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Melatonin stimulates growth hormone secretion through pathways other than the growth hormone-releasing hormone

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Summary

OBJECTIVE There is evidence that melatonin plays a role in the regulation of GH secretion. The aim of this study was to investigate the neuroendocrine mechanisms by which melatonin modulates GH secretion. Thus we assessed the effect of oral melatonin on the GH responses to GHRH administration and compared the effects of melatonin with those of pyridostigmine, a cholinergic agonist drug which is likely to suppress hypothalamic somatostatin release.

DESIGN The study consisted of four protocols carried out during the afternoon hours. Study 1: oral melatonin (10 mg) or placebo were administered 60 minutes prior to GHRH (100 μg i.v. bolus). Study 2: GHRH (100 μg i.v. bolus) or placebo were administered at 0 minutes; oral melatonin or placebo were given at 60 minutes and were followed by a second GHRH stimulus (100 μg i.v. bolus) at 120 minutes. Study 3: placebo; oral melatonin (10 mg); oral pyridostigmine (120 mg); melatonin (10 mg) plus pyridostigmine (120 mg) were administered on separate occasions. Study 4: placebo; oral melatonin (10 mg); oral pyridostigmine (120 mg); melatonin (10 mg) plus pyridostigmine (120 mg) were administered on separate occasions 60 minutes prior to a submaximal dose (3 μg i.v. bolus) of GHRH.

SUBJECTS Four groups of eight normal male subjects, ages 22–35 years, were randomly assigned to each protocol.

MEASUREMENTS Growth hormone was measured by RIA at 15-minute intervals.

RESULTS Oral melatonin administration had a weak stimulatory effect on GH basal levels. Prior melatonin administration approximately doubled the GH release induced by supramaximal (100 μg) or submaximal (3 μg) doses of GHRH. Melatonin administration restored the GH response to a second GHRH challenge, given 120 minutes after a first GHRH i.v. bolus. The GH releasing effects of pyridostigmine, either alone or followed by GHRH, were greater than those of melatonin. However, the simultaneous administration of melatonin and pyridostigmine was not followed by any further enhancement of GH release, either in the absence or in the presence of exogenous GHRH.

CONCLUSIONS Our data indicate that oral administration of melatonin to normal human males increases basal GH release and GH responsiveness to GHRH through the same pathways as pyridostigmine. Therefore it is likely that melatonin plays this facilitatory role at the hypothalamic level by inhibiting endogenous somatostatin release, although with a lower potency than pyridostigmine. The physiological role of melatonin in GH neuroregulation remains to be established.

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There is evidence that the pineal hormone melatonin plays a role in the regulation of growth and GH secretion. In rodents, melatonin injections have a marked inhibitory effect on GH secretion (Attanasio *et al.*, 1986) and could block 5-hydroxytryptophan-induced GH release (Smythe & Lazarus, 1973). Thus blind rats or normal rats kept in constant darkness, in which melatonin secretion is increased, show reduced body weight, reduced tibial length and reduced pituitary GH content (Smythe & Lazarus, 1973; Sorrentino *et al.*, 1971a,b; Osman *et al.*, 1972). This antisomatotrophic activity of melatonin is, however, controversial. Melatonin injected hamsters show increased body weight (Tamarkin *et al.*, 1976; Reiter *et al.*, 1977), and in male hamsters daily evening melatonin injections increase serum IGF-I and GH levels (Vriend *et al.*, 1990).

