Role of Galanin in the Regulation of Somatotrope and Gonadotrope Function in Young Ovulatory Women

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The aim of the study was to elucidate the role of the neuropeptide galanin in the regulation of somatotropic and gonadotropic function in normal women. Thirteen normally ovulating (aged 28 to 40 years), non-obese (body mass index, 18.4 to 27.1 kg/m^2) women with infertility due to a tubal or male factor were studied. Each woman underwent three tests: (1) bolus intravenous (IV) injection of growth hormone (GH)-releasing hormone (GHRH) (1-29)NH_2 1 μg/kg plus gonadotropin-releasing hormone (GnRH) 100 μg at time 0; (2) IV infusion of porcine galanin 500 μg in 100 mL saline from −10 minutes; and (3) bolus IV injection of GHRH(1-29)NH_2 1 μg/kg plus GnRH 100 μg at time 0 plus IV infusion of porcine galanin 500 μg in 100 mL saline from −10 to +30 minutes. All results are expressed as the mean ± SEM. GH peak after GHRH was 14 ± 5 μg/L; porcine galanin significantly increased serum GH (GH peak, 7.3 ± 1.2) with respect to baseline levels. No significant differences were observed between either GH peak or GH absolute values after galanin as compared with GHRH alone. Porcine galanin significantly enhanced GH response to GHRH (peak, 31.4 ± 4.4 μg/L) with respect to either GHRH or galanin alone. Luteinizing hormone (LH)/follicle-stimulating hormone (FSH) peaks after GnRH were 16.5 ± 5.3 and 17.4 ± 4 IU/L, respectively. Porcine galanin did not cause significant increases in serum LH and FSH levels with respect to baseline. Galanin coadministration slightly increased LH (peak, 25 ± 6.9 IU/L) but not FSH (peak, 17.1 ± 3.2) maximal responses to GnRH. In conclusion, galanin plays a significant role in the regulation of GH secretion in women, as well as in men. Moreover, this report suggests a minor role of galanin in the regulation of the hypothalamic-pituitary-ovarian axis in women.

GONADOTROPINS are episodically secreted with specific characteristics throughout the menstrual cycle.[1] In fertile women, stimulatory actions of gonadotropins determine follicle growth and differentiation, as well as sex steroid secretion from the ovary.[2]

Growth hormone (GH) synthesis and secretion are regulated by the hypothalamic peptides GH-releasing hormone (GHRH), which has an excitatory role, and somatostatin, which has an inhibitory role.[3] Estrogens affect GH secretion in humans, probably acting directly at the pituitary level, although a hypothalamic site of action has also been hypothesized.[4] A co-gonadotropic effect of GH has been demonstrated in vitro on human granulosa cells.[5]

Galanin is a 29–amino acid peptide with a wide distribution in central neurons of several mammalian species, including humans.[6] Synthetic porcine galanin elicits GH secretion when given alone[7] and enhances GH response to GHRH in normal adults.[8] The stimulatory action of galanin has been hypothesized to be mediated either by an increase in endogenous GHRH secretion[9,10] or by a decrease in hypothalamic somatostatin secretion.[11] Galanin has also been hypothesized to have a stimulatory role in the regulation of luteinizing hormone (LH) secretion in the rat, which is exerted both at the hypothalamic level and at the pituitary level. In fact, in male rats a stimulatory effect of rat galanin on gonadotropin-releasing hormone (GnRH) secretion from arcuate nucleus–median eminence fragments in vitro has been demonstrated. This effect is apparent at doses as low as 50 nmol/L and requires a functional noradrenergic neurotransmission.[12] Galanin also induces a small dose-dependent increase in LH secretion from cultured, dispersed anterior pituitary cells and enhances the ability of GnRH to stimulated LH secretion.[13] Our previous data showed that galanin did not influence either baseline or LH-releasing hormone (LHRH)-stimulated LH and follicle-stimulating hormone (FSH) secretion in young males[8]; however, it cannot be excluded in humans, as has been hypothesized for the rat,[12] that circulating estrogens play a crucial permissive role in regulating the action of galanin on pituitary gonadotropic function.

The aim of our study was (1) to elucidate the physiologic role of the neuropeptide galanin in the regulation of somatotrophic and gonadotropic function in eumenorrheic women with ovulatory cycles; and (2) to evaluate the relationship between somatotrope and gonadotrope secretory responses to their specific hypothalamic-releasing factors and to galanin.

