Different Effects of Naloxone on the Growth Hormone Response to Melatonin and Pyridostigmine in Normal Men

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The effect of melatonin (MEL) (12 mg orally), pyridostigmine (60 mg orally), the combination of MEL and pyridostigmine, or placebo on growth hormone (GH) secretion was tested in seven normal men. In addition, MEL tests and pyridostigmine tests were repeated after pretreatment with naloxone (1.2-mg bolus followed by intravenous [IV] infusion of 1.6 mg/h for 3 hours). Serum GH levels increased fivefold after MEL and sixfold after pyridostigmine administration. The concomitant administration of MEL did not change the GH response to pyridostigmine. In the presence of naloxone, the GH response to MEL was completely abolished, whereas naloxone did not modify the pyridostigmine-induced GH increase. These data suggest that MEL and pyridostigmine stimulate GH secretion through a common mechanism, which is probably represented by the inhibition of somatostatin activity. However, in contrast to pyridostigmine, the action of MEL appears to be exerted through a naloxone-sensitive opioid mediation.

THE ADMINISTRATION of the pineal hormone melatonin (MEL) to normal human subjects in basal conditions is known to produce a slight, but significant increase in growth hormone (GH) secretion.[1-6] The GH-stimulating effect of MEL is thought to be exerted through inhibition of hypothalamic somatostatin release[3]; in addition, evidence has been provided of an opioid mediation of this MEL action.[4] To achieve better insight into this matter, in this study, we tested the GH response to MEL in the presence of the cholinergic agent pyridostigmine, a well-known stimulator of GH secretion through somatostatin inhibition.[2,5,7,8] Furthermore, we tested the effects of MEL and pyridostigmine either in the presence or in the absence of the opioid antagonist naloxone to establish whether endogenous opioids exert similar effects in modulation of the stimulating actions of these drugs on GH secretion.

MATERIALS AND METHODS

Seven young male subjects (mean age, 34 ± 2.3 [SE] years) were studied after giving informed consent. None of them were taking any medication and all were within 10% of their ideal body weight. They were fully ambulatory, well nourished, and without clinical or laboratory evidence of endocrine, metabolic, renal, hepatic, or neoplastic diseases. None of them were taking drugs before or during the period of the study, or were engaged in excessive alcohol consumption (<300 g ethanol/wk).

On the experimental day, subjects had breakfast and then fasted until the experiment. They remained recumbent throughout the experiment, which started in the afternoon at 2:30 to 3 PM. This period of the day was chosen because biological systems are most sensitive to MEL in the afternoon.[9] In fact, downregulation of MEL receptors might occur in the morning after exposure to endogenous MEL during the night.[10]

Six tests were performed in random order at weekly intervals. At time 0 (30 minutes after the insertion of a cannula into the left antecubital vein for naloxone or saline administration and of a double-lumen indwelling catheter into the right antecubital vein for blood withdrawal), subjects were given MEL (Melatonin Caps; Twinlab Speciality Corp, Ronkonkoma, NY) (12 mg orally), MEL plus naloxone (1.2 mg as an intravenous [IV] bolus, followed by an IV infusion of 1.6 mg/h for 3 hours), pyridostigmine (60 mg orally), pyridostigmine plus naloxone, MEL plus pyridostigmine, or placebo. In all experiments, the same amount and route of administration of drugs was used. In the MEL, MEL plus pyridostigmine, pyridostigmine, and placebo tests, normal saline was infused instead of naloxone. The dose of 12 mg MEL was chosen because our preliminary studies in men have shown that this dose produces a maximal GH response. Naloxone dose and route of administration were the same as those that completely inhibited the GH response to MEL stimulation in previous studies.[5]

In a preliminary study performed in other subjects, 120 mg pyridostigmine induced a large GH response (mean peak, 15.5 times higher than baseline). In view of the small GH response to MEL, conclusions about the enhancement by MEL of the GH response to pyridostigmine could not be reliable in these experimental conditions. We therefore decised to use a smaller dose (60 mg) of pyridostigmine.

Samples were taken at time 0 and thereafter at 15-minute intervals from 0 to 180 minutes. GH was evaluated with a specific radioimmunoassay (RIA) using commercial kits (Biodata, Milan, Italy). The sensitivity of the GH assay was 0.5 ng/mL; the intraassay and interassay coefficients of variation were 3.6% and 8%, respectively.

Results are expressed as the mean ± SE. The GH secretory responses are presented both as absolute values and as areas under the curve (AUC) variable from 0 to +180 minutes, evaluated by trapezoid integration. Statistical analyses were performed using Wilcoxon's matched-pair rank-sum test, and two-way ANOVA, as appropriate.

RESULTS

GH responses in the various tests are presented in Fig 1 as response curves and in Fig 2 as incremental AUCs.

Placebo Test

Serum GH levels did not change at any time point after placebo administration.

MEL Test

The administration of MEL induced a slight, but significant increase in serum GH levels, with a mean peak response five times higher than baseline at 45 minutes ($P < .01$).