In man the interpretation of the available evidence is even more intriguing. Although idiopathic GH deficiency has been associated with the absence of melatonin circadian rhythm (Grugni *et al.*, 1990), in normal healthy individuals no relationship has been found between endogenous nocturnal melatonin and GH levels (Vaughan *et al.*, 1978; Rao & Mager, 1987). Oral melatonin treatment (2 mg at 1700 hour) had no effect on the levels or 24-hour rhythms of GH in adult individuals (Wright *et al.*, 1986), while a significant decrease in plasma GH levels was observed in prepubertal children after melatonin injection (0·2 mg/kg b.w.) (Lissoni *et al.*, 1986). On the other hand, large doses (1 g) of oral melatonin

(Smythe & Lazarus, 1974a; Valcavi *et al.*, 1987) or injected melatonin (0·4 mg/kg b.w.) (Esposti *et al.*, 1988) had a stimulatory action on serum GH in adult individuals. Weinberg *et al.* (1981), however, failed to find any effect on basal GH levels of melatonin infusion (2·1 mg/min over 4 hours). Furthermore, the effects of melatonin pretreatment on stimulated GH release are controversial. Melatonin administration reduced the GH responses to insulin-induced hypoglycaemia (Smythe & Lazarus, 1974b) and L-tryptophan (Koulu & Lammintausta, 1979), but it did not affect the GH response to L-dopa (Weinberg *et al.*, 1981) and it enhanced the GHRH-stimulated GH release (Valcavi *et al.*, 1987).

In order to gain further insight into the mechanisms by which melatonin modulates GH secretion, we assessed the effect of oral melatonin pretreatment on the GH responses to i.v. administration of GHRH. Furthermore, the effects of melatonin were compared with the effects of pyridostigmine, a cholinergic agonist drug which is assumed to suppress endogenous hypothalamic somatostatin release (Richardson *et al.*, 1980; Massara *et al.*, 1986a; Ross *et al.*, 1987).

Materials and methods

Thirty-two normal male subjects, aged 22–35 years (mean ± SE 27·3 ± 0·8), were recruited for the study which consisted of four protocols. Tests were performed double-blind in random order, at least 1 week apart, using four groups of eight subjects randomly assigned to each experimental protocol. The volunteers weighed 72·3–83·9 kg (mean ± SE 78·2 ± 0·7); all of them were within 10% of ideal body weight and were taking no medication. Informed consent was obtained from each subject following ethical approval from the local health authorities. There were no differences in the mean age and body weight among the four groups. The subjects had breakfast and then fasted. They remained recumbent throughout the studies which commenced in the afternoon between 1430 and 1500 h, 30 minutes after the insertion of forearm intravenous catheters for blood withdrawal and/or drug administration. We chose this period of the day since in the afternoon hours the biological systems are most sensitive to melatonin (Tamarkin *et al.*, 1976). Down regulation of melatonin receptors might occur in the morning, after exposure to endogenous melatonin during the night (Vacas & Cardinali, 1979).

Study 1

On four separate occasions and in random order, each of the eight subjects received either melatonin (10 mg orally) or placebo, 60 minutes before the administration of a maximal dose of GHRH (GHRH 1–44 100 μg i.v. bolus at 0 min) or 0·9% physiological saline (2 ml i.v.). Samples for GH assay were taken at −60 and −30 minutes and then at 15-minute intervals up to 120 minutes.

Study 2

A second group of eight subjects received GHRH 1–44 (100 μg i.v. bolus) or placebo at time 0 on separate occasions, followed by melatonin 10 mg p.o. or placebo at 60 minutes and a second GHRH i.v. bolus (100 μg) at 120 min. Three tests were carried out: placebo + placebo + placebo; GHRH + placebo + GHRH; GHRH + melatonin + GHRH. Samples for GH assay were taken at −30 minutes and thereafter at 15-minute intervals from 0 to 240 minutes.

Study 3

A third group of eight subjects received on separate occasions: placebo; oral melatonin (10 mg); pyridostigmine (120 mg orally); oral melatonin (10 mg) plus pyridostigmine (120 mg). Samples for GH assay were taken at 15-minute intervals up to 180 minutes.

