Stress-Induced Secretion of Human Growth Hormone in Transgenic Mice (42988)

RONALD PORTANOVA, JEUNG S. YUN,* AND THOMAS E. WAGNER*
Department of Zoological and Biomedical Sciences and the College of Osteopathic Medicine and the Edison Animal Biotechnology Center, Ohio University, Athens, Ohio 45701*

Abstract. **The effect of stress on human growth hormone (hGH) secretion was studied in transgenic mice. Experiments were conducted on fourth, fifth, and sixth generation male mice carrying a fusion gene, consisting of the promoter sequence of the mouse metallothionein *I* gene ligated to the hGH structural gene (mMT-*I*/hGH). In animals adapted to a controlled photoperiod, basal (unstimulated) levels of plasma hGH exhibited a diurnal cycling, with peak values occurring during the later half of the light period (15.5 ± 1.0 vs 10.7 ± 0.9 ng/ml, mean ± SE, light versus dark, respectively). Food deprivation (5 days) led to elevated levels of plasma hGH (11.0 ± 0.7 vs 32.0 ± 4.2 ng/ml, pre- versus post-fast, respectively) accompanied by weight loss (49.5 ± 0.8 vs 34.3 ± 0.7 g), and hypoglycemia (7.8 ± 0.2 vs 5.0 ± 0.3 m*M*); glucose administration (5% drinking solution *ad libitum*) blocked the changes in levels of plasma hGH (12.2 ± 1.1 vs 13.8 ± 0.8 ng/ml) and plasma glucose (7.4 ± 0.3 vs 7.9 ± 0.5 m*M*), although the animals still sustained significant weight loss (44.9 ± 1.6 vs 35.2 ± 1.1 g). Vigorous exercise (swimming, 4 hr) produced a small but significant increase in plasma hGH, 12.1 ± 1.1 ng/ml (1 hr pre-swim) vs 16.7 ± 0.6 ng/ml (immediately post-swim). These findings indicate that the mMT-*I*/hGH transgene is responsive to the physiologic status of the host animal. Taken together with information regarding the heterologous components of the fusion gene, these data are consistent with the view that the hGH (structural) sequence may play a role in the response to stress.** [P.S.E.B.M. 1990, Vol 193]

It has become possible to introduce well-characterized genetic information into the chromosomal complement of mammals (1, 2). Mouse lines have been established containing gene constructs which code for a number of substances including human growth hormone (hGH) (see 3). The transgenes are heritable and expressed *in utero*, and the gene products are recognized "as self" by the immune system of the recipient animal (4). The transgenic (TG) animal thus represents a unique model system for studying the effects of gene products on the host animal and for studying the regulation of foreign gene expression. The present study was conducted using TG mice carrying a fusion gene comprised of the mouse metallothionein I (mMT-*I*) promoter, ligated to the hGH structural gene. To date, the mMT-I/hGH fusion gene has been shown to be induced by heavy metals (4, 5) and glucocorticoids (GC) (6). Since endogenous MT genes are also induced by heavy metals (5, 7) and GC (8, 9), these findings appear to indicate that the expression of the fusion gene resembles that of the MT gene. However, recent findings suggest that GC induction of the fusion gene is not mediated via the mMT-*I* promoter (7, 10, 11), but rather by the hGH sequence (6), i.e., the expression of the fusion gene is not a simple extrapolation of MT gene expression. In order to reassess the transgene's activity and the factors that control it, we studied the autonomous (basal) activity of the gene and the effect of a physiologic challenge (stress) on the expression of the gene. A preliminary report of this work has appeared (12).

Received May 16, 1989. [P.S.E.B.M. 1990, Vol 193]
Accepted September 11, 1989.