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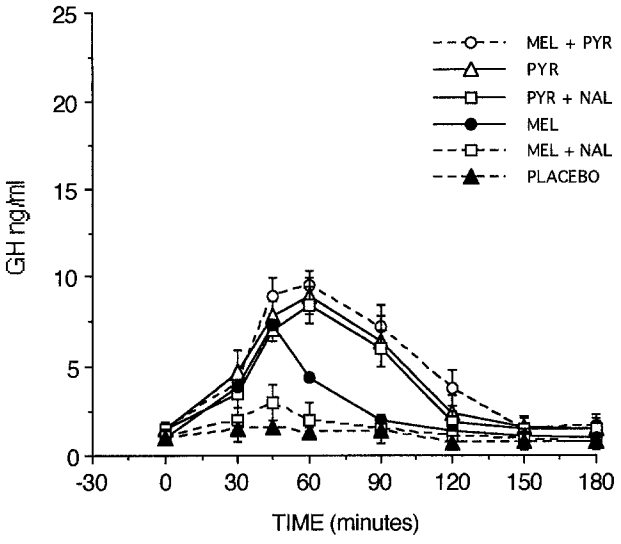

Fig 1. Effect of MEL, pyridostigmine, MEL plus pyridostigmine, MEL plus naloxone, pyridostigmine plus naloxone, or placebo in 7 normal men. Each point represents the mean ± SE of the observations.

MEL Plus Naloxone Test

In the presence of naloxone, the administration of MEL did not change the circulating concentrations of GH ($F = 8.99$, $P < .02$ *v* MEL test; not significant [NS] *v* placebo test).

Pyridostigmine Test

Pyridostigmine administration induced a significant increase in serum GH levels, with a mean peak response six times higher than baseline at 60 minutes ($P < .01$).

Pyridostigmine Plus Naloxone Test

Naloxone administration did not change the GH response to pyridostigmine (NS *v* pyridostigmine test).

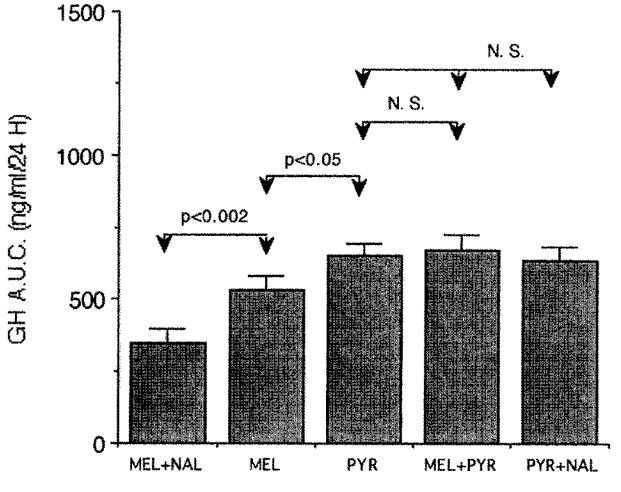

Fig 2. Serum GH responses (area under the curve [AUC]; mean ± SE) in 7 normal men during MEL, pyridostigmine, MEL plus pyridostigmine, MEL plus naloxone, and pyridostigmine plus naloxone tests. N.S., not significant.

MEL Plus Pyridostigmine Test

The concomitant administration of MEL did not change the GH response to pyridostigmine (NS *v* pyridostigmine test).

Side Effects

Pyridostigmine administration induced mild abdominal pain in all subjects. Symptoms due to pyridostigmine were similar both in the presence and absence of MEL or naloxone. No side effects were observed after MEL or naloxone treatment.

DISCUSSION

The results of the present study confirm previous observations of other investigators[4] about the involvement of naloxone-sensitive endogenous opioids in the mechanism underlying the GH response to MEL administration.

MEL induced a significant increase in GH secretion when it was given alone; in contrast, the pineal hormone was unable to produce any further increase in pyridostigmine-stimulated GH secretion, suggesting that MEL and pyridostigmine stimulate GH secretion through a common pathway. It is unlikely that MEL stimulates GH secretion at the anterior pituitary level, because studies in vitro failed to show significant effects of MEL on basal and GH-releasing hormone (GH-RH)–stimulated GH secretion.[3] A direct action of pyridostigmine on the somatotrophs is also unlikely, because addition of atropine to anterior pituitary cell cultures did not change the GH response to GH-RH, arguing against direct cholinergic effects on the pituitary somatotrophs.[9] More likely, MEL and pyridostigmine stimulate GH by acting at hypothalamic level.[2,5,7,8] GH-RH and the GH-inhibitor somatostatin are the major hypothalamic regulators of GH secretion. Previous studies have shown that both pyridostigmine[6] and MEL[3,6] enhance the GH response to GH-RH, suggesting that the effect of both drugs is mediated by somatostatin. Further observations support this hypothesis. Pyridostigmine inhibits acetylcholinesterase,[10] and thus activates endogenous cholinergic neurotransmission, which produces a tonic inhibition of hypothalamic somatostatin release.[2,5,7,8] On the other hand, somatostatin release is also inhibited by serotonin[8] and MEL is known to exert various serotonin-like effects.[11-14]

Even though a reduction in the somatostatinergic inhibitory action on GH secretion might explain the GH-releasing effects of both pyridostigmine and MEL, the underlying neurotransmitter mechanisms appear to be different. Opioid receptors have been shown to modulate somatostatin release from mediobasal hypothalamic neurons.[15] The data presented here show that the GH response to MEL is inhibited by naloxone, whereas pyridostigmine-stimulated GH secretion is not reduced by the concomitant administration of naloxone. Therefore, endogenous opioids may be supposed to be involved in the mechanism of action of MEL, but not in mediation of pyridostigmine activity. In agreement with this hypothesis, several studies indicate interactions between opioids and serotonin at various

levels in the CNS,[16-19] and particularly in the stimulatory control of GH secretion.[20]

In conclusion, our data support the hypothesis that both MEL and pyridostigmine increase the basal secretion of GH through the same pathway, probably by inhibiting hypothalamic somatostatin activity. However, in contrast with pyridostigmine, the action of MEL is exerted through naloxone-sensitive opioid mediation.

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