Study 4

A fourth group of eight subjects received on separate occasions: placebo; oral melatonin (10 mg); pyridostigmine (120 mg orally); oral melatonin (10 mg) plus pyridostigmine (120 mg) 60 minutes before the administration of a submaximal dose of GHRH (GHRH 1–44, 3 μg i.v. bolus at 0 minute). This dose is about the smallest capable of releasing GH from the pituitary (Vance *et al.*, 1984; Gelato *et al.*, 1984). As in study 1, samples for GH assay were taken at 15-minute intervals from −60 minutes to 120 minutes.

Hormone assay

Serum growth hormone was measured by liquid-phase, double antibody radioimmunoassay (RIA) and by magnetic bound/free separation (Ares Serono, Milano, Italy) with a sensitivity of 0·4 mU/l. The intra-assay coefficient of variation (CV) was 2·5 and 3·9% at dose levels of 8·0 and 40·0 mU/l, respectively; the interassay CV was 5·8 and 8·5% at the same dose levels.

Statistical analysis

Statistical analysis was performed by non-parametric analysis of variance for repeated measurements (Friedman's test). Multiple comparison tests were computed by Wilcoxon's

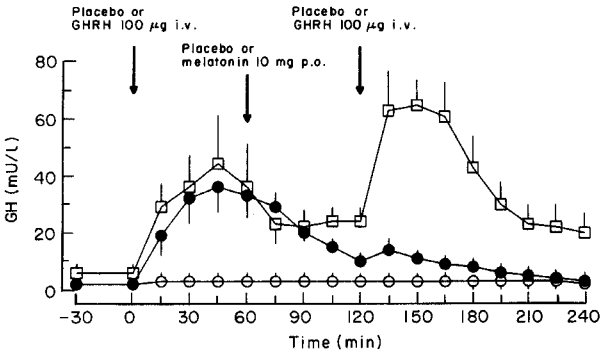

Fig. 1 Effect of oral melatonin treatment on the GH responses to GHRH preceded by GHRH injection 2 hours before. Tests were performed at 1430–1500 hours. Mean ± SE in eight normal male subjects. ○, Placebo + placebo + placebo; ●, GHRH + placebo + GHRH; □, GHRH + melatonin + GHRH.

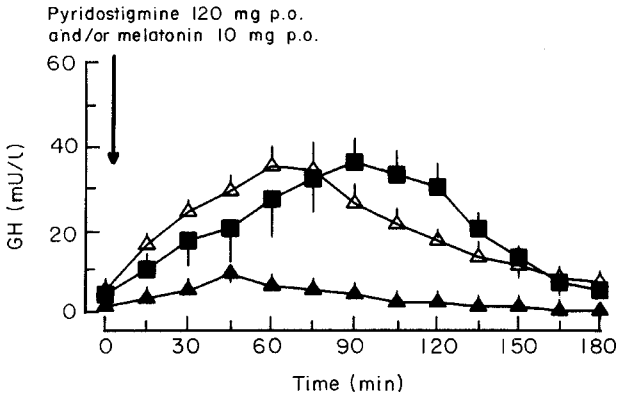

Fig. 2 Serum GH levels following the administration of either oral ▲, melatonin; △, pyridostigmine; ■, or the combination of both drugs at 1430–1500 hours. Mean ± SE in eight normal male subjects.

matched-pairs signed-rank test, using the Bonferroni correction. Areas under the curve (AUC) were calculated by the trapezoidal method. Data are presented as the mean ± SE.

Results

Study 1

Melatonin administration caused a small increase in mean plasma GH levels. GH levels were significantly greater following melatonin compared with placebo, with a peak 45 minutes after melatonin administration (8·4 ± 1·2 *vs* 2·8 ± 0·4 mU/l at −15 minute, $P < 0{\cdot}01$). This GH releasing effect of melatonin lasted for 1 hour.