SUBJECTS AND METHODS

Patients

Thirteen normally ovulating (aged 28 to 40 years), non-obese (body mass index, 18.4 to 27.1 kg/m^2) women with infertility due to a tubal or male factor were studied. All were referred to the Division of Gynecology at our hospital to be submitted to in vitro fertilization procedures. None of the patients had a family history of endocrine or metabolic diseases or were taking any medications at the beginning of the study. The study protocol was approved by the local Ethics Committee, and patients provided informed consent before entering the study.

Methods

All the subjects were admitted to our Clinical Research Unit and rested in a recumbent position with an antecubital vein catheter inserted percutaneously throughout the test. Each woman under-

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went the following tests during the first 7 days of the follicular phase of the menstrual cycle in random order after an overnight fast: (1) bolus intravenous (IV) injection of GHRH (1-29)NH_2 (Geref, Serono, Italy) 1 μg/kg plus GnRH (Relisorm, Serono, Italy) 100 μg at time 0; (2) IV infusion of sterile, endotoxin-free synthetic porcine galanin (Inalco, Milano, Italy) 500 μg in 100 mL saline from time −10 to +30 minutes; and (3) bolus IV injection of GHRH(1-29)NH_2 1 μg/kg plus GnRH 100 μg at time 0, plus IV infusion of sterile, endotoxin-free synthetic porcine galanin 500 μg in 100 mL saline from time −10 to +30 minutes. The study protocol was designed based on previous reports showing that galanin elicits significant pituitary GH responses in humans only when infused over periods of 40 to 60 minutes.[7,8,10,11]

Blood samples for determination of GH, LH, and FSH were drawn at −15, 0, +15, +30, +45, +60, +90, and +120 minutes after an IV bolus of GHRH and LHRH, and at −15, −10, 0, +15, +30, +45, +60, +90, and +120 minutes when galanin was infused alone or in combination with the releasing hormones. Basal blood samples at −15 minutes of each test were drawn for 17β-estradiol, progesterone, and prolactin assays.

Assays

All samples from the same subject were assayed together in duplicate. Commercial kits were used for determination of GH (immunoradiometric assay [IRMA], Allegro hGH; Nichols Institute, San Juan Capistrano, CA; interassay and intraassay coefficients of variation [CVs], 7% and 4%; sensitivity limit, 0.02 μg/L), prolactin (IRMA; Ares, Serono, Italy; interassay and intraassay CVs, 6.4% and 2.1%; sensitivity limit, 0.3 μg/L), LH (IRMA, LH; MAIACLONE, Serono, Italy; interassay and intraassay CVs, 8.5% and 6.4%; sensitivity limit, 0.22 mU/L), FSH (IRMA, FSH; MAIACLONE, Serono, Italy; interassay and intraassay CVs, 3.7% and 2.6%; sensitivity limit, 0.26 mU/L), 17β-estradiol (Estradiol Coat-A-Count; DPC, Los Angeles, CA; interassay and intraassay CVs, 7.4% and 5.8%; sensitivity limit, 8.6 pg/mL), and progesterone (Progesterone Coat-A-Count; DPC; interassay and intraassay CVs, 5.1% and 8.8%; sensitivity limit, 2 ng/mL).

Statistical Analysis

Results are expressed as the mean ± SEM. Hormonal secretory responses to the various stimuli were expressed either as absolute values or as peak values. Absolute and peak values of each hormone after the three different stimuli were compared with Student's *t* test for paired data. Correlations between the various endocrine parameters were found with linear regression analysis. *P* less than .05 was considered statistically significant.

RESULTS

GH peak after GHRH was 14 ± 5 μg/L; porcine galanin induced a significant increase in serum GH (GH peak, 7.3 ± 1.2 μg/L) with respect to baseline levels. No significant differences were observed for either GH peak or GH absolute values after galanin as compared with GHRH alone. Porcine galanin significantly enhanced GH response to GHRH (peak, 31.4 ± 4.4 μg/L) with respect to either GHRH or galanin alone (Fig 1).

LH/FSH peaks after GnRH were 16.5 ± 5.3 and 17.4 ± 4 IU/L, respectively. Porcine galanin did not cause significant increases in serum LH and FSH with respect to baseline levels. Galanin coadministration slightly but not significantly increased mean LH (peak, 25 ± 6.9 IU/L) but not

