Effects of Cholinergic Blockade on Nocturnal Thyrotropin and Growth Hormone (GH) Secretion in Type I Diabetes Mellitus: Further Evidence Supporting Somatostatin's Involvement in GH Suppression

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To investigate the influence of cholinergic pathways on somatostatin (SS) tone in type I diabetes mellitus, we studied the effect of the muscarinic receptor antagonist pirenzepine ([PZP] 100 mg orally) on spontaneous nocturnal growth hormone (GH) and thyrotropin (TSH) secretion and on their response to GH-releasing hormone (GHRH) in the morning in a group of nine insulin-dependent diabetic patients with poor diabetic control. When the nocturnal period was divided into two phases (11:00 PM to 2:30 AM and 3:00 AM to 6:00 AM), both GH and TSH mean concentrations during the first phase were higher than those seen in the second half of the night following placebo administration (GH, 13.4 ± 1.1 *v* 4.15 ± 0.9 ng/mL, $P < .001$; TSH, 1.9 ± 0.21 *v* 1.57 ± 0.1 μU/mL, $P < .05$). Pretreatment with PZP induced a significant reduction of GH secretion (3.17 ± 1.1 *v* 13.4 ± 1.1 ng/mL, $P < .001$) and TSH secretion (1.61 ± 0.2 *v* 1.9 ± 0.21 μU/mL, $P < .05$) in the first phase of the night, accounting for a 64% and 11% reduction in the GH and TSH nocturnal peak, respectively. PZP reduced the GH response to GHRH in the morning (17.9 ± 2.7 *v* 36.7 ± 6.3 ng/mL, $P < .05$), but did not induce any change in TSH values at that time. A positive relationship ($r = .73$, $P < .01$) was observed between the percent reduction of the GH response to GHRH and that of the nocturnal GH peak following PZP administration. PZP caused a significant reduction in glucose levels during the second phase of the night (6.4 ± 0.92 *v* 9.81 ± 0.85 mmol/L, $P < .05$). These results demonstrate that administration of PZP reduces GH and TSH secretion, providing further support for the involvement of SS in the inhibition of GH secretion induced by cholinergic antagonists in type I diabetics. The inhibitory effect of PZP on GHRH-induced GH secretion may help to predict nocturnal GH behavior following administration of the drug.

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OVER THE LAST TWO DECADES, a large amount of evidence has accumulated demonstrating significant abnormalities of growth hormone (GH) neuroregulation in patients with type I diabetes mellitus. Both basal GH hypersecretion and exaggerated and paradoxical GH responses to several stimuli have frequently been found in this type of patient.[1-4] Although alterations in the hypothalamic control of GH secretion presenting as a primary defect or mediated by metabolic factors, such as hyperglycemia and low insulin-like growth factor-I (IGF-I) levels, have been proposed as an etiologic hypothesis,[5,6] the exact underlying mechanisms involved in this phenomenon remain unclear.

Normal GH secretion by the anterior pituitary results from the balanced effects of GH-releasing hormone (GHRH), which is the main stimulatory factor, and somatostatin (SS), which has an inhibitory influence. The synthesis and release of these hypothalamic peptides are regulated in turn by central neurotransmitters and peripheral signals.[7] In particular, cholinergic pathways play a significant role in GH modulation, an effect thought to be mediated by changes in SS release.[8,9] Previous studies have shown that administration of muscarinic receptor antagonists in type I diabetics leads to a significant reduction of GH levels,[10-12] suggesting that the excessive amplitude and frequency of GH pulses may be related to hypothalamic SS deficiency.

It is well known that thyrotropin (TSH) secretion is also modulated by SS in a negative way.[13,14] Estimation of the TSH concentration might therefore represent another valuable functional marker of SS tone manipulation, thus helping to clarify the mechanisms implicated in GH variations that result from pharmacological interventions. However, TSH secretion displays a significant circadian rhythm,[15] showing maximal values during the first part of the night. Therefore, the suppressive effects on TSH levels might be better tested at that time.

In an attempt to confirm the involvement of SS in the inhibitory effect of cholinergic antagonists on GH secretion in type I diabetics and to investigate the relationships between TSH and GH behavior following acute cholinergic blockade, we assessed the effects of administration of the muscarinic receptor blocker pirenzepine (PZP) on the nocturnal secretion of both hormones, as well as on their response to GHRH.

