Differential GH-releasing hormone regulation of GHRH receptor mRNA expression in the rat pituitary

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Lasko, Catherine M., Andrew I. Korytko, William B. Wehrenberg, and Leona Cuttler. Differential GH-releasing hormone regulation of GHRH receptor mRNA expression in the rat pituitary. Am J Physiol Endocrinol Metab 280: E626–E631, 2001.—To understand the capacity of growth hormone-releasing hormone (GHRH) to regulate expression of the GHRH receptor, we studied the effects of GHRH on GHRH receptor mRNA expression in immature and adult rats by use of pituitary cell culture and immunoneutralization approaches. Pituitary cell cultures from neonatal (2-day-old) and adult (70-day-old) rats were treated with GHRH for 4, 24, or 72 h. The effect of GHRH on GHRH receptor mRNA expression depended on the duration of GHRH exposure in both age groups; short-term (4 h) GHRH treatment significantly reduced GHRH receptor mRNA expression (P < 0.05), whereas intermediate treatment (24 h) restored GHRH receptor mRNA to basal levels, and long-term treatment (72 h) stimulated GHRH receptor mRNA expression (P < 0.02). The long-term stimulatory effect of GHRH on GHRH receptor mRNA expression required the presence of serum in the culture medium, and, in the absence of serum, the stimulatory effect was completely abolished. Moreover, the capacity of the pituitary to increase GHRH receptor mRNA expression in response to 72-h GHRH treatment was age dependent, with neonatal pituitaries exhibiting a much greater stimulatory effect than adult pituitaries (P < 0.025). Immunoneutralization of endogenous GHRH significantly reduced GHRH receptor mRNA expression in neonatal (P < 0.004), juvenile (P < 0.003), and mature (P < 0.004) pituitaries compared with age-matched controls. Taken together, these results indicate that GHRH is a potent regulator of GHRH receptor gene expression in immature and mature pituitaries; however, the nature and direction of GHRH regulation of its receptor depend significantly on several variables, including the duration of GHRH exposure, the presence of permissive components in serum, and the developmental stage of the pituitary.

growth hormone-releasing hormone; development; neonate

THE GROWTH HORMONE-RELEASING HORMONE (GHRH) receptor plays a critical role in somatotroph function by mediating the stimulatory effects of GHRH on GH synthesis and secretion (3, 14, 15). Although there are several clear examples of hypothalamic secretagogues influencing expression of their pituitary receptors (27, 36, 37), data on the GHRH/GHRH receptor axis have been difficult to interpret due to apparent incongruities in findings. For example, in perinatal rats, short-term GHRH antiserum treatment of adult rats has been reported to increase GHRH receptor mRNA expression (30), whereas long-term GHRH antisera treatment has been found to decrease GHRH receptor mRNA expression (19). Furthermore, short-term GHRH treatment downregulates GHRH receptor mRNA expression of pituitary cells cultured in serum-free medium (1). However, animals that chronically overexpress GHRH in vivo maintain high circulating levels of GH and continue to secrete GH in response to exogenous GHRH treatment (26). These apparently disparate findings raise the possibility of a complex regulatory system in which the capacity of GHRH to influence expression of its pituitary receptor may depend on the duration of GHRH exposure, the ambient cell culture conditions, and/or the age of the animal studied. Although these factors are potentially critical in determining the effect of GHRH on its pituitary receptor, they have not been systematically assessed.

This study focuses on defining the regulation of GHRH receptor gene expression by GHRH in developing and mature pituitaries by means of in vitro and in vivo approaches. The specific aims were to 1) define whether the effect of GHRH on GHRH receptor mRNA expression is determined by the duration of exposure to GHRH, 2) determine whether the capacity of the pituitary to regulate GHRH receptor in response to GHRH is developmentally regulated, 3) determine whether the effect of GHRH on GHRH receptor mRNA expression is influenced by the presence of serum in the culture medium, and 4) investigate the role of GHRH in maintaining GHRH receptor mRNA expression during key stages of development.