Following i.v. GHRH administration at the conventional maximal dose of 100 μg, the expected increase in plasma GH was observed in all subjects, with a mean peak of 30·6 ± 2·4 mU/l at 30 minutes ($P < 0{\cdot}01$ *vs* placebo) and a mean AUC of 1916 ± 202 mU/l/2 h (320 ± 40 mU/l/2 h in the placebo study, $P < 0{\cdot}01$). Prior administration of melatonin led to enhancement of the GH responses to GHRH both in terms of peak (mU/l, 56·4 ± 8·8 at 30 minutes, $P < 0{\cdot}05$ *vs* GHRH alone) and AUC (mU/l/2 h, 4292 ± 614, $P < 0{\cdot}01$ *vs* GHRH alone).

Study 2 (Figure 1)

In all the eight subjects who received i.v. supramaximal GHRH boluses at 0 and 120 minutes, GH levels rose after the first dose of GHRH with peak levels being attained at 45 minutes (35·2 ± 7·6 mU/l). Following the second bolus of GHRH, GH levels rose only slightly from 10·0 ± 2·0 mU/l at 120 minutes to 13·8 ± 4·6 mU/l at 135 minutes. This rise was not statistically significant. However, GH AUC 120–240 minutes was greater when GHRH instead of placebo was administered at 120 minutes (1002 ± 188 *vs* 352 ± 20 mU/l/2 h, $P < 0{\cdot}01$). On the other hand, when the test was repeated with oral administration of melatonin preceding the second GHRH bolus, the GH peak after the second GHRH bolus was greatly enhanced (GH peak, 65·0 ± 9·0 mU/l at 150 minutes, $P < 0{\cdot}01$ *vs* placebo/GHRH; GH AUC 120–240 minutes, 4940 ± 1040 *vs* 1002 ± 188 mU/l/2 h in the placebo/GHRH study, $P < 0{\cdot}01$).

However, at the time of the second GHRH bolus (120 minutes), plasma GH levels were greater when melatonin was administered instead of placebo at 60 minutes (24·0 ± 4·6 *vs* 10·0 ± 2·0 mU/l, $P < 0{\cdot}05$). This was probably due to the GH releasing effects of melatonin already observed in study 1. All data were thus recalculated as the difference of the two curves and reassessed by Friedman's test. This analysis confirmed that the GH response to the second GHRH i.v. bolus was definitely enhanced by melatonin administration ($P < 0{\cdot}001$).

Study 3 (Figure 2)

As observed in study 1, melatonin had a weak stimulatory effect on plasma GH levels, reaching a peak of 9·0 ± 1·0 mU/l 45 minutes after melatonin administration. Pyridostigmine administration was followed by the expected GH peak (35·8 ± 5·2 mU/l at 60 minutes), significantly greater than that observed after oral melatonin ($P < 0{\cdot}01$). The simultaneous administration of melatonin and pyridostigmine had no additive effects (GH peak, 36·4 ± 6·4 mU/l at 90 minutes, NS *vs* pyridostigmine alone). Analysis of AUCs (0–120 minutes) confirmed that melatonin released a smaller amount of GH than did pyridostigmine (628 ± 60 *vs*

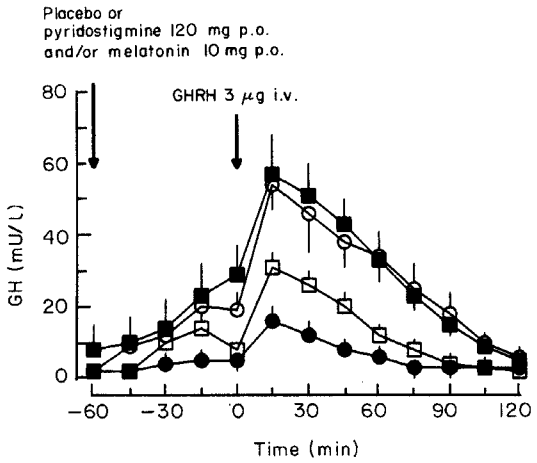

Fig. 3 Effects of the administration of either oral □, melatonin; ○, pyridostigmine; ■, or the combination of both drugs; or ●, placebo on the GH responses to a small GHRH dose. Tests were performed at 1430–1500 hours. Mean ± SE in eight normal male subjects.