SUBJECTS AND METHODS

Patients

Nine insulin-dependent diabetics (seven men and two women) aged 26.3 ± 1.11 years (range, 21 to 32) were studied. All of them showed undetectable C-peptide levels following intravenous glucagon injection. The duration of diabetes was 8.6 ± 1.8 years. All patients were taking a mixture of short- and intermediate-acting insulin by subcutaneous injections twice daily before breakfast (8:00 AM) and dinner (8:30 PM). The mean daily insulin dosage was 0.6 ± 0.05 U/kg (range, 0.4 to 0.8). Body mass index was in the normal range in all cases (23.06 ± 0.66 kg/m^2; range, 20.5 to 26.2). Metabolic control was poor as assessed by hemoglobin A_1 levels (11.5% ± 1.1%; range, 6% to 16%), but no patient had clinical features or laboratory evidence of ketosis. Three patients had mild background retinopathy, and microalbuminuria was detected in one of them. None had any other diabetic complications or any signs of associated endocrine or intercurrent disease. All subjects were clinically and biochemically euthyroid, and none were taking any medication other than insulin. The study protocol was approved by the local ethics committee.

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Methods

After the subjects had dinner at 8:30 PM, nocturnal sampling was started at 11:00 PM in all patients via an indwelling catheter placed in an antecubital vein 30 minutes before. Blood samples were taken every 30 minutes for GH and glucose measurement and hourly for TSH measurement from 11:00 PM to 6:00 AM. Plasma specimens were collected at 11:00 PM, 3:00 AM, and 6:00 AM for cortisol, IGF-I, and glucagon determination. This experiment was performed on two occasions following oral administration of placebo or PZP (Gastrozepin; Boehringer, Barcelona, Spain; 100 mg) at 10:30 PM. Both tests were separated by at least 4 days and were performed according to a randomized double-blind protocol. Blood samples were collected through an indwelling catheter connected to a long narrow-lumen tube to avoid sleep disturbances.

A GHRH test was also performed on different days in all subjects. After an overnight fast, an indwelling catheter was inserted into an antecubital vein at 8:30 AM. Following three basal samples, a single bolus of GHRH (GHRH 1-29, Geref; Serono, Madrid, Spain; 50 μg) was intravenously injected. Blood samples were taken at −60, −20, 0, 20, 40, 60, 80, and 100 minutes for GH, TSH, and glucose determination. Subjects were studied on two separate occasions in random order after oral pretreatment with placebo or PZP (100 mg), which was administered 60 minutes before GHRH injection. An interval of at least 72 hours was observed between both tests. The morning insulin dose was withheld until the GHRH test was completed.

The daily insulin dosage was kept constant over the period of study in all patients.

Samples were centrifuged immediately, and the serum was separated and stored at −20°C until assayed. All samples from a single patient were analyzed in the same assay.

Assays

Glucose levels were measured by a hexokinase method. GH levels were estimated by immunoradiometric assay (Serono Diagnostics, Rome, Italy). Intraassay and interassay coefficients were 3.7% and 8.4%, respectively. TSH concentrations were measured by an immunoradiometric assay (CIS, Gif-sur-Yvette, France). Intraassay and interassay coefficients were 3.5% and 4.7%, respectively. IGF-I was assessed by immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). Intraassay and interassay coefficients were 2.8% and 7%, respectively. Cortisol and glucagon were measured by radioimmunoassay (Serono Diagnostics, Rome, Italy). Intraassay and interassay variations were 4.6% and 9.5% for cortisol and 4.2% and 6.3% for glucagon, respectively.

To obtain an integrated perspective from a chronological point of view of the effect of placebo and PZP administration on GH and TSH secretion, the whole night was divided into two phases (first phase, 11:00 PM to 2:30 AM; second phase, 3:00 AM to 6:00 AM) to enable grouping of the nocturnal GH maximum peak from all subjects in the first phase. Results are expressed as the mean ± SEM. Areas under the curve (AUCs) were calculated by the trapezoidal method. Comparison between PZP and placebo studies was made by Student's two-tailed paired *t* test. The correlation between two variables was calculated by Pearson's method.