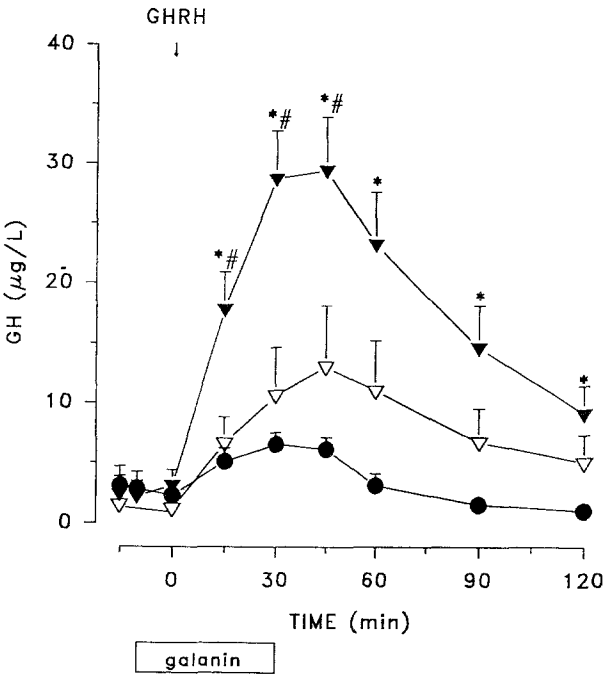

Fig 1. Serum GH levels (mean ± SEM) in 13 normal women after (●) galanin IV infusion from −10 to +30 minutes, (▽) GHRH IV bolus at 0 minutes, and (▼) galanin IV infusion from −10 to +30 minutes + GHRH IV bolus at 0 minutes. *Galanin *v* galanin + GHRH, *P* < .05; #GHRH *v* galanin + GHRH, *P* < .05.

FSH (peak, 17.1 ± 3.2) maximal responses to GnRH. In fact, nine of 13 subjects showed greater LH responses to galanin plus GnRH as compared with GnRH alone; two subjects had identical responses to the two stimuli, and the remaining two subjects had greater GH responses to GnRH alone as compared with galanin plus GnRH. Finally, no significant differences in either LH or FSH absolute values after galanin plus GnRH as compared with GnRH alone were observed at any time point of the curves (Fig 2).

A significant positive relationship was observed between GH peak after GHRH and LH peak after GnRH ($r = .796$, $P < .05$). No significant correlations between GH and LH peaks were observed when galanin was coadministered with GHRH and GnRH (Fig 3). GH peak after GHRH was significantly correlated with 17β-estradiol baseline levels ($r = .733, P < .05$).

DISCUSSION

Our findings demonstrate that in young women, GH responsiveness to GHRH is significantly increased by galanin coadministration. In addition, galanin enhances the maximal LH response elicited by GnRH in young women. Finally, our data show that the GH response to GHRH is significantly correlated with LH response to GnRH and with baseline 17β-estradiol levels; however, these correlations do not reach statistical significance after galanin coadministration.

Galanin is a potent and specific GH secretagogue in men.[7,8,10,11] This neuropeptide has been proposed to act at the hypothalamic level in eliciting GH secretion, since it

