Galanin Counteracts the Inhibitory Effects of Glucocorticoids on Growth Hormone Secretion in the Rat

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The aim of our study was to investigate the effect of galanin on baseline and growth hormone (GH)-releasing hormone (GHRH)-stimulated GH concentrations in conscious, freely moving rats receiving long-term glucocorticoid treatment. Animals were treated for 7 days with an intraperitoneal injection of either vehicle or dexamethasone ([dex] 40 μg/d). Rats underwent the following experimental trials: at −15 minutes animals received an intravenous injection of saline or galanin (12.5 μg/kg), and at 0 minutes rats received a second intravenous injection of saline or rat GHRH (500 ng/kg). Blood samples were drawn every 5 minutes from −15 to +15 minutes and then at 30 minutes. The GH response to saline + GHRH alone was significantly higher ($P < .05$) in chronically vehicle-treated rats as compared with chronically dex-treated ones. In contrast, galanin + saline increased serum GH levels in a similar fashion in both chronically vehicle- and dex-treated rats. The response to galanin + GHRH was similar to galanin + saline in chronically vehicle-treated rats, but was significantly enhanced in chronically dex-treated rats. These results suggest that galanin-mediated GH release in rats may involve somatostatinergic pathways.
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ELEVATED LEVELS of the glucocorticoids, whether due to exogenous or endogenous sources, interfere with normal somatic growth in laboratory animals[1] and humans.[2,3] Numerous studies have demonstrated that both rats[4] and normal men[5] treated with pharmacological doses of glucocorticoids have reduced growth hormone (GH) responses to exogenous GH-releasing hormone (GHRH). On the other hand, glucocorticoids enhance GH release in vitro,[6,7] and GH deficiency is resolved in hypoadrenal patients during glucocorticoid replacement therapy.[8]

GH is regulated by the hypothalamic peptides, GHRH, which has an excitatory role, and somatostatin, which has an inhibitory role. Recently, several studies performed in rats have shown that the inhibitory effects of glucocorticoids on GH secretion in vivo[4] are due to enhancement of hypothalamic somatostatin release. Specifically, the inhibitory effects of glucocorticoids are reversed in vivo by passively immunizing rats against somatostatin[4] or by using pentobarbital anesthesia.[9] Both procedures are known to decrease endogenous somatostatin tone.[10]

Galanin is a 29–amino acid peptide originally isolated from porcine intestine.[11] It is thought to be a neurotransmitter in the central nervous system of several mammalian species, including rats.[12] Considerable evidence exists to suggest that galanin selectively stimulates GH secretion in different animal species.[13,14] The mechanism of this stimulatory action of galanin is unclear. Experimental studies suggest that the action of galanin may be mediated either by a decrease in hypothalamic somatostatin secretion[15] or by an increase in endogenous GHRH secretion.[16] The aim of our study was to investigate the effect of galanin administration on baseline and GHRH-stimulated GH secretion in rats receiving long-term glucocorticoid treatment, an animal model hypothesized to have elevated somatostatin tone.[4]

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MATERIALS AND METHODS

All procedures were reviewed and approved by the University of Wisconsin-Milwaukee Animal Care and Use Committee before conducting the studies. Male Sprague–Dawley rats (225 to 250 g) were housed in a temperature- and humidity-controlled environment and exposed to a 14-hour light, 10-hour dark lighting schedule (lights on at 7:30 AM). Food and water were available ad libitum.

Animals were treated daily (intraperitoneally, 9:00 AM) for 7 days, including the day of experimentation, with either vehicle (0.5 mL) or the synthetic glucocorticoid dexamethasone sodium phosphate ([dex] 40 μg/d; Decadron; Merck, Sharp, & Dohme, West Point, PA). This dose was selected based on its ability to inhibit somatic growth[17] and the GH response to GHRH in conscious rats[4] and to enhance the GH response to GHRH in rats with reduced somatostatin tone.[9] The duration was selected based on the study by Seifert et al[18] that showed changes in pituitary GHRH receptor binding sites within 24 hours of glucocorticoid treatment in rats.