RESULTS

Nocturnal Study

Nocturnal GH levels showed a significant circadian variation following placebo administration (Fig 1). GH values during the first part of the night were higher than those found in the second phase (13.4 ± 1.1 *v* 4.15 ± 0.9 ng/mL, $P < .001$; Fig 2). From an individual point of view, all patients had the same chronological pattern of secretion, with maximum GH peaks detected in the first phase in all cases.

PZP treatment induced a clear reduction of mean GH levels with respect to placebo values, which was especially evident in the first phase (3.17 ± 1.1 *v* 13.4 ± 1.1 ng/mL, $P < .001$; Fig 2), representing a 77.5% reduction in the value seen at the same time following pretreatment with placebo. Accordingly, the nocturnal GH peak also decreased by 64.2% compared with the peak in control conditions (11.9 ± 3.2 *v* 34.5 ± 3.4 ng/mL, $P < .001$). In contrast to the placebo study, the GH peak following pretreatment with PZP was delayed to the second phase of the night in six patients. Pretreatment with PZP did not induce any change in GH levels during the second phase of the night compared with the values obtained after placebo administration (3.1 ± 0.7 *v* 4.1 ± 0.9 ng/mL). Treatment with PZP flattened the GH circadian variation, with both night phases showing GH values in a similar range when the cholinergic antagonist was given (Fig 2).

Following placebo administration, TSH levels over the first phase were higher than those found during the second part of

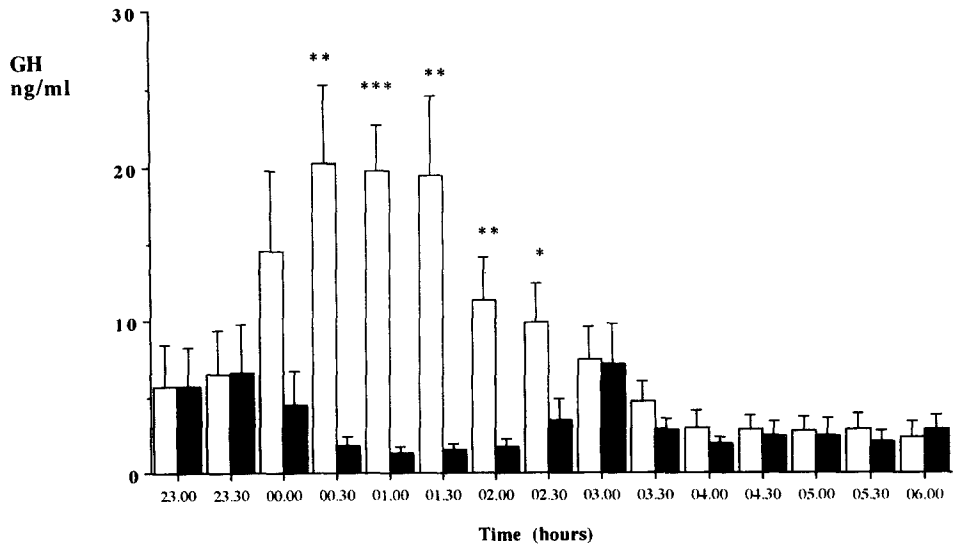


Fig 1. Mean ± SEM GH levels from 23.00 h (11:00 PM) to 06.00 h (6:00 AM) in 9 type I diabetic patients following placebo (□) and PZP (■). *$P < .05$, **$P < .01$, ***$P < .001$.

