Physiological Role of the Opioid-Cholinergic Interaction in Growth Hormone Neuroregulation: Effect of Sex and Food Intake

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Studies performed in animals and humans have suggested a functional interaction between opioid and cholinergic systems in the control of growth hormone (GH) secretion. Moreover, the sex-dependent modulation of GH secretion in humans is well established. To investigate the role of sex and food intake in the regulation of the reciprocal influences of opioids and acetylcholine in the modulation of GH secretion, we studied the GH response to pyridostigmine (PYR) alone and during a naloxone (NAL) infusion in a group of normal men and women before a meal (at 1:00 PM) and postprandially. In women, the response of GH to PYR alone before the meal was significantly lower than in the men (area under the curve [AUC], mean ± SEM, 320.18 ± 87.16 *v* 1,031.06 ± 333.21 μg/L/90 min, $P < .01$). Before the meal, NAL completely abolished the response of GH to PYR in men (AUC, 1,031.06 ± 333.21 *v* 16.50 ± 7.50 μg/L/90 min, $P < .01$), whereas infusion of NAL did not significantly modify the GH response to PYR in women. Consumption of the meal significantly decreased PYR-induced GH release in both women (AUC, 21.75 ± 12.75 *v* 320.18 ± 87.16 μg/L/90 min, $P < .05$) and men (AUC, 45.75 ± 18.75 *v* 1,031.06 ± 333.21 μg/L/90 min, $P < .01$). Conversely, food intake did not change the effects of NAL infusion on the GH response to PYR either in women or in men. We conclude that the sex-dependent opioid modulation of PYR-induced GH secretion is observed before a meal but not in the postprandial state. Food intake may be hypothesized to influence the cholinergic regulation of GH secretion and the sex-dependent opioid modulation of central cholinergic tone.

IT IS KNOWN THAT GROWTH HORMONE (GH) RELEASE is modulated by a large number of neurotransmitters, which act through the mediation of the two final pathways of GH-releasing hormone (GHRH) and somatostatin.[1] Studies performed on animals and humans have suggested that the action of opiate analogs on GH secretion is mediated by cholinergic neurons[2] via somatostatin.[3-5] It is also well known that GH neuroregulation is sexually dimorphic both in animals[6] and in humans.[7,8] However, it remains unclear whether significant gender influences may exist in the effect of cholinergic agonists on GH secretion.

Similarly, it is at present unclear if the opioid effects on GH secretion are different in male and female human subjects. On the other hand, we have previously demonstrated that food intake may significantly influence the regulation of GH secretion in normal humans, presumably via somatostatin.[9]

The aim of the present study was to investigate the reciprocal influences of opioids and acethylcholine in the modulation of GH secretion in the two sexes and the effect of food intake on this interaction.

SUBJECTS AND METHODS

Twenty-one healthy volunteers, 10 men aged 20 to 42 years and 11 women aged 19 to 44, participated in this study after provision of informed consent (Table 1). Body weight was within 10% of the ideal according to sex and age. None of the subjects were taking drugs. All women menstruated normally and were tested during the early or midfollicular phase of their cycle as assessed by estrogen and gonadotropin levels and ultrasonography monitoring. All subjects underwent four tests in randomized order and on different days (two before and two after a meal).

Preprandial Tests

The subjects had a continental breakfast (400 kcal) at 8:00 AM and did not consume food again until the tests were performed. At 12:30 PM in a quiet room, subjects underwent the following tests.

Pyridostigmine test. Normal saline (100 mL/h) was infused into an arm vein through a 19-gauge needle. Pyridostigmine ([PYR] Mestinon; Roche, Milan, Italy) was administered orally (60 mg) 30 minutes later, time 0, and blood samples for GH determination were collected at −30, 0, 30, 45, 60, and 90 minutes.

PYR test during naloxone infusion. Naloxone ([NAL] Narcan; Crinos, Como, Italy) was infused (1.6 mg/h), after dilution in normal saline (1.6 mg/100 mL), into an arm vein via a 19-gauge needle. PYR (Mestinon; Roche) was administered orally (60 mg) 30 minutes later, time 0, and blood samples for GH determination were collected at −30, 0, 30, 45, 60, and 90 minutes.

Postprandial Tests

All subjects had a continental breakfast (400 kcal) at 8:00 AM. At 12:30 PM in a quiet room, the subjects underwent the same tests after a standard 800-kcal meal (assumed at 12:00 PM) composed approximately of 55% carbohydrate, 30% lipid, and 15% protein. Infusion of NAL or saline was started at time −30 minutes (15 minutes after the end of the meal). PYR was administered at time 0, 1 hour after starting the meal. Blood samples were collected at the same times. Different tests were performed in randomized order on different days, with an interval of at least 3 days. Subjects did not know whether they were receiving NAL or saline.