One day before testing, animals were prepared with an intravenous catheter under ether anesthesia as previously described.[19] At approximately noon on the day of experimentation, a control blood sample (0.4 mL) was drawn at −15 minutes, followed immediately by an intravenous injection of saline (0.5 mL) or galanin (12.5 μg/kg). Subsequent blood samples were drawn at −10, −5, and 0 minutes. Following the 0-minute blood sample, rats received a second intravenous injection of saline (0.5 mL) or rat GHRH (500 ng/kg). Subsequent blood samples were drawn at 5, 10, 15, and 30 minutes. Sample sizes were between six and 10 animals per group (see figures for specific group sizes). We ensured that changes in GH concentrations reflected treatment effects by (1) initiating experiments at approximately noon, a predicted time of a GH trough based on our lighting schedule, and (2) not including any animals in the study that had baseline (15 minutes) GH concentrations greater than 50 ng/mL. The effectiveness of these criteria in eliminating animals with spontaneous GH pulses is best illustrated in Fig 2.

Serum GH concentrations were determined by radioimmunoassay using a double-antibody method and reagents provided by the

National Institutes of Health. The within-assay coefficient of variation was 8%, and the between-assay coefficient of variation was 10%. The area under the response curve (AUC) was calculated by trapezoid analysis. Data were subjected to log transformation to correct for heterogeneity of variance. For clarity of presentation, data are presented as the arithmetic mean ± SEM. Significant treatment effects were identified by multifactorial ANOVA for repeated measures.[20]

RESULTS

Galanin significantly increased ($P < .05$) serum GH with respect to baseline concentrations in both chronically vehicle-treated and dex-treated rats. Moreover, GH levels after galanin administration were not different between the two treatment groups (Fig 1). The AUC was 1.61 ± 0.30 μg/mL/45 min in the vehicle-treated group and 1.46 ± 0.31 in the dex-treated group.

The injection of GHRH increased ($P < .05$) GH concentrations in chronically vehicle-treated rats. As illustrated in Fig 2, serum GH levels in rats chronically treated with dex were significantly lower ($P < .05$) than in chronically vehicle-treated animals. The AUC was 0.95 ± 0.22 ng/mL/45 min in the latter group and only 0.31 ± 0.12 in the former group.

When GHRH was administered 15 minutes after galanin treatment, the results were the opposite. That is, chronically dex-treated rats demonstrated an enhanced GH response to GHRH administration as compared with chronically vehicle-treated rats (Fig 3). Moreover, galanin injection enhanced the GH response to GHRH only in chronically dex-treated rats. The AUC was similar in the two groups, 1.16 ± 0.35 μg/mL/45 min in chronically vehicle-treated animals and 1.22 ± 0.31 in chronically dex-treated rats.

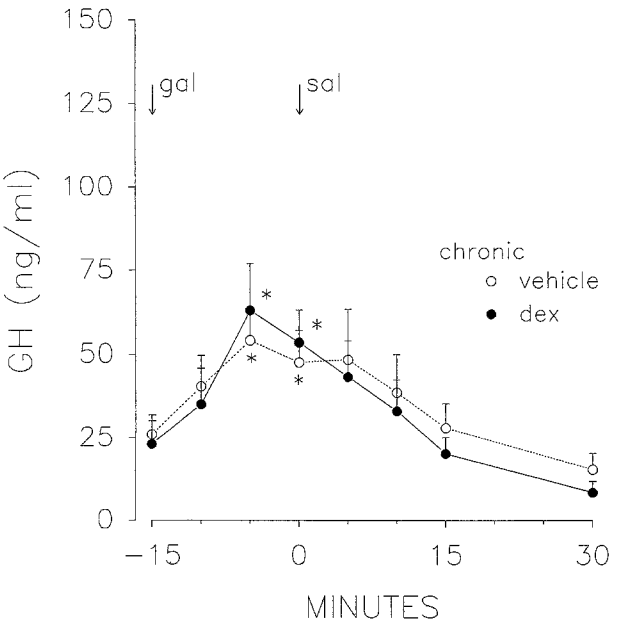

Fig 1. Serum GH levels (ng/mL, mean ± SEM) after intravenous administration of galanin ([gal] 12.5 μg/kg) at −15 minutes followed by saline ([sal] 0.5 mL) at 0 minutes in chronically vehicle-treated rats (n = 7) and chronically dex-treated rats (n = 8). *$P < .05$ *v* baseline value (−15 minutes) of that respective group.