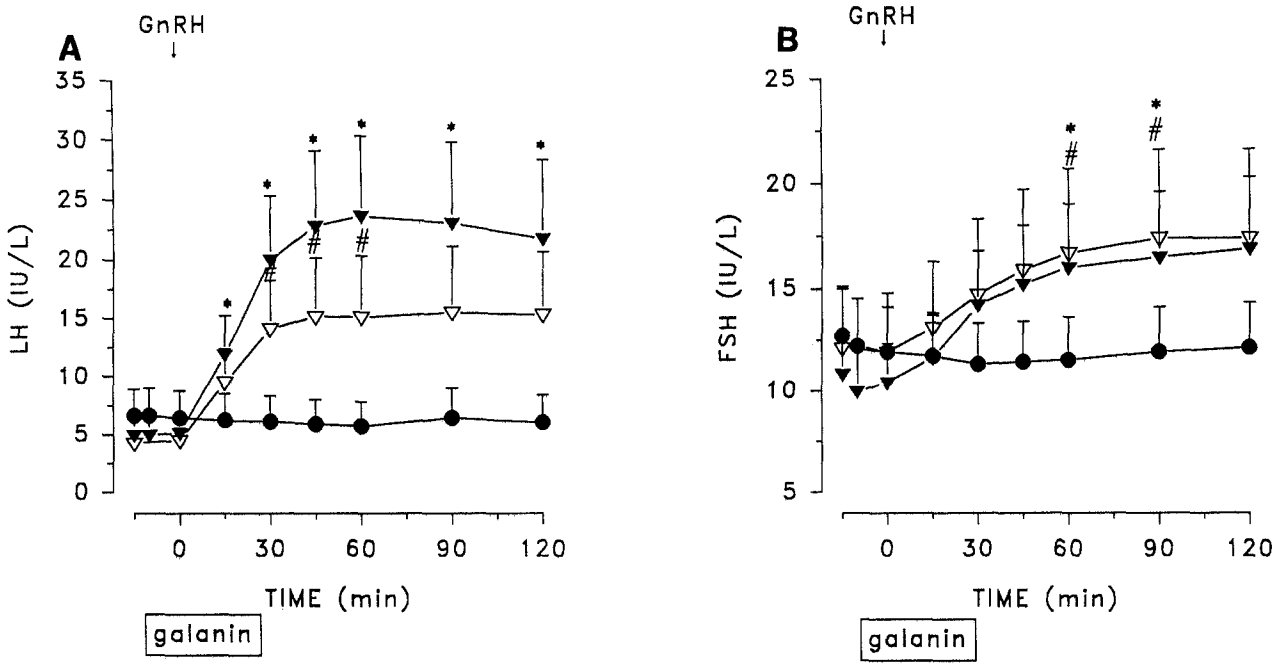

Fig 2. Serum (A) LH and (B) FSH levels (mean ± SEM) in 13 normal women after (●) galanin IV infusion from −10 to +30 minutes, (▽) GnRH IV bolus at 0 minutes, and (▼) galanin IV infusion from −10 to +30 minutes + GnRH IV bolus at 0 minutes. *Galanin *v* galanin + GnRH, $P < .05$; #galanin *v* GnRH, $P < .05$.

was effective when injected into the rat third ventricle (intracerebroventricularly [ICV]), but had no effect when added to rat anterior pituitary cells in vitro.[14] Moreover, ICV injection of specific antiserum to galanin markedly decreased plasma GH concentration, leading to a major reduction of GH pulse amplitude but with an increase in pulse frequency[15]: this latter evidence also suggested that galanin had a physiologic role in regulating GH release. Episodic GH secretion is regulated by interaction between GHRH and somatostatin.[3] Most of the neurotransmitters modulating GH secretion are believed to act through either GHRH and/or somatostatin.[4] An involvement of GHRH in the GH-releasing action of galanin has been proposed after observing that treatment of male rats with GHRH antiserum markedly inhibits GH response to either IV or ICV galanin administration.[16] The demonstration of the coexistence of galanin and GHRH in the same neurons in the arcuate nucleus[17] further suggests the possibility of interaction between the two neuropeptides. However, experimental observations indicate innervation of somatostatin neurons by galanin cells in the hypothalamus.[18] Therefore, it has also been suggested that galanin may stimulate GH secretion via a decrease in hypothalamic somatostatin.[11] Our recent data provided clear evidence that galanin

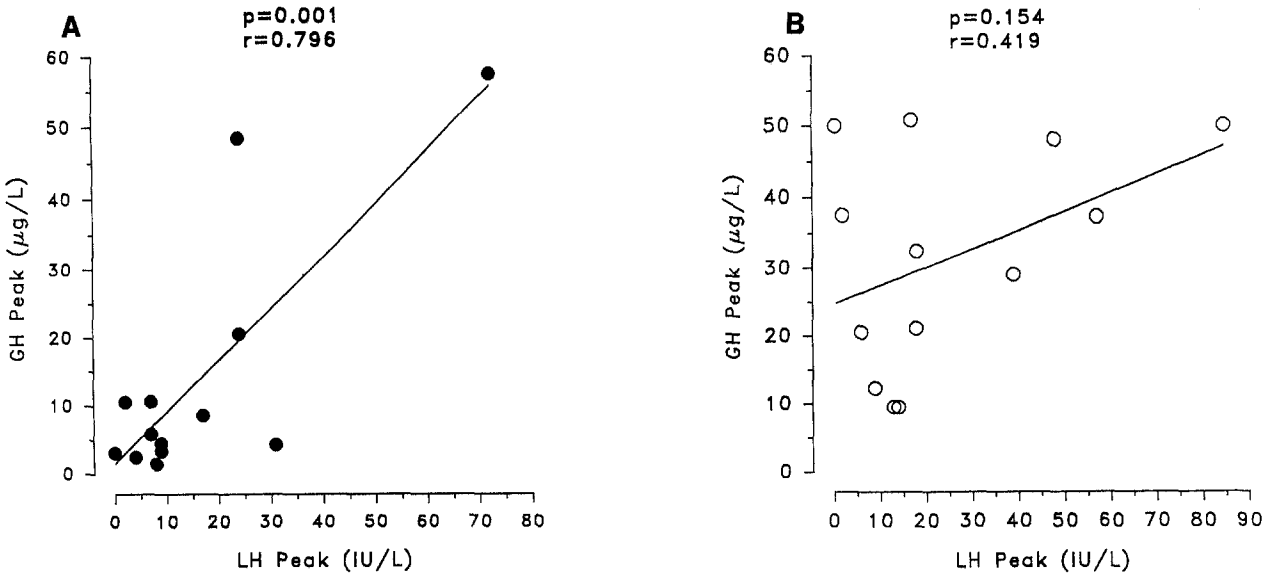

Fig 3. Relationship between GH and LH peaks after (A) GHRH + GnRH and (B) galanin + GHRH + GnRH in 13 normal women.