Blood samples were centrifuged within 2 hours, and plasma for GH determination was frozen at −20°C until assayed. GH level was measured by a specific radioimmunoassay. All samples were assayed in duplicate using kits supplied by Radim (Pomezia-Rome, Italy). The sensitivity of the methods was 0.05 μg/L. Intraassay coefficient of variation were 6.5% in a sample with a mean value of 5.13 ± 0.66 μg/L and 5% in a sample with a mean value of 15.76 ± 1.60 μg/L. Interassay coefficients of variation were 6.7% in a sample with a mean value of 3.99 ± 0.54 μg/L and 8.9% in a sample with a mean value of 19.23 ± 2.42 μg/L. The normal range (in adult subjects) was 0.2 to 5 μg/L. All samples from each test were measured in the same assay.

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Submitted April 27, 1996; accepted January 23, 1997.

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0026-0495/97/4607-0005$03.00/0

Table 1. Age and Body Mass Index in the Men and Women (mean ± SEM)

Group	Age (yr)	Body Mass Index (kg/m²)
Men (n = 10)	29.7 ± 9.9	23.8 ± 1.7
Women (n = 11)	28.5 ± 8.9	22.6 ± 1.4

Statistical Evaluation

Statistical evaluation was performed using Wilcoxon's rank-sum test for intragroup comparison and the Mann-Whitney rank-sum test for intergroup comparison. The level of statistical significance was set at P less than .05. Areas under the curve (AUCs) were calculated by trapezoidal rule, relative to baseline GH concentrations. All results are expressed as the mean ± SEM.

RESULTS

Mean basal GH values were not significantly different between men and women (respectively, 0.40 ± 0.10 *v* 0.47 ± 0.11 μg/L).

When administered alone in the fasting state, PYR induced an increment in GH levels in both men and women.

The mean GH response to PYR and NAL + PYR is shown in Fig 1 (before meal) and Fig 2 (postprandially). Figure 3 shows the mean peak of the response. Table 2 shows AUCs in the different groups.

Men

Preprandial tests. During NAL infusion, the response of GH to PYR was completely abolished (Fig 1A). The mean values for AUC were significantly different (1,031.06 ± 333.21 *v* 16.50 ± 7.50 μg/L/90 min, $P < .01$) (Table 2).

Postprandial tests. Administration of PYR failed to induce GH release. No differences were observed during NAL infusion (Fig 2A).

We observed a significant inhibition in AUC values (Table 2) when comparing the GH response to PYR alone preprandially and postprandially (AUC, 1,031.06 ± 333.21 *v* 45.75 ± 18.75 μg/L/90 min, $P < .02$).

Women

Preprandial tests. In women, the fasting response of GH to PYR alone was significantly lower than that of the men. Infusion of NAL did not significantly modify the GH response to PYR (Fig 1B).

The mean AUC values (Table 2) were also significantly different (320.18 ± 87.16 *v* 1,031.06 ± 333.21 μg/L/90 min, $P < .01$). Otherwise, no differences were observed in the mean AUC during NAL infusion (308.09 ± 129.91 *v* 320.18 ± 87.16 μg/L/90 min) (Table 2).

Postprandial tests. GH release in response to PYR and to NAL + PYR was abolished postprandially in the subjects tested (Fig 2B). A significant difference was observed for AUCs between preprandial and postprandial PYR tests (Table 2). No significant difference was observed postprandially between men and women in both PYR and NAL + PYR tests.

DISCUSSION

In this study, we investigated the effect of NAL infusion on PYR-induced GH release both in men and in women to clarify whether opioid-cholinergic control of GH secretion is sex-dependent.

The first important finding of our study is that PYR-induced GH release is significantly higher in men than in women. This finding is in contrast to a previous report in which no gender-related effects on the GH response to PYR + GHRH were observed.[10] In fact, Arvat et al[10] found no differences between males and females; the latter were tested after an overnight fast and in the follicular phase of the menstrual cycle. Conversely, Barbarino et al[11] showed a sexual dimorphism of the PYR effect, testing women also in the morning, but in the luteal phase of the cycle.[12] This difference could be ascribed not only to the different experimental protocol of the present study, in which the response to PYR alone was studied, but also to the time of day at which the tests were performed and/or the nutritional status (lunchtime at 4 hours after breakfast *v* postabsorptive state).[6]

Different data indicate that the steroid environment profoundly affects GH dynamics in both sexes. O'Keane and Dinan[12] indicated that the GH response to PYR alone in normal females is profoundly influenced by the phase of the cycle: in fact, it increases from the early to the late phase of the cycle. Estrogen augments the GH response to GHRH.[13] Moreover, progesterone may have a significant priming effect on GH secretion and may represent an amplification of an estrogen-induced response.[14] The effects of sex steroids on the cholinergic system have been investigated by Lucey et al,[15] who observed (after PYR administration) a greater increase of GH levels in females versus males, a close correlation with estradiol