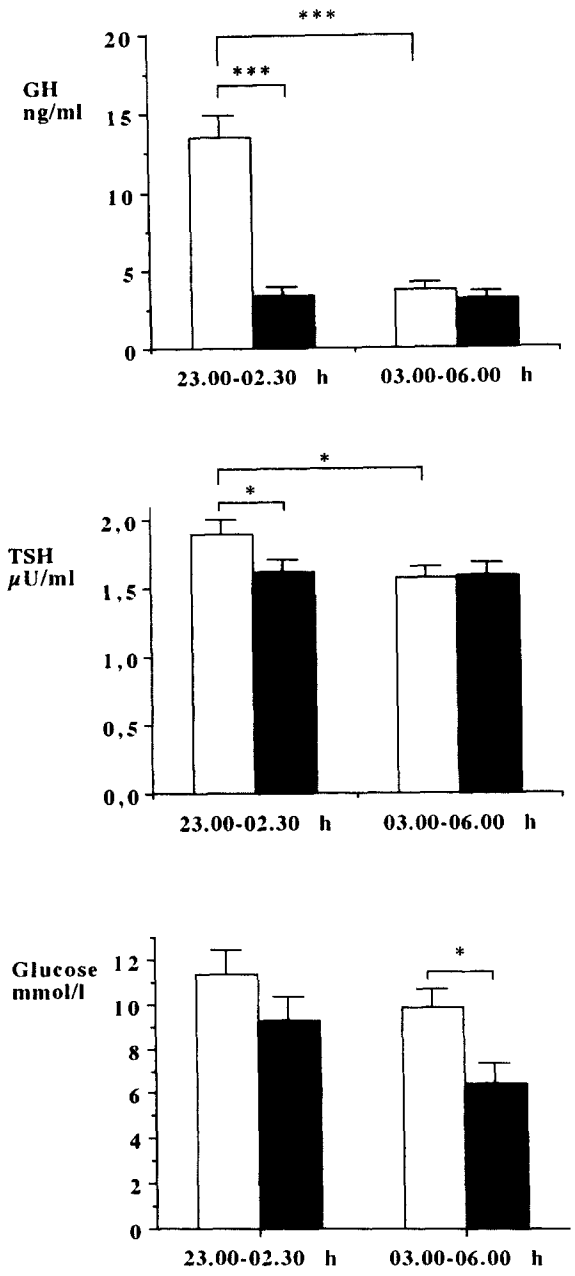


Fig 2. Mean GH, TSH, and glucose values in both nocturnal phases following placebo (□) and PZP administration (■). *$P < .05$, ***$P < .001$.

the night (1.9 ± 0.21 *v* 1.57 ± 0.1 μU/mL, $P < .05$; Fig 3), according to a normal circadian variation. However, two patients had similar values in both phases. Maximum TSH values were observed between 11:00 PM and midnight in all subjects. Thereafter, a progressive reduction in TSH levels was seen through the entire sampling period (Fig 3).

PZP administration induced a slight reduction in mean nocturnal TSH concentrations compared with those found in control conditions (1.60 ± 0.12 *v* 1.73 ± 0.13 μU/mL, NS). When considering night phases, a significant decrease in TSH levels was observed during the first phase (1.61 ± 0.2 *v* 1.90 ± 0.21 μU/mL, $P < .05$; Fig 2), just when control TSH values were at the highest point. However, no changes in TSH levels were seen over the last part of the night (1.58 ± 0.16 *v* 1.57 ± 0.16 μU/mL). The maximum TSH nocturnal peak following PZP treatment was detected in the second phase in six patients, and the magnitude was similar to that found in control conditions (2.06 ± 0.22 *v* 2.23 ± 0.22 μU/mL, NS). There was no relationship between the percent reduction in nocturnal levels of GH versus TSH ($r = .12$, NS) after pretreatment with PZP.

All glucose values following PZP treatment were lower than after placebo administration. However, glycemic values were especially reduced from 4:00 AM (Fig 4). Accordingly, a significant decrease in glucose levels was also demonstrated in the second phase of the night (6.4 ± 0.92 *v* 9.81 ± 0.85 mmol/L, $P < .05$; Fig 2).

Cortisol levels during the control night exhibited significant circadian changes, reaching the lowest concentration at 3:00 AM (72 ± 7.1 nmol/L) and the highest at 6:00 AM (324.4 ± 59.5 nmol/L). These variations were unaffected by treatment with PZP (3:00 AM, 70.3 ± 7.7 nmol/L; 6:00 AM, 380.7 ± 67.3 nmol/L). IGF-I and glucagon levels were similar at all times and did not change following PZP administration (Table 1).

GHRH Test

Following placebo administration, GHRH injection elicited a marked GH response, reaching a maximum peak of 36.7 ± 6.3 ng/mL (Fig 5).