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MATERIALS AND METHODS

In Vitro Studies

Animals and primary pituitary cell culture. Neonatal (2-day-old, day 0, day of birth) and young adult male (70-day-old) Sprague-Dawley rats (Zivic Miller, Zelienople, PA) were studied. These ages correspond to major changes in circulating GH levels (35, 40). All rats were maintained on a 12:12-h light (0600–1800)-dark cycle at constant ambient temperature and were provided food (standard lab chow) and water ad libitum. Animals were killed between 0900 and 1100 by decapitation. Whole pituitaries were removed, immediately placed in ice-cold Dulbecco’s Modified Eagle Medium (DMEM) with 25 mM HEPES and 0.3% bovine serum albumin (BSA), and prepared for primary cell culture as previously described (40). Cells were maintained in serum-containing medium (DMEM supplemented with MEM non-essential amino acids, l-glutamine, 10% horse serum, 2.5% fetal bovine serum, nystatin, and gentamicin) as described (16), and plated in 35-mm wells at a density of 3 x 10⁶ cells/well for 24 h before treatment.

Experimental protocol. To assess the influence of duration of exposure to GHRH on expression of GHRH receptor in neonatal and adult rats, pituitary cell cultures from both age groups were treated with 10 nM GHRH (Peninsula Laboratories, Belmont, CA) or vehicle (controls) for 4, 24, or 72 h, beginning 1 day after cell plating. The treatment dose of GHRH (10 nM) elicits a maximal GH secretory response in both perinatal and adult rat pituitary tissues; a representative experiment is shown. (controls) in serum-containing medium. Total RNA was analyzed for GHRH receptor mRNA expression to 66% of controls (153 ± 28% of controls). However, 72-h treatment period, medium (containing 10 nM GHRH or vehicle) was replaced every 24 h. After the treatment periods, total RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction (11) and retained at −70°C for analysis. GHRH receptor mRNA expression was determined in equal amounts of total RNA by ribonuclease protection assay, as previously described (25). Protected bands were quantified by radioimaging (AMBIS, San Diego, CA), and data were expressed as counts per minute relative to that of controls.

To assess the role of serum on basal and GHRH-mediated GHRH receptor mRNA expression, pituitary cells from adult rats were prepared and maintained in serum-containing medium for 24 h as described above. Cells were subsequently washed and maintained in either the serum-containing medium or a defined serum-free medium (DMEM with 0.2% BSA, 10 mM HEPES, parathyroid hormone 200 ng/l, glucagon 10 ng/l, transferrin 10 mg/l, penicillin-streptomycin, and nystatin [24, 45, 46]) and treated with either 10 nM GHRH or vehicle (controls) for 72 h. After the treatment period, total RNA was extracted, and GHRH receptor mRNA expression was assessed as described above.

In Vivo Studies

Animals and experimental protocol. Rats were studied at postnatal day 1 (neonate), day 25 (juvenile), or day 70 (adult). In each age group, rats were treated with a highly specific antisera to GHRH, in a dose known to be immunoneutralizing (10 µl/10 g for neonates and 250 µl for juveniles and adults; kindly provided by Dr. W. Wehrenberg, Clemson University) or an equal volume of normal rabbit serum (controls) subcutaneously daily for 14 days (42). Body weights were measured daily. After the 14-day treatment period, animals were killed by decapitation, and the pituitaries were removed and weighed to the nearest milligram, and total RNA was processed as described above. GHRH receptor mRNA expression was assessed in 20 µg of total pituitary RNA by ribonuclease protection assay. GH mRNA was assessed in 2 µg of total RNA by Northern blot analysis, as described previously (25). All protected or hybridized bands were quantified by radioimaging, and data were expressed as counts per minute per milligram of total RNA relative to that of controls.

Statistical Analysis

A minimum of three totally independent experiments was conducted for each treatment and age group for both in vitro and in vivo study protocols. For each outcome measure, the mean value for GHRH receptor mRNA expression in each experiment was utilized in the data analysis. GHRH receptor mRNA expression under experimental conditions was expressed as a percentage of that under vehicle-treated (control) conditions. Comparisons between groups were evaluated by paired or independent Student’s t-test, as appropriate. A P value <0.05 was equated with a significant statistical difference.

RESULTS

In Vitro Studies

Effect of GHRH treatment duration on GHRH receptor mRNA expression in neonates. GHRH receptor mRNA expression in neonatal pituitary cells was independent of the duration of GHRH treatment (Fig. 1). Short-term (4 h) exposure to GHRH reduced GHRH receptor mRNA expression to 66 ± 12% of controls (P < 0.045), and 24-h exposure to GHRH restored GHRH receptor mRNA expression to levels equivalent to those of controls (153 ± 28% of controls). However, 72-h
GHRH treatment of neonatal pituitary cells resulted in a dramatic increase in GHRH receptor mRNA levels over vehicle-treated controls (363 ± 65%, P < 0.015).