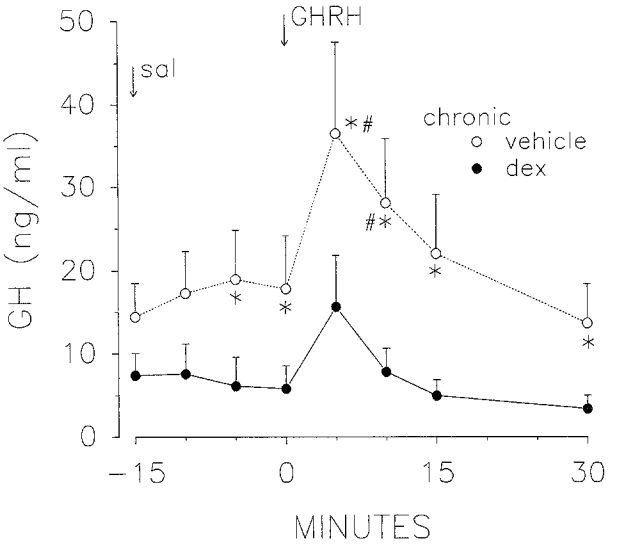

Fig 2. Serum GH levels (ng/mL, mean ± SEM) in chronically vehicle-treated rats (n = 8) and chronically dex-treated rats (n = 10) following intravenous administration of saline ([sal] 0.5 mL) at −15 minutes and rat GHRH (500 ng/kg) at 0 minutes. *$P < .05$ *v* baseline value (−15 minutes) of that respective group. #$P < .05$ *v* that time point of the other treatment group.

DISCUSSION

Numerous studies have established the inhibitory effects of glucocorticoids on somatic growth.[1,2] This inhibition has been explained in part by a glucocorticoid-mediated alteration in GH secretion.[5,17,21] A primary mechanism involved

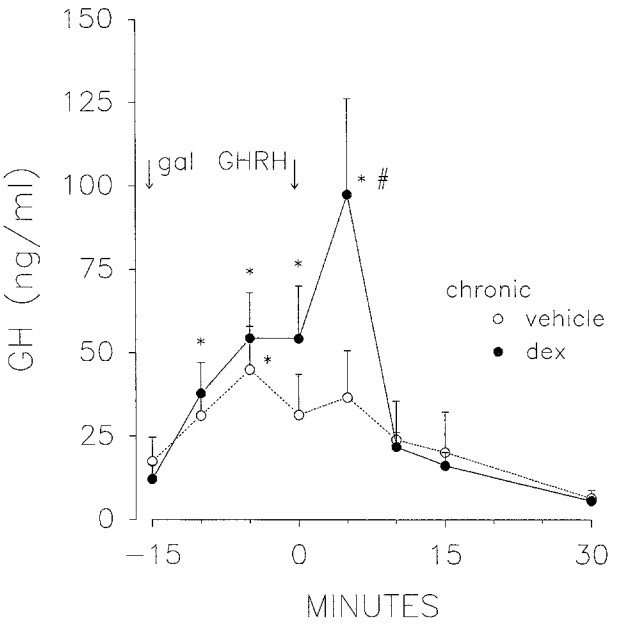

Fig 3. Serum GH levels (ng/mL, mean ± SEM) in chronically vehicle-treated rats (n = 6) and chronically dex-treated rats (n = 6) following intravenous administration of galanin ([gal] 12.5 μg/kg) at −15 minutes and rat GHRH (500 ng/kg) at 0 minutes. *$P < .05$ *v* baseline value (−15 minutes) of that respective group. #$P < .05$ *v* that time point of the other treatment group.