0037-9727/90/1931-0046$2.00/0

Materials and Methods

TG mice. TG mice were produced as described previously (1). In brief, fertilized mouse eggs were recovered in cumulus from the oviducts of (C57 × C3H)F_1 females that had previously mated with F_1 males. The mMT-I/hGH gene construct was microinjected into the male pronucleus of fertilized eggs (approximately 1000 copies/egg) which were then implanted into the oviducts of 1-day pseudopregnant ICR foster mothers and carried to term. The transgene is permanently incorporated into the genome of these animals and is transmitted in a Mendelian fashion to

approximately half the offspring (4, 13). The TG mice produce hGH-mRNA and hGH in ectopic sites, have high levels of circulating hGH, and attain adult weights greater than 1.5 times that of non-TG littermates (3, 13, 14).

The TG mice used in this study were adult (4–8 months) males (fourth through sixth generation descendants of a single line) bred from matings between TG males and non-TG ($B6C3F_1$) females (The Jackson Laboratory, Bar Harbor, ME). Animals were housed singly (basal secretion and exercise experiments) or two per cage (fasting experiment) at controlled temperature (21°C) and light cycle (6 AM:6 PM, light:dark) and provided a balanced diet (Purina 5001 Rodent Chow; Purina Mills, Inc., St. Louis, MO) and tap water *ad libitum*.

Assays. Blood samples were collected from a tail vein and hGH levels were determined on 100-μl aliquots of heparinized plasma using a solid-phase double monoclonal antibody radioimmunoassay (Hybridtech, Inc., San Diego, CA) according to the manufacturer's protocol. The concentration of glucose in plasma was measured using a YSI model 23 Analyzer fitted with a YSI 2365 Glucose Membrane Kit (YSI Co., Inc., Yellow Springs, OH).

Basal (Unstimulated) Levels of Plasma hGH. TG mice (n = 12) were bled four times (each) at approximately 30-day intervals. Because of the time period (3 months) involved, the protocol was designed to minimize confounding effects (seasonal variation, age). The mice (4–5 months old at the start of the experiment) were divided at random into two equal size groups and bled according to a crossover design: initial blood samples were obtained from one group between 12 PM and 6 AM and from the second group between 12 AM and 6 PM; successive samples were taken sequentially from each animal at a clock time approximately 12 hr later than that of the previous sample. This protocol allows comparisons between the group values; however, due to the nonrandom sampling (sequential) it does not allow comparison of hGH values within the groups.

Fasting. Series 1: TG mice (n = 32) were weighed, bled (about 10 AM), and returned to their home cage. Twenty-four hours later, all food was removed and the animals were fasted for 5 days. At the end of the fast, the animals were again weighed and bled. During the fast the animals appeared to be somewhat sluggish but were otherwise healthy and endured the fast without apparent distress. Series 2: TG mice (n = 9) were weighed and bled as described above. Twenty-four hours later all food was removed and the drinking water was replaced with 5% glucose *ad libitum*. After 5 days the mice were again weighed and bled.

Strenuous Exercise. TG mice (n = 9) were bled (about 10 AM) in order to determine pre-swim levels of plasma hGH, and the animals were returned to their home cage. One hour later the mice were placed individually in polycarbonate tanks containing water maintained at 27–29°C and were forced to swim for a total of 4 hr with a 5-min rest after each 30 min of swimming. The water depth was approximately 18 cm so that the animals could not touch the bottom without being submerged. Immediately after the swimming period, the animals were again bled in order to obtain a post-exercise sample.

Statistical Analysis. Group data are expressed as mean ± SE and Student's t test was used to compare group effects. In some cases (as noted), individual estimates of plasma hGH are also presented.

Results

Basal (Unstimulated) Levels of Plasma hGH. The 24-hr profile of basal levels of plasma hGH is shown in Figure 1. Individual estimates of plasma hGH vary considerably. However, the group data reveal that plasma levels of hGH are elevated ($P < 0.001$) during the later half of the light period compared with the later half of the dark period (15.5 ± 1.0 vs 10.7 ± 0.9 ng/ml).