3018 ± 398 mU/l/2 h, $P < 0.01$), and that the addition of melatonin did not change the GH responses to pyridostigmine (2898 ± 704 mU/l/2 h, NS).

Study 4 (Figure 3)

As expected, baseline GH levels (time 0) were increased by prior administration of melatonin (7·8 ± 2·2 *vs* 4·8 ± 1·2 mU/l in the placebo study, $P < 0.05$); or pyridostigmine (20·0 ± 4·8 mU/l; $P < 0.05$ *vs* placebo; $P < 0.05$ *vs* melatonin); or pyridostigmine plus melatonin (29·0 ± 8·4 mU/l, $P < 0.02$ *vs* placebo; $P < 0.05$ *vs* melatonin; NS *vs* pyridostigmine).

An i.v. GHRH dose as small as 3 μg was able to increase plasma GH levels with a mean peak of 16·4 ± 4·2 mU/l at 15 minutes. The GH AUC in the 2 hours following GHRH 3 μg i.v. was 838 ± 186 mU/l/2h.

Pretreatment with oral melatonin led to enhanced GH release by this small GHRH dose. Peak GH at 15 minutes was 31·4 ± 4·4 mU/l ($P < 0.05$ *vs* GHRH alone) and GH AUC increased up to 1642 ± 280 mU/l/2 h ($P < 0.01$ *vs* GHRH alone). However, when pyridostigmine was administered instead of melatonin, a greater enhancement of GH responses was observed. Peak GH at 15 minutes was 53·8 ± 6·6 mU/l ($P < 0.01$ *vs* placebo plus GHRH; $P < 0.02$ *vs* melatonin plus GHRH). GH AUC increased up to 3520 ± 600 mU/l/2 h ($P < 0.01$ *vs* placebo plus GHRH; $P < 0.05$ *vs* melatonin plus GHRH). Finally, when melatonin was administered together with pyridostigmine before GHRH, GH peak and AUC responses were similar to those observed after pyridostigmine plus GHRH (GH peak, 56·2 ± 10·6 mU/l, NS; GH AUC, 3768 ± 608, mU/l/2 h, NS).

The GH AUCs responses in all experiments are summarized in Figure 4.

Discussion

In accordance with previous studies (Smythe & Lazarus, 1974a; Valcavi *et al.*, 1987; Esposti *et al.*,1988), these data confirm that melatonin administration causes a small increase in basal GH levels in normal human subjects. In addition, the present study shows that the oral dose of melatonin (10 mg) releases the same amount of GH as is released by oral doses as great as 1 g (Smythe & Lazarus, 1974a,b; Valcavi *et al.*, 1987) or following melatonin injection (0·4 mg/kg b.w.) (Esposti *et al.*, 1988). The latter doses produce concentrations of circulating melatonin several thousand times above the physiological range (Waldhauser *et al.*, 1984). In the present study we aimed at a more physiological experimental model by giving smaller doses of melatonin in the afternoon hours, i.e. at a time when biological systems are most sensitive to it (Tamarkin *et al.*, 1976). However, the first study showed that the effects of 10 mg oral melatonin were essentially the same as those we observed previously with a dose a hundredfold greater (Valcavi *et al.*, 1987). This indicates that 10 mg melatonin is itself a maximal dose for GH stimulation.

The site where melatonin acts in the control of GH secretion is still not clear. Melatonin is capable of reducing GH production by GH4 rat pituitary cell strain (Griffiths *et al.*, 1987). However, we have previously demonstrated that in monolayer cultures of rat anterior pituitary cell, neither basal nor GHRH-stimulated GH release was affected by melatonin administration, thus indicating that melatonin acts primarily at central level (Valcavi *et al.*, 1987). In incubated fragments of rat hypothalamus, melatonin stimulates somatostatin release (Richardson *et al.*, 1981), suggesting that the GH inhibitory effect of melatonin on GH secretion may be mediated by hypothalamic somatostatin activity in the rat. It should be emphasized, however, that there are important differences in neuroregulation of GH secretion between rodents and primates (Dieguez *et al.*, 1988).