in this inhibition is increased hypothalamic somatostatin tone.[10] This has been demonstrated by the enhanced GH response to GHRH obtained in chronically dex-treated rats passively immunized against somatostatin[4] or anesthetized with sodium pentobarbital.[9] Data showing that pharmacological doses of glucocorticoids increase somatostatin synthesis and release from the rat 44-2c cell line[22] and increase hypothalamic somatostatin content[23] and mRNA in the rat[24] also support this conclusion.

One of the most prominent effects of galanin is its ability to increase GH secretion.[13,14] Our understanding of the mechanism underlying this action is still developing. Studies performed in man suggest that the actions of galanin may be mediated either by a decrease in hypothalamic somatostatin[15,25] or by an increase in hypothalamic GHRH.[21,26] In support of the latter, Murakami et al[14] have shown that rat GHRH antiserum markedly attenuates the GH response to galanin, and galanin has been shown to stimulate immunoreactive GHRH secretion from perfused rat hypothalamic slices.[16] Therefore, current evidence seems to suggest that hypothalamic GHRH may be important in mediating the action of galanin. However, no data are available on the possible interactions between galanin and somatostatin.

Our observations confirm that galanin significantly stimulates GH secretion in the normal rat.[14,27] Our data also demonstrate that a 7-day high-dose treatment of glucocorticoids does not impair the GH response to galanin. Most interestingly, galanin reversed the blunted GH response to GHRH in chronically dex-treated rats. Administration of pharmacological doses of glucocorticoids is thought to decrease the GH response to GHRH through an increase in hypothalamic somatostatin tone.[10] That galanin stimulates GH secretion in a similar fashion in both chronically vehicle- and dex-treated rats suggests that the mechanism of the GH-releasing activity of galanin in the rat may, in part, involve a decrease in somatostatin release by the hypothalamus. The present study is well designed to illustrate this, since the somatostatin-inhibiting activity of galanin may counteract the glucocorticoid-mediated increase in hypothalamic somatostatin tone. This conclusion is also supported by the other observation in this experiment showing that after galanin pretreatment there was an enhanced GH response to GHRH in chronically dex-treated rats as compared with chronically vehicle-treated animals. We have previously demonstrated that an enhanced GH response to GHRH occurs when endogenous somatostatin is neutralized.[9] Other mechanisms participating most likely include enhanced pituitary GHRH receptors[18] and GH synthesis.[28]

The fact that galanin did not enhance the GH response to GHRH in animals pretreated with vehicle was unexpected. This most likely reflects the interaction of numerous factors. First, it is known that glucocorticoids enhance the GH response to GHRH in vitro and in vivo when endogenous somatostatin secretion is neutralized.[4,7] This is accomplished via increased GH synthesis[28] and pituitary GHRH receptors.[18] Therefore, the glucocorticoid-treated animals had a greater GH response to galanin and GHRH because their pituitary secretory capacity was simply higher than that in vehicle-pretreated animals. Two, it is possible that the dose of galanin resulted in a maximum response in vehicle-pretreated animals, and therefore, GHRH would have no additional effect.

Recently, it has been reported that galanin is unable to normalize the GH response to GHRH in human subjects after long-term treatment with glucocorticoids.[26] Due to the fact that the decrease in GH secretion also has been hypothesized to be mediated via somatostatin,[10] it can be suggested either that galanin activates different hypothalamic pathways in the rat and man or that changes in the relative sensitivity of hypothalamic somatostatin neurons to glucocorticoids and galanin may occur between species.

In summary, galanin is able to counteract the glucocorticoid-mediated inhibition of baseline and GHRH-stimulated GH secretion in the rat. We hypothesize that this GH-releasing action of galanin occurs at the hypothalamic level and involves both GHRH and somatostatin pathways.

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