Fasting. TG mice deprived of food for 5 days (Table I, Series 1) had increased $P < 0.001$) levels of

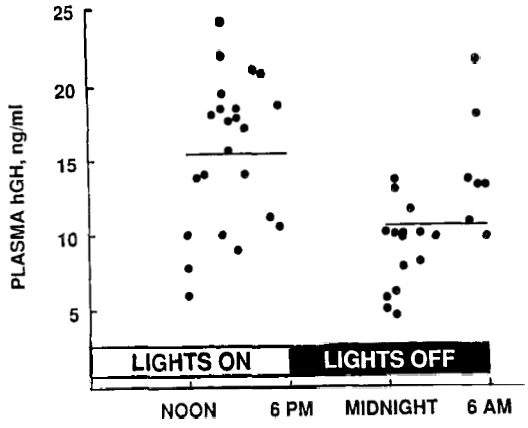

Figure 1. Daily variation of plasma hGH levels. Blood samples were obtained from transgenic mice (n = 12) on four occasions, at 30-day intervals. Data points represent individual estimates of plasma hGH on samples obtained at indicated clock time; horizontal bars represent the group means.

Table I. Effect of Fasting on Plasma Levels of hGH[a]

Treatment	hGH (ng/ml)	Plasma glucose (m*M*)	Body weight (g)
Series 1 (n = 32)			
Normal diet	11.0 ± 0.7	7.8 ± 0.2	49.5 ± 0.8
Food deprived (5 days)	32.0 ± 4.2[b]	5.0 ± 0.3[b]	34.3 ± 0.7[b]
Series 2 (n = 9)			
Normal diet	12.2 ± 1.1	7.4 ± 0.3	44.9 ± 1.6
5% Glucose (5 days)	13.8 ± 0.8	7.9 ± 0.5	35.2 ± 1.1[b]

[a] *Values are mean* ± SE.
[b] Indicates significant group effect ($P < 0.001$).

plasma hGH accompanied by reductions ($P < 0.001$) in plasma glucose and body weight. When the TG mice were deprived of food but provided a 5% glucose drinking solution (Table I, Series 2), body weight was reduced ($P < 0.001$) but no significant change ($P > 0.05$) in plasma concentrations of hGH or glucose was observed.

Strenuous Exercise. Strenuous exercise (swimming, 4 hr) led to elevated levels of plasma hGH (1.2- to 1.8-fold) in eight of the nine TG mice tested. As the data in Table II indicate, the group effect (pre- versus post-swim) is highly significant ($P < 0.001$).

Discussion

The expression of the mMT-I/hGH transgene resembles that of endogenous MT genes in that both are induced by heavy metals (4, 5, 7) and by GC (6, 8, 9). However, recent evidence indicates that GC regulation of the fusion gene is not mediated via the mMT promoter (7, 10, 11) but rather by the hGH sequence (6). Significantly, a GC receptor binding site identified within the hGH gene (15) is also present in the mMT-I/hGH gene (6). Since the hGH region of the fusion gene may contribute to its regulation, we decided to assess the transgene's activity in terms of physiologic condition known to affect the secretion of endogenous hGH.

Our data indicate that the basal (unstimulated) activity of the mMT-I/hGH transgene cycles over a 24-hr period; in animals acclimated to a controlled photoperiod, plasma levels of hGH are elevated during the later half of the light period. These data do not establish that the transgene is influenced by photic cues or by secondary signals (e.g., physical activity) entrained to the light cycle; i.e., it is possible that the observed effect is due to factors coincident with, but causally independent of the photoperiod. We are unaware of evidence linking the activity of endogenous MT genes to photoperiod. However, elevated levels of physical activity induce MT genes (16), which suggests that photoperiod-entrained levels of daily activity might also influence expression of the gene. With regard to the hGH portion of the gene, it should be noted that endogenous GH secretion is known to occur in a cyclic fashion. Daily variations are prominent in unstressed adolescent humans and higher frequency episodic secretion occurs in adolescent humans, in other primates, and in lower species including rats (17). The rhythmic secretion of pituitary GH is probably driven by hypothalamic influences and it is likely to reflect hormone release from storage pools rather than events at the genomic level. Although it seems unlikely that the hGH portion of the fusion gene contributes to the cyclic secretion of hGH in TG mice, this possibility cannot be ruled out in view of data that GC interacts with the hGH sequence (6).