Pretreatment with PZP did not induce any change in basal GH levels with respect to the control levels (0.82 ± 0.17 *v* 3.2 ± 2.1 ng/mL, NS). However, the maximum GH peak (17.9 ± 2.7 *v* 36.7 ± 6.3 ng/mL, $P < .05$) and the AUC value (107.8 ± 13.7 *v* 214.5 ± 35.9 ng/mL/160 min, $P < .05$) were clearly reduced when compared with the values calculated following placebo administration. The magnitude of the GH peak and AUC values were suppressed by 31.4% and 50.2%, respectively, when PZP was given.

The percent suppression of the GH response to GHRH after PZP administration was lower than at the spontaneous nocturnal GH peak ($P < .01$). Interestingly, a positive relationship was observed between the two parameters ($r = .73$, $P < .01$; Fig 6).

Morning TSH values remained unchanged after GHRH injection following pretreatment with either placebo or PZP (Fig 5).

Mean basal glucose levels when subjects received placebo were similar to those measured following PZP (11.07 ± 0.8 *v* 10.16 ± 1.38 mmol/L, NS). Mean glucose AUC values following GHRH stimulation under both conditions were also within the same range (160.8 ± 11.1 *v* 146.3 ± 18.2 mmol/L/160 min, NS).

DISCUSSION

Several mechanisms have been suggested to explain the uncontrolled GH release that takes place in patients with type I diabetes mellitus. Among them, the hypothesis of a reduced SS action related to deficient secretion or to somatotrope resistance has emerged as the most convincing possibility.[16] According to this, pharmacological manipulations intended to increase somatostatinergic tone have been attempted to reduce GH levels and improve metabolic control.[10,12] Although type I diabetics ex-

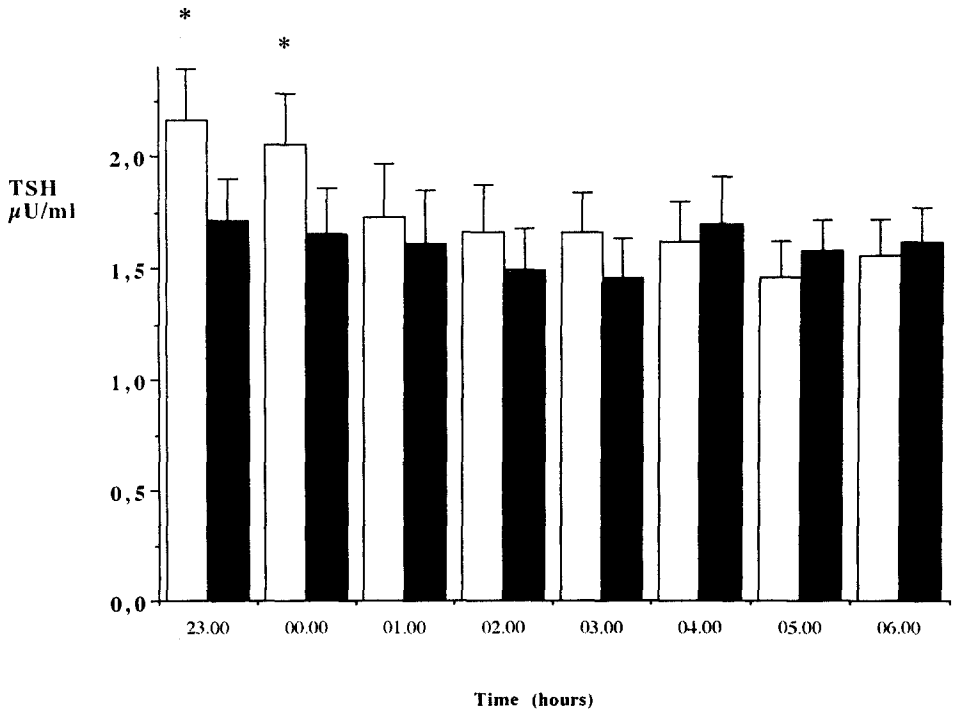


Fig 3. Mean ± SEM TSH levels from 23.00 h (11:00 PM) to 06.00 h (6:00 AM) in 9 type I diabetic patients following placebo (□) and PZP (■). *$P < .05$.