**Effect of GHRH treatment duration on GHRH receptor mRNA expression in adults.** The effect of GHRH on GHRH receptor mRNA expression was also markedly dependent on the duration of GHRH treatment in adult pituitaries (Fig. 2). Short-term (4 h) treatment with GHRH reduced expression of GHRH receptor mRNA to 62 ± 10% of controls (P < 0.02). However, 24-h GHRH treatment reestablished GHRH receptor mRNA expression to levels similar to those of controls. Moreover, 72-h GHRH treatment stimulated GHRH receptor mRNA expression significantly (176 ± 21% of controls, P < 0.01). The induction of GHRH receptor mRNA expression in neonatal pituitaries, however, far exceeded that in adult pituitaries treated identically (363 ± 65 and 176 ± 21% of controls, respectively, P < 0.025; Fig. 3).

**Effect of serum in the culture medium on GHRH receptor mRNA expression.** GHRH receptor mRNA expression was markedly affected by the presence of serum in the culture medium (Fig. 4). Basal expression of GHRH receptor mRNA by adult cells cultured in serum-free medium was 65 ± 5% of that by cells cultured in serum-containing medium (P < 0.002). When cells were cultured in serum-containing medium, 72-h GHRH treatment increased GHRH receptor mRNA expression (P < 0.01), but the stimulatory effect of GHRH was completely abolished when cells were cultured in serum-free medium (Fig. 4).

**In Vivo Studies**

Treatment with GHRH antiserum exerted pronounced effects on the GH axis in neonatal, juvenile, and adult rats. GHRH antiserum significantly reduced GHRH receptor mRNA abundance in all age groups (Fig. 5). GHRH receptor mRNA expression fell to 62 ± 5% of controls in neonates (P < 0.004), 59 ± 8% of controls in juveniles (P < 0.003), and 60 ± 9% of controls in adults (P < 0.004). In addition, treatment with GHRH antiserum decreased pituitary weight in neonatal (79 ± 3% of controls, P < 0.002) and juvenile rats (65 ± 4% of controls, P < 0.001) and decreased body weight in juvenile (59 ± 3% of controls, P < 0.001) and adult rats (43 ± 7% of controls, P < 0.0013). Pituitary GH mRNA levels were significantly reduced by GHRH antiserum treatment in all groups relative to controls (55 ± 4% of controls in neonates, P < 0.0062; 26 ± 5% of controls in juveniles, P < 0.0001; 64 ± 4% of controls in adults, P < 0.036).

**DISCUSSION**

GH secretion from pituitary somatotrophs is regulated primarily by two hypothalamic hormones, GHRH and somatostatin. GHRH stimulates GH synthesis and secretion after its binding to the GHRH receptor, whereas somatostatin inhibits GH secretion via its interaction with one or more somatostatin receptors (8, 38). In addition, the recent cloning of the GH-secretagogue receptor and identification of an endogenous ligand strongly implicate another endogenous regulator of GH secretion (20–22, 29). Collectively, these hormones are believed to establish normal circulating GH levels and to direct rhythmic pulses of GH secretion (41). Central to each of their actions are their respective pituitary receptors.
This study focuses on the homologous regulation of pituitary GHRH receptor by GHRH. Precedents show that control of receptors by their endogenous ligands represents a fundamental mechanism for biological regulation and frequently has therapeutic ramifications (13, 27, 36). The current data indicate that GHRH influences expression of its pituitary receptor in a multifaceted manner, with duration of exposure to GHRH, ambient culture medium, and age as key determinant variables.