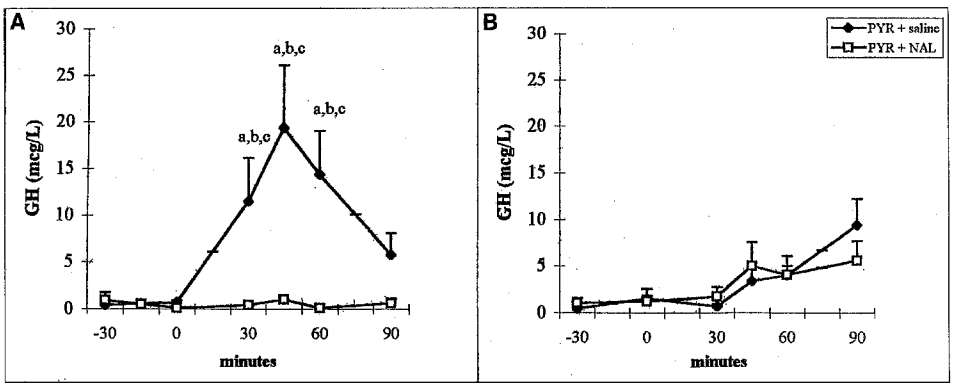

Fig 1. GH response to PYR alone and PYR + NAL (mean ± SEM) before a meal in normal men (A) and normal women (B). [a]$P < .03$, PYR *v* PYR + NAL; [b]$P < .01$, men *v* women; [c]$P < .02$, preprandial *v* postprandial.

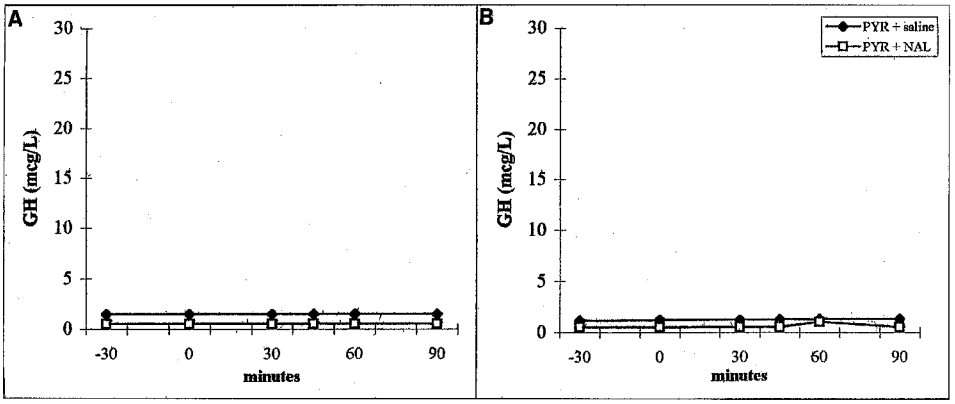

Fig 2. GH response to PYR and PYR + NAL (mean ± SEM) after a meal in normal men (A) and normal women (B).

levels, and a negative correlation between GH response and age. In female rats, it has been demonstrated that estrogen treatment induces an increase in choline acetyltransferase activity in the basal forebrain area.[16] This observation may explain why the cholinergic influence on GH regulation may be more pronounced in females.

The second finding of our study is that blockade of opioid receptors, obtained with NAL, blunted the PYR-induced GH release in men but failed to induce this effect in women, in the fasting state at 1:00 PM. This suggests a sex-dependent interaction between the opioid and the cholinergic system in the modulation of GH secretion. Although cholinergic receptors have been found at the pituitary level in rodents,[17] the effectiveness of cholinergic agonists and antagonists in modulating the GH response to GHRH supports the hypothesis of an action exerted via somatostatinergic neurons.[3] In contrast, the action of endogenous opioids on GH secretion is believed to be mediated by hypothalamic GHRH.[18,19] However, some groups have reported data in favor of an opioid activity mediated by somatostatin.[20] The observation that DAMME, a met-enkephalin analog, is able to increase GHRH-induced GH release in humans (even when a maximally stimulatory dose of GHRH is used) suggests that opioids also act through inhibition of somatostatin release.[21] Moreover, the existence of interactions between endogenous opioid and cholinergic systems is supported by observations that pretreatment with cholinergic blocking agents in dogs[4,5] and humans[2] abolishes the GH-releasing effects of DAMME. In agreement with these data, intraventricular administration of NAL in rats increases GH release, but this effect is blocked by atropine.[22]

However, the present data underline the concept that the GH response to different secretagogues can be differentially modulated by endogenous opioids. In our previous study,[23] a sex-related NAL influence on GHRH-induced GH release was discovered. In that case, NAL was capable of blunting GH release in women but not in men; other tests, such as stress-related GH release, are not influenced by NAL,[24-26] even if most such studies have been performed in male subjects.