hibit a certain degree of resistance to the effects of SS, administration of SS analogs on a short-term basis has been followed by a variable reduction in GH and glucose concentrations.[17,18] Treatment with cholinergic muscarinic receptor antagonists represents another therapeutic approach, but, as with SS, a reduced sensitivity to the GH-suppressive effects of these drugs in diabetics has also been described.[19] In agreement with previous reports, our study also shows that insulin-dependent diabetics display a significant GH response to GHRH, as well as evident nocturnal GH elevations, despite the fact that they exhibit marked hyperglycemia.[3,4] Furthermore, pretreatment with the muscarinic receptor blocker PZP attenuated both GH excursions, as found by others.[10,11] This study also demonstrates that an oral dose of 100 mg PZP at 10:30 PM is effective enough to suppress GH levels during the first part of the night, just when maximum concentrations are attained, making it unnecessary to use higher doses. In fact, PZP administration abolished the differences in absolute GH levels seen between the two night phases, leading to a significant delay in the occurrence of GH peak. This different effect of PZP on both night phases could be ascribed either to the progressive reduction in drug bioavailability related to its pharmacokinetic properties[20] or to a special sensitivity to GH suppression related to the high GH level. Pretreatment with PZP at night reduced the nocturnal GH peak by 64%, a magnitude similar to that found in a previous report,[21] whereas after GHRH the maximum GH response was decreased by 31%, suggesting that the drug is more active in inhibiting spontaneous versus pharmacologically stimulated GH secretion. Nevertheless, the reduction in the GH peak in both tests showed a significant relationship, indicating that PZP-induced suppression of the GH response to GHRH might help to predict nocturnal GH behavior following acute treatment with the cholinergic antagonist.

Glycemic values were reduced by PZP administration, especially in the second part of the night, even though there were no changes in insulin dosage. Although more prolonged sampling

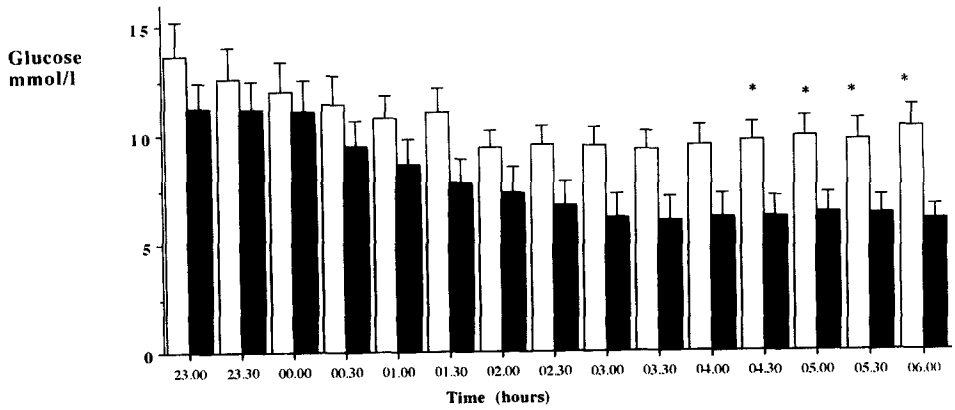
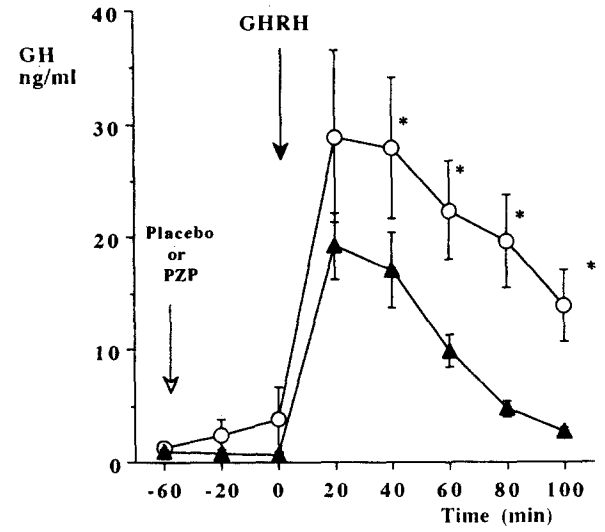


Fig 4. Mean ± SEM glucose levels from 23.00 h (11:00 PM) to 06.00 h (6:00 AM) in 9 type I diabetic patients following placebo (□) and PZP (■). *$P < .05$.

