parlow for the RIA kits used in this investigation, and Drs. J. Hyde and K. Mayo for the MT-h-GH-RH transgenic mice used to establish our breeding colony of these animals.

This work was supported by NIH grants HD 34255 and HD 20001.

References

1. Advis, J. P., White, S. S., & Ojeda, S. R.(1981). *Endocrinology* **108,** 1343–1352.
2. Arsenijevic, Y., Wehrenberg, W. B., Conz, A., Eshkol, A., Sizonenko, P. C., and Aubert, M. L. (1989). *Endocrinology* **124,** 3050–3059.
3. Wilson, M. E. and Tanner, J. M. (1991) *J. Endocrinol.* **130,** 435–441.
4. Bartke, A. (1964). *Anat. Rec.* **149,** 225–235.
5. Cecim, M., Kerr, J., and Bartke, A. (1995). *Biol. Reprod.* **52,** 1162–1166.
6. Zhou, Y., Xu, B. C., Maheshwari, H. G., He, L., Reed, M., Lozykowski, M., Okada, S., Cataldo, L., Coschigamo, K., Wagner, T. E., Baumann, G., and Kopchik, J. J. (1997). *Proc. Natl. Acad. Sci. USA* **94,** 13215–13220.
7. Steger, R. W., Bartke, A., Yun, J. S., and Wagner,T. E. (1993). Transgene **1,** 19–26.
8. Tang, K., Bartke, A., Gardiner, C. S., Wagner, T. E., and Yun, J. S. (1994). *J. Androl.* **15,** 9–14.
9. Cecim, M., Ghosh, P. K., Esquifino, A. I., Began, T., Wagner, T. E., Yun, J. S., and Bartke, A. (1991). *Neuroendocrinology* **53,** 313–316.
10. Meliska, C. J. and Bartke, A. (1997). *J. Androl.* **18,** 305–311.
11. Smith, P. E. and MacDowell, E. C. (1931). *Anat. Rec.* **50,** 85–93.
12. Cheng, T. C., Beamer, W. G., Phillips, J. A., and Bartke, A. (1983). *Endocrinology* **113,** 1669–1678.
13. Chubb, C. (1987). *Biol. Reprod.* **37,** 564–569.
14. Nogami, H. and Takeuchi, T. (1993). *Biol. Reprod.* **45,**178–187.
15. Bartke, A., Chandrashekar, V., Turyn, D., Steger, R. W., Debeljuk, L., Winters, T. A., Mattison, J. A., Danilovich, N. A., Croson,W., Wernsing, D. R., and Kopchick, J. J. (1999). *Proc. Soc. exp. Biol. Med.,* in press.
16. Hammer, R. E., Brinster, R. L., Rosenfeld, M. G., Evans, R. M., and Mayo, K. E. (1985). *Nature* **315,** 413–416.
17. Mayo, K. E., Hammer, R. E., Swanson, L. W., Brinster, R. L., Rosenfeld, M. G., and Evans, R. M. (1988). *Mol. Endocrinol.* **2,** 606–612.
18. Stefaneanu, L., Kovacs, K., Asa, S. L., Losinski, N. E., Billestrup, N., Price, J. and Vale, W. (1989). *Endocrinology* **125,** 2710–2718.
19. Barraclough, C. A. and Wise, P. M. (1982). *Endocr. Rev.* **3,** 91–119.
20. Akema, T. and Kimura, F. (1993). *Neuroendocrinology* **57,** 28–33
21. Brann, D. W. and Mahesh, V. (1994). *Front. Neuroendocrinol.* **15,** 3–49.
22. Kalra, S. P. and Crowley, W. R. (1992). *Front. Neuroendocrinol.* **13,** 1–46.
23. Debeljuk, L. and Lasaga, M. (1999). *Peptides* **20,** 285–299.
24. Bartke, A., Cecim, M., Tang, K., Steger, R. W., Chandrashekar, V., and Turyn, D. (1994). *Proc. Soc. exp. Biol. Med.* **206,** 345–359.
25. Weiner, R. I., Findell, P. R., and Kordon, C. (1988). Role of Classic and Peptide Neuromediators in the Neuroendocrine Regulation of LH and Prolactin, in *The Physiology of Reproduction*, (Knobil, E. and Neill, J. eds.), Raven, New York, pp. 1235–1281.
26. Stefaneanu, L., Kovacs, K., Horvath, E., Losinski, N. E., Mayerhofer, A., Wagner, T. E., and Bartke, A. (1990). *Endocrinology* **126,** 608–615.
27. Moore, J. P., Cai, A., Maley, B. E., Jennes, L., and Hyde, J. F. (1999). *Endocrinology* **140,** 1789–1799.
28. Debeljuk, L., Wright, J. C., Phelps, C., and Bartke, A. (1999). *Neuroendocrinology* **70,** 107–116.
29. Vrontakis, M. E., Torsello, A., and Friesen, H. G. (1991). *J. Endocrinol. Invest.* **14,** 785–794.
30. Debeljuk, L., Khar, A., and Jutisz, M. (1978). *J. Endocrinol.* **77,** 409–415.
31. Steger, R. W., De Paolo, L., Asch, R. H., and Silverman, A. Y. (1983). *Neuroendocrinology* **37,** 361–370.
32. Steger, R. W., De Paolo, L., and Shephers, A. M. (1985). *Neurobiol. Aging* 6:113–116.