In our previous report (Valcavi *et al.*, 1987) and in the first study of the present paper, melatonin enhanced GH release induced by supramaximal doses (100 μg i.v.) of GHRH. This suggests that melatonin facilitates pituitary GH release through pathways other than GHRH, possibly by inhibiting hypothalamic somatostatin release. However, we could not exclude the possibility that melatonin enhanced the pituitary GH release by stimulating endogenous GHRH release.

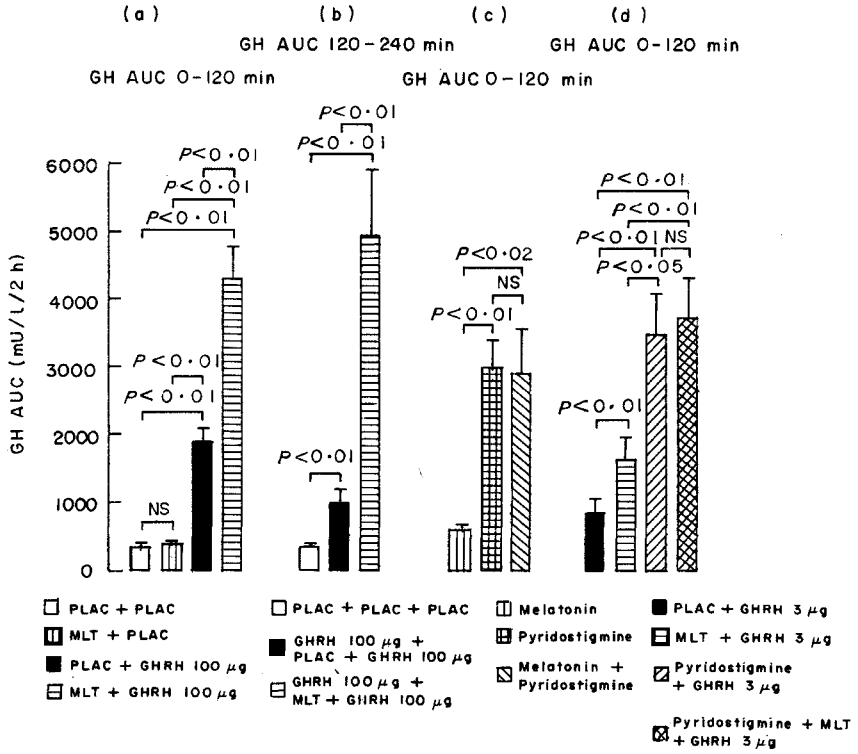

Fig. 4 Influence of prior oral melatonin or pyridostigmine treatment on mean (± SE) GH AUC responses to i.v. GHRH injections in four groups of 8 normal male subjects. All experiments were carried out in the afternoon between 1430 and 1500 hours. a, Study 1: melatonin (MLT) or placebo (PLAC) were administered 60 minutes prior to maximal GHRH i.v. stimulus (0 minutes). b, Study 2: a maximal GHRH i.v. bolus was given at 0 minutes, oral melatonin or placebo were then administered at 60 minutes and a second GHRH i.v. injection was repeated at 120 minutes. The histograms represent the GH AUC following the second GHRH challenge (120–240 minutes). c, Study 3: either oral melatonin, or pyridostigmine, or the combination of both drugs were administered at 0 minutes. d, Study 4: either oral melatonin, or pyridostigmine, or the combination of both drugs, or placebo were administered 60 minutes prior to an i.v. GHRH small dose (0 minutes).

Therefore we investigated the mechanisms of melatonin actions on GH release by means of other experimental approaches in an attempt to shed light on this issue.