Table 1. Glucagon, Cortisol, and IGF-I Levels Following Treatment With Placebo or PZP (Mean ± SEM)

Parameter	Total Mean Value	11:00 PM	3:00 AM	6:00 AM
Glucagon (pg/mL)				
Placebo	113.2 ± 16.4	134 ± 17.7	101.8 ± 19	104 ± 21
PZP	108 ± 17.1	107.8 ± 20.6	102.4 ± 20.1	114 ± 15.9
Cortisol (nmol/L)				
Placebo	166.3 ± 19.9	100.6 ± 16.2	72 ± 7.1	324.4 ± 59.5
PZP	190.3 ± 30.1	117.2 ± 29.8	70.3 ± 7.7	380.7 ± 67.3
IGF-I (U/mL)				
Placebo	0.24 ± 0.04	0.26 ± 0.06	0.23 ± 0.04	0.21 ± 0.03
PZP	0.21 ± 0.04	0.21 ± 0.05	0.23 ± 0.05	0.2 ± 0.04



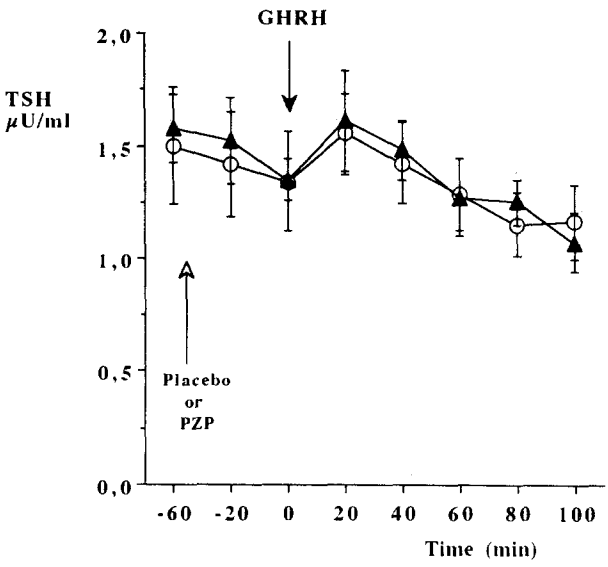


Fig 5. GH and TSH levels following GHRH injection in the morning after pretreatment with placebo (○) and PZP (▲). *$P < .05$.

and clamp techniques would be needed to study the effects of PZP on carbohydrate metabolism, this finding points to the contributory role of GH hypersecretion to nocturnal hyperglycemia, in agreement with other studies.[21,22] The possibility that a lower glycemic concentration could account for the reduction in GH levels after PZP treatment is unlikely, since short-term changes in metabolic control do not result in any significant variation in nocturnal GH secretion.[23,24] Moreover, nocturnal IGF-I levels were similar following both placebo and PZP, and this is one of the factors that could be involved in the reduction of GH levels following improvement of metabolic control in some situations.[6,16]

The mechanism by which PZP induces GH suppression is believed to be based on stimulation of endogenous SS release.[9] The inhibitory influence of SS on TSH secretion in man is a well-established phenomenon.[13,14] However, only a few data have been reported regarding the effects of cholinergic blockade on TSH secretion. Oral or intravenous PZP administration to patients with diabetes, liver cirrhosis, or depression does not result in any variation of TSH values.[25-27] Our study demonstrates that cholinergic antagonist administration, besides reducing GH levels, induces a significant decrease in TSH concentra-