In humans and other primates, plasma levels of GH are elevated in response to a variety of stresses including, starvation, insulin induced hypoglycemia, and vigorous exercise (17). This response may be mediated (in part) at the genomic level since similar nonspecific stresses increase plasma GC (18), and since GC has been found to increase GH biosynthesis (19) and to induce native GH genes (20). Endogenous MT genes are also induced by stress (strenuous exercise, starvation) and as reported here the mMT-I/hGH fusion gene is regulated by similar stimuli. In response to vigorous physical activity (swimming), plasma levels of hGH in TG mice were elevated approximately 1.4-fold. This is compatible with the finding that swimming increased tissue levels of endogenous MT about 3-fold (16). Bremmer and Davies (21) reported that food restriction increased tissue levels of MT in the rat and noted that the magnitude of the effect is related to the degree of starvation. In TG mice, food deprivation led to elevated levels of plasma hGH (about 3-fold), weight loss, and hypoglycemia. Significantly, TG mice administered glucose (5%) as their only caloric intake sustained weight loss ($P < 0.001$), but had levels of blood glucose and hGH that did not differ from pretreatment values ($P > 0.05$). We interpret this to indicate that hypoglycemia, rather than weight loss (tissue catabolism), is the stimulus to hGH secretion.

The molecular mechanisms by which stress induces MTs are not well defined but tend to involve heavy metals (e.g., zinc) or GC. Oh *et al.* (16) suggested that in response to stress, MTs are induced by zinc mobilized as a result of tissue catabolism. As noted above, our data in TG mice do not support this view. Karin and Herschman (9) suggested that the induction of MTs by stress is mediated by GC. If GC mediates the stress response in TG mice, it is likely that the steroid interacts with the hGH sequence (6, 15), rather than the mMT sequence (7, 10, 11). Durnam *et al.* (7) reported that induction of MTs by the inflammatory agent lipopolysaccharide involves neither GC nor heavy metals and proposed that a third (unidentified) type of molecule may regulate MT genes during stress. Whether such molecules play a role in the TG animal cannot be evaluated at this time.

Our data indicate that the mMT-I/hGH transgene is responsive to the physiologic status of the host animal. Although the fusion gene is regulated by stimuli which are known to induce endogenous MT genes, our data are not fully explained by the molecular mechanisms known to control MT gene activity. Taken in

Table II. Effect of Exercise on Plasma Levels of hGH[a]

Pre-swim hGH (ng/ml)	Post-swim hGH (ng/ml)	*P*
12.1 ± 1.1	16.7 ± 0.6	<0.001

[a] Blood samples were obtained from transgenic animals ($n = 9$) 1 hr before (pre-swim) and again immediately after (post-swim) swimming (4 hr); values are mean ± SE.

combination with information regarding pituitary GH secretion, our findings are consistent with the view that the hGH sequence may play a role in the response to stress.

This work was supported by a grant from the U.S. Department of Agriculture (85-CRCR-1-1834) and the Ohio University College of Osteopathic Medicine. We are grateful to Soonhee Choe, Cindy Gemma and Beth Portanova for excellent technical assistance.