The administration of 200 μg GHRH to normal human subjects completely abolishes the GH responses to GHRH given 2 hours later, while GH responses to insulin-induced hypoglycaemia are maintained (Shibasaki *et al.*, 1985). In addition, in a similar experimental setting, the administration of pyridostigmine, a cholinergic agonist drug which is likely to suppress hypothalamic somatostatin release (Richardson *et al.*, 1980; Massara *et al.*, 1986a; Ross *et al.*, 1987), restores and potentiates the GH responsiveness to the second GHRH challenge (Massara *et al.*, 1986b). These data indicate that somatotroph responsiveness to repeated GHRH is maintained and enhanced when non-GHRH dependent mechanisms are activated by insulin-induced hypoglycaemia or by cholinergic enhancement with pyridostigmine. According to the established view, it is likely that these pathways independent of GHRH involve the inhibition of hypothalamic somatostatin release. Our finding of restored GH responses to GHRH by melatonin after GHRH pretreatment, at a time when GH responses to GHRH were suppressed, provides further evidence that melatonin exerts its stimulatory effects on serum GH by inhibiting hypothalamic somatostatinergic tone.

The available evidence suggests that doses of GHRH as low as 3 μg/subject can elicit a rise in plasma GH levels in a few normal subjects, with maximal GH release attained at doses above 20 μg/subject (Wood *et al.*, 1983; Vance *et al.*, 1984; Gelato *et al.*, 1984; Boissel *et al.*, 1986). Therefore we studied the effects of prior melatonin administration on the GH releasing activity of submaximal (3 μg/subject) doses of GHRH. As for supramaximal (100 μg/subject) doses, we found that the respective quantity of GH released by i.v. GHRH was approximately doubled by oral melatonin pretreatment, as assessed by both GH plasma peak levels and AUCs. It is possible that melatonin enhanced the pituitary GH release by stimulating endogenous GHRH release when submaximal doses of GHRH (3 μg) were administered. However, since the same enhancing effect of melatonin pretreatment on pituitary GH release was observed following supramaximal exogenous concentrations of GHRH (100 μg), it is unlikely that melatonin acted through mechanisms mediated via endogenous GHRH. On the contrary, these findings provide further support for the hypothesis that melatonin facilitates GH pituitary release through pathways other than GHRH.

The effects of melatonin on GH secretion were then compared to the effects of pyridostigmine. The GH releasing effects of pyridostigmine, either alone or followed by GHRH

administration, appeared more powerful than those of melatonin. However, when melatonin and pyridostigmine were administered together no further enhancement of GH release occurred, either in the absence or presence of exogenous GHRH. This suggests that melatonin acts through the same pathways as pyridostigmine by inhibiting hypothalamic somatostatin, although at a lower level of potency. Our studies in humans, however, cannot rule out a possible additional stimulatory effect of melatonin on endogenous GHRH release, although this is unlikely. Another mechanism, which is impossible to assess with the present studies, is that melatonin could act on GH secretion by changing somatotroph responsiveness either to GHRH or to somatostatin.

In humans GH shows a noticeable inverse association with melatonin. Stimulation of GH, due either to insulin hypoglycaemia, arginine infusion (Gupta *et al.*, 1983), or GHRH administration (Gupta, 1986) was consistently found to be associated with the inhibition of circulating melatonin. Therefore the interaction between GH and melatonin might operate through somatostatinergic pathways, with reciprocal negative feedback regulation. However our data can not provide direct evidence to substantiate this hypothesis, and we do not know the pathways through which this interaction between melatonin and somatostatin might occur.

To summarize, our data indicate that oral administration of melatonin to normal men increases basal GH release and GH responses to GHRH through the same pathways as the cholinergic agonist drug pyridostigmine. It is likely that melatonin plays this facilitating role at hypothalamic level by inhibiting endogenous somatostatinergic tone, although with a lower potency than pyridostigmine. The physiological role of melatonin on GH neuroregulation, however, remains to be established.

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