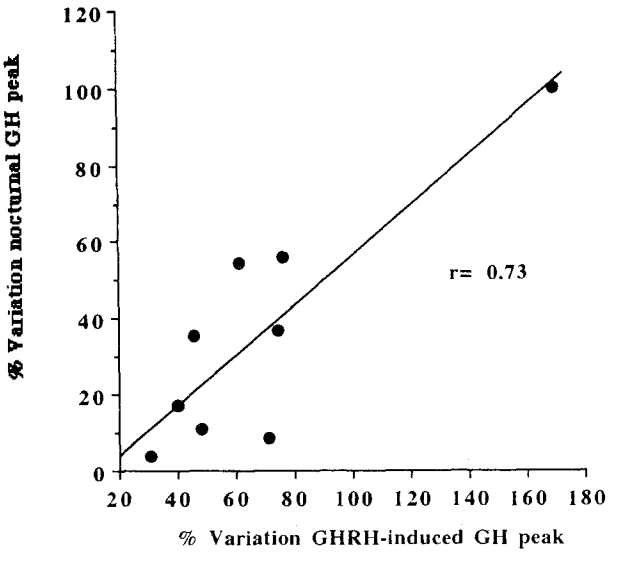


Fig 6. Relationship between the percent change in nocturnal and GHRH-stimulated GH peak induced by PZP versus values found after treatment with placebo ($r = .73$, $P < .01$).

tions during the first part of the night. However, as was the case with GH secretion, TSH concentrations over the second phase of the night were unaffected by PZP. Thus, the significant difference in circulating levels exhibited by both hormones in the two night phases was flattened by treatment with PZP. The apparent discrepancy with previous studies can be explained by the fact that between 11:00 PM and 2:30 AM TSH levels are at the highest value, making the inhibitory action of SS on TSH feasible and enabling it to be more easily detected than at any other time of day. The lack of a PZP effect on morning TSH levels also suggests that the suppressive action of the drug on TSH release can only be demonstrated at night. This finding is compatible with the observations made by Ghigo et al,[28] who demonstrated that, unlike the morning period, the somatostatinergic tone controlling GH secretion is almost absent at night in normal subjects, providing an explanation for the nocturnal dominance of the PZP-suppressive effect on GH and TSH levels. In contrast, the majority of studies designed to test cholinergic modulation of TSH secretion were performed in the morning, when TSH levels are physiologically low and a further suppressive action may be difficult to detect. On the other hand, most patients were tested following stimulation with high doses of TRH, which results in massive TSH release, masking other weaker influences operating on thyrotrope function such as that mediated by cholinergic antagonism. Therefore, the inhibitory effect of PZP on both TSH and GH levels provides further support for the involvement of SS in the suppressive effect of PZP on GH secretion in insulin-dependent diabetic patients. On the other hand, these findings suggest that SS has a role in the modulation of nocturnal TSH secretion, and hence in its circadian rhythm.

As reported previously, the suppressive effect of SS on GH is stronger than that exerted on TSH secretion.[29] This characteristic is also present in our group of diabetic patients, who showed a 77% decrease in nocturnal mean GH levels over the first night phase, whereas the magnitude of TSH reduction was 11% at the same time. Differences were still larger in the morning, when stimulated GH levels were reduced by 60%, whereas TSH concentrations were unchanged. No relationship could be found between nocturnal decrements of GH and TSH levels, suggesting that previous observations pointing to a different action of SS on GH and TSH secretion in normal subjects[30] also apply to type I diabetics.

Changes in GH or TSH levels following PZP administration were evident even though IGF-I levels remained unmodified. Although variations in IGF binding proteins cannot be ruled out, these results do not support the participation of this peptide, through feedback mechanisms, in the effects of PZP on TSH and GH secretion observed in this group of patients.

Although cholinergic pathways play a modulatory role in the activity of the hypothalamic-pituitary-adrenal axis in human subjects,[31] muscarinic receptor blockade induced by PZP administration did not modify the pattern of cortisol secretion in diabetic patients. Glucagon levels also remained unchanged following PZP treatment, suggesting a selective effect of the drug on GH secretion, with preservation of the integrity of other elements of the counterregulatory system.

In summary, these data show that administration of muscarinic antagonists to type I diabetic patients results in a reduction of TSH and GH levels over the first part of the night, providing further support for the involvement of SS as the main mechanism by which PZP decreases GH secretion in this group of subjects. The effects of PZP on GHRH-induced GH release may predict the nocturnal GH response to the drug, a finding with interesting future implications when considering diabetic patients for long-term treatment with cholinergic antagonists.

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