1. Wagner TE, Hoppe P, Jollick J, Scholl D, Hodinka R, Gault J. Microinjection of a rabbit beta-globin gene into zygotes and its subsequent expression in adult mice and their offspring. Proc Natl Acad Sci USA **78**:6376–6380, 1981.
2. Pursel VG, Rexroad RL, Bolt DJ, Miller RJ, Wall RJ, Hammer RE, Pinkert CA, Brinster RL. Progress on gene transfer in farm animals. Vet Immunol Immunopathol **17**:303–312, 1987.
3. Wagner TE. Introduction and regulation of cloned genes for agricultural livestock improvement. In: Evans JW, Hollander A, Eds. Genetic Engineering of Mammals: An Agricultural Perspective. New York: Plenum Press, pp151–161, 1986.
4. Palmiter RD, Norstedt G, Gelinas E, Hammer RE, Brinster RL. Metallothionein human GH fusion genes stimulate growth of mice. Science **222**:809–814, 1983.
5. Pavlakis GN, Hamer DH. Regulation of a metallothionein-growth hormone hybrid gene in bovine papilloma virus. Proc Natl Acad Sci USA **80**:397–401, 1983.
6. Selden RF, Yun JS, Moore DD, Rowe M, Malia M, Wagner TE, Goodman HM. Glucocorticoid regulation of human growth hormone expression in transgenic mice and transiently transfected cells. J Endocrinol **122**:49–60, 1989.
7. Durnam DM, Hoffman JS, Quaife CJ, Benditt EP, Chen HY, Brinster RL, Palmiter RD. Induction of mouse metallothionein-I mRNA by bacterial endotoxin is independent of metals and glucocorticoid hormones. Proc Natl Acad Sci USA **81**:1053–1056, 1984.
8. Hager LJ, Palmiter RD. Transcription regulation of mouse liver metallothionein-I gene by glucocorticoids. Nature **291**:340–342, 1981.
9. Karin M, Herschman HR. Dexamethasone stimulation of metallothionein synthesis in HeLa cell cultures. Science **204**:176–177, 1979.
10. Palmiter RD, Chen HY, Brinster RL. Differential regulation of metallothionein-thymide kinase fusion genes in transgenic mice and their offspring. Cell **29**:701–710, 1982.
11. Mayo KE, Warren R, Palmiter RD. The mouse metallothionein-I gene is transcriptionally regulated by cadmium following transfection into human or mouse cells. Cell **29**:99–108, 1982.
12. Portanova R, Yun JS, Wagner TE. Expression of the hGH transgene in response to physiological challenges in mice. 70th Annual Meeting of the Endocrine Society, p1157, 1988.
13. 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.
14. Dudley GA, Portanova R. Histochemical characteristics of soleus muscle in hGH transgenic mice. Proc Soc Exp Biol Med **185**:403–408, 1987.
15. Moore DD, Marks AR, Buckley DI, Kapler G, Payvar F, Goodman HM. The first intron of the human growth hormone gene contains a binding site for glucocorticoid receptor. Proc Natl Acad Sci USA **82**:699–702, 1985.
16. Oh SH, Deagen JT, Whanger PD, Weswig PH. Biological function of metallothionein. V. Its induction in rats by various stresses. Am J Physiol **234**:E282–E285, 1978.
17. Reichlin S. Regulation of somatotropic hormone secretion. In: Greep RO, Astwood EB, Eds. Handbook of Physiology. Baltimore: American Physiology Society, Sec 7, Vol **IV**: p405, 1974.
18. Sayers G, Portanova R. Regulation of the secretory activity of the adrenal cortex: Cortisol and corticosterone. In: Greep RO, Astwood EB, Eds. Handbook of Physiology. Baltimore: American Physiology Society, Sec 7, Vol **VI**: p41, 1975.
19. Brattin WJ, Portanova R. Effect of glucocorticoid levels in vivo on growth hormone biosynthesis. Mol Cell Endocrinol **15**:19–27, 1979.
20. Mellon SH. Hormonal regulation of growth hormone gene expression. In: Raiti S, Tolman RA, Eds. Human Growth Hormone. New York: Plenum Press, p179, 1986.
21. Bremner I, Davies NT. The induction of metallothionein in rat liver by zinc injection and restriction of food intake. Biochem J **149**:733–738, 1975.