infusion causes a consistent increase in baseline GH levels in both males and females,[7,19] and is also a potent enhancer of the GH response to GHRH, at least in males.[8] However, to our knowledge, limited information is available on the interaction between galanin and GHRH in normal women. Our data show that in young women, as well as in men, galanin exerts a potent synergistic action with GHRH on GH secretion. GH response to GHRH in our study subjects was correlated with baseline estrogen levels measured on the morning of the test; on the other hand, no significant correlations were observed between GH peaks after either GHRH plus galanin or galanin alone and baseline 17β-estradiol levels. Galanin gene expression is greater in female rats and is regulated by estrogens; therefore, the peptide may be hypothesized to mediate the effects of ovarian steroids on both hypothalamic and anterior-pituitary hormones.[20] It can be hypothesized that the stimulatory action of estrogens on GH response to GHRH may be mediated in physiologic conditions by the endogenous production of galanin, which in turn could be thought to decrease hypothalamic somatostatin tone and enhance endogenous GH responsivity to GHRH. In the past 5 years, animal experiments on the role of galanin in the regulation of gonadotropins, particularly LH, have produced contradictory results. Some reports have shown negative results,[21] whereas others have shown that galanin, after ICV administration, exerts a stimulatory effect on LH release in ovariectomized, estrogen/progesterone-primed rats.[12] The discrepancies observed between these reports might be explained in part by the distinct steroid environment, suggesting that estradiol and progesterone priming is necessary for porcine galanin to exert an effect on gonadotrope cells.

The action of galanin on gonadotropin secretion has been explained at two levels of integration. On one hand, galanin could enhance LH secretion by increasing hypothalamic GnRH output.[22] On the other hand, galanin could stimulate LH secretion or enhance GnRH action at the pituitary level.[13] Previous data from our group show that galanin does not influence either baseline or GnRH-stimulated LH and FSH secretion in men. However, our study subjects were all young males; therefore, it was suggested that in humans, as has been hypothesized for the rat,[12] circulating estrogens may play a crucial permissive role in regulating the action of galanin on pituitary gonadotropic function. The present study confirms this previously suggested hypothesis by showing that in normal women galanin slightly enhances the maximal LH response to GnRH, even if the neuropeptide does not affect baseline LH levels when administered alone. Therefore, we can hypothesize that galanin may play a minor estrogen-dependent role in the regulation of LH secretion in women, likely by acting directly on the pituitary, facilitating the stimulatory action of LHRH.

Our data also demonstrate a strong correlation between GH and LH peaks after concomitant administration of their releasing hormones, GHRH and GnRH. The co-gonadotropic effect of GH has been demonstrated on human granulosa cells.[5] Moreover, GH treatment has been shown to be effective in increasing the number of mature follicles induced by gonadotropin induction in women.[23,24] Similarly, stimulation of endogenous GH by GHRH treatment increases the ovulation induction by exogenous gonadotropin administration.[25] All these data support the role of GH as a cofactor in follicle maturation and in the induction of ovulation. Recently, it has also been reported that 7 days of GH administration significantly modified integrated LH plasma levels and the LH pulse frequency and amplitude in normogonadotropinemic amenorrheic patients.[26] It can be hypothesized that endogenously secreted GH may influence, with a direct effect on the gonadotropes, LH responsiveness to GnRH. Therefore, it could be suggested that reduced endogenous GH secretory capacity may negatively influence the function of the hypothalamic-gonadotropic-ovarian axis. This observation provides interesting experimental support for the role of GH in the treatment of ovulation induction.[23,24] On the basis of our data, it can also be hypothesized that the stimulatory effect of galanin on gonadotropin secretion may occur indirectly via an increase in endogenous GH secretion. The fact that after exogenous galanin coadministration a significant correlation between GH and LH peaks after GHRH and GnRH is no longer observed may suggest that endogenous levels of galanin may play a significant role in regulating this GH-LH interaction.

In conclusion, galanin plays a significant role in the regulation of GH secretion in women, as well as in men. Moreover, this report suggests a minor role for galanin in the regulation of the hypothalamic-pituitary-ovarian axis in women.

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