Our results indicate that the duration of GHRH exposure influences the effect of GHRH on GHRH receptor mRNA expression. Although short-term exposure to GHRH reduces GHRH receptor mRNA expression, extended GHRH exposure (24 h) restores receptor mRNA expression to levels comparable to those of controls. Furthermore, more prolonged exposure to GHRH (72 h) stimulates GHRH receptor mRNA expression. These results suggest that GHRH exerts a short-term suppressive effect and a longer-term inductive effect on GHRH receptor mRNA levels. Earlier studies indicate that continuous GHRH treatment in vivo or in vitro initially stimulates GH levels but that GH responses then decline considerably (2, 5–7, 17, 43). In previous work, we also found that, with prolonged exposure of pituitary cell cultures to GHRH, GH concentrations in the medium rise and, in neonates, continue above basal; however, the GH secretory response to GHRH declines (12). Although depletion of GH stores may play a role in the reduction of GHRH-stimulated GH release after prolonged exposure to GHRH (9, 12), it has been suggested that a concomitant decrease in GHRH receptor mRNA expression or function (4, 5, 44) contributes to the fall in GH secretory response. The current results suggest, however, that, based on the stimulatory response of GHRH receptor mRNA expression exhibited after prolonged GHRH exposure, a decrease in GHRH-mediated stimulation of GH secretion after prolonged exposure to GHRH is not likely due to a direct decrease in gene expression of GHRH receptor.

The results also indicate that the presence of serum in the culture medium plays a permissive role in maintaining basal levels of the GHRH receptor and in mediating the stimulatory effect of GHRH on GHRH receptor mRNA expression. The precise serum components that are required for GHRH to exert its stimulatory effect are not known. Studies from our laboratory and others (24, 28, 31–33) have demonstrated that thyroid hormone and glucocorticoids, both present in serum, can stimulate GHRH receptor mRNA expression. We have previously found, however, that the stimulatory effect of thyroid hormone and glucocorticoids on the GHRH receptor is similar in neonates and adults (23), suggesting that these hormones are not likely responsible for the observed differential effect of age on GHRH-induced GHRH receptor mRNA expression. It is further unlikely that standard nutrients in serum-free medium inhibited the stimulatory effect of GHRH or that serum-free medium damaged the cells, because the defined medium is standard for studies of somatotroph function, and other regulatory agents alter GHRH receptor mRNA expression in such medium (24, 45, 46). Taken together with the previous data, the current results suggest a combinatorial process in the physiological regulation of GHRH receptor gene expression.

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Several in vivo and in vitro studies (10, 12, 18, 34, 39, 40) have demonstrated that pituitaries of perinatal animals exhibit heightened GH secretory response to GHRH compared with adult pituitaries and that perinatal pituitaries are relatively resistant to GHRH desensitization compared with mature pituitaries. We have previously demonstrated that expression of rat pituitary GHRH receptor mRNA is developmentally determined, with high levels in neonates and declining levels later in life; this pattern of GHRH receptor mRNA expression may contribute to classic developmental changes in circulating GH levels and to age-dependent pituitary responsiveness to GHRH (25). Therefore, it was of considerable interest to explore whether the capacity of GHRH to modulate GHRH receptor mRNA expression is developmentally determined. The results indicate that neonatal pituitaries exhibit a much greater induction of GHRH receptor mRNA expression after 72 h of GHRH treatment than adult pituitaries. The heightened capacity of the immature pituitary to stimulate GHRH receptor gene expression in response to GHRH may contribute to the previously observed differential responsiveness of neonatal and adult pituitaries to GHRH.

Although in vitro models provide important information on the capacity of hormones to regulate gene expression, a potential drawback is that they involve an artificial environment that may not be applicable to in vivo effects. Therefore, we also sought to determine whether GHRH is a mediator of GHRH receptor mRNA expression throughout development in vivo. Previous studies (19) have shown that passive immunonutralization of endogenous GHRH decreases GHRH receptor mRNA expression in the immature rat pituitary. The current findings demonstrate that GHRH is necessary and permissive for maintaining normal GHRH receptor mRNA expression throughout development. Of particular interest was the potent effect of GHRH antiserum on decreasing GH mRNA expression in juvenile rats. The dramatic decrease in GH mRNA, coupled with the decrease in GHRH receptor mRNA, suggests that GHRH is critically important in mediating pituitary function during sexual maturation.

In summary, the results of this study indicate that GHRH is a key mediator of GHRH receptor mRNA expression both in vitro and in vivo. Moreover, the results demonstrate that the nature and direction of GHRH regulation of its receptor depend significantly on several variables, including the duration of GHRH exposure, the ambient pituitary cell environment, and the age of the animal. Recognition of the combinatorial process by which GHRH influences expression of its pituitary receptor is essential to developing an understanding of the physiological regulation of the GHRH receptor and the GH axis.

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REFERENCES