Finally, other groups showed an augmenting effect of NAL on GH release in humans, but with the use of larger doses of the opiate-antagonist, which could be related rather to a stimulatory effect on opioid receptors.[22] The discrepancies in these different studies can therefore be reduced to the difference in the dose of opiate antagonist and different mechanism of GH stimulation by the various secretagogues.

Moreover, in vitro data showed that GHRH may actually increase somatostatin release via β-endorphin, and therefore NAL may diminish somatostatin release in response to GHRH. In the case of PYR treatment, somatostatinergic tone is depressed, so this speculative effect of NAL would not be apparent.

The reduction of cholinergic tone was similar in men and women, suggesting that the sex-related differences observed in the fasting state were completely abolished by the meal. In this experiment, we cannot establish what component of the standard meal is responsible for the observed effect, but it has been

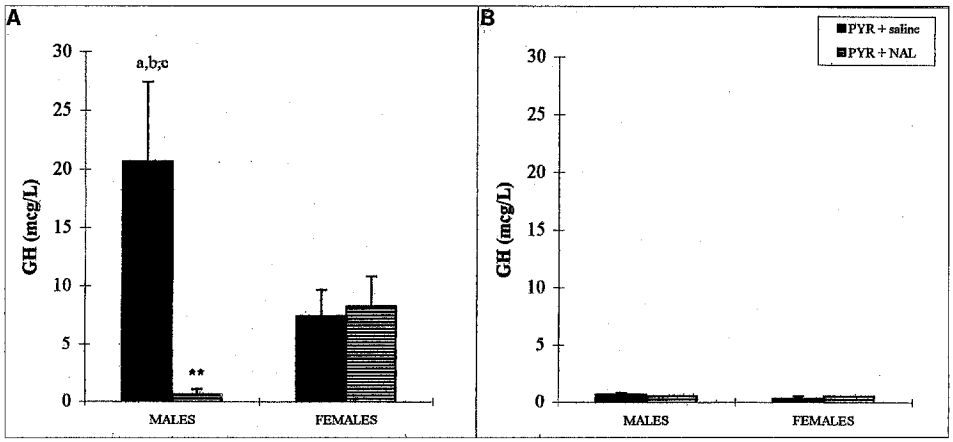

Fig 3. Basal and peak response to PYR and PYR + NAL (mean ± SEM) preprandially (A) and postprandially (B). [a]$P < .03$, PYR *v* PYR + NAL; [b]$P < .03$, men *v* women (PYR test); [c]$P < .01$, before meal *v* after meal; **$P < .05$, men *v* women (PYR + NAL test).

Table 2. AUC for Preprandial and Postprandial GH Response to PYR and NAL + PYR in Men and Women (mean ± SEM)

Group	Before Meal	After Meal
Men (n = 10)		
PYR	1,031.06 ± 333.21‡	45.75 ± 18.75†
NAL + PYR	16.50 ± 7.50*§	45.50 ± 4.30
Women (n = 11)		
PYR	320.18 ± 87.16	21.75 ± 12.75†
NAL + PYR	308.09 ± 129.91	45.20 ± 3.20†

*$P < .01$, NAL + PYR *v* PYR alone.
†$P < .02$, after *v* before meal.
‡$P < .01$, men *v* women.
§$P < .05$, men *v* women.

previously demonstrated[27,28] that both glucose and free fatty acids are capable of blunting the GH response to GHRH.

The data reported herein seem to confirm the existence of a significant opioid-cholinergic interaction in GH regulation in normal man; moreover, these results suggest that the role played by the endogenous opioid tone is significantly modulated by the sex steroid environment. Experimental data seem to confirm this finding. In fact, in rats estrogens induce an increase of the medial preoptic area central mu-receptor density.[29] Moreover, in postmenopausal women treated with transdermal 17β-estradiol, the sex steroid thermoregulatory effect has been found to depend on endogenous opioid activity.[30]

The third significant finding reported herein is the postprandial inhibition of PYR-induced GH release.

The data in the present study confirm that PYR-induced GH release is abolished postprandially in men and women, in agreement with other studies.[31] These data suggest that endogenous cholinergic tone may be reduced in the postprandial state. Alternatively, food consumption may augment peripheral or central somatostatin release, as other experimental in vivo models suggest.[32]

In conclusion, we confirm that there is a significant interaction between the opioid and cholinergic systems in regulating GH secretion in normal humans. Moreover, we demonstrate that this interaction is significantly influenced by the sex difference and food intake.

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