CLINICAL STUDY

Exercise-induced GH secretion is enhanced by the oral ingestion of melatonin in healthy adult male subjects

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Abstract

There is evidence that melatonin may play a role in modulating pituitary secretion, although the mechanisms are unclear. We examined the effects of a single dose of oral melatonin (5 mg) on exercise-induced GH secretion. In a randomised, double-blind, placebo-controlled study, seven healthy male subjects undertook an initial period of graded bicycle ergometric exercise to determine maximum workload and oxygen uptake ($\text{VO}_{2\text{max}}$). Subjects were subsequently studied on two further occasions, receiving either melatonin or placebo in random order at the onset of each study (–60 min). At 0 min a period of bicycle exercise was performed for 8 min at a workload corresponding to 70 % of that achieved at $\text{VO}_{2\text{max}}$. Serum GH and IGF-binding protein-1 (IGFBP-1) concentration was measured at 15-min intervals from the onset of the study until 120 min post-exercise. Blood was also sampled for the measurement of plasma glucose, insulin, non-esterified fatty acids, IGFBP-3, melatonin and vasopressin concentration. There was an exercise-induced increase in GH concentration following melatonin which was greater compared with placebo as assessed by both area under the curve ($P < 0.01$) and peak increase in GH levels ($P < 0.01$). The peak increase in IGFBP-1 levels post-exercise was also significantly greater following melatonin compared with placebo ($P < 0.01$) but did not quite reach levels of significance as measured by area under the curve ($P = 0.07$). Since exercise-induced GH secretion is thought to be mediated predominantly through a hypothalamic pathway, it seems likely that melatonin facilitates GH secretion at a hypothalamic level.

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Introduction

The relationship between melatonin and growth hormone (GH) secretion is poorly understood. Melatonin, the main hormonal product secreted by the pineal gland, follows a circadian rhythm and is principally controlled by the light:darkness environment. Melatonin interacts with many endocrine and non-endocrine tissues to influence their metabolic activity and there is increased interest in the role that it may play in modulating GH secretion.

Small doses of oral melatonin (2 mg) have not been shown to affect the 24-h profile of GH concentration (1), although much larger doses (1 g orally and 0.4 mg/kg intravenously) have been shown to have a stimulatory effect on basal GH levels (2–4). Results from studies which have investigated the effect of melatonin on stimulated GH secretion have been contradictory. Melatonin has been shown to enhance the GH response to GH-releasing hormone (GHRH) (2, 5) but not to stimulation with L-dopa (6). A reduction in GH response has been observed following insulin-induced hypoglycaemia (3) and L-tryptophan administration (7).

Acute physical exercise is a potent stimulus for endogenous GH secretion. The GH response increases with both duration (8) and intensity of exercise (9, 10). It is likely that exercise stimulates GH secretion through similar pathways to those that operate in the resting state (see 11 for references), through the hypothalamic secretion of GHRH and somatostatin, which stimulate and inhibit GH release respectively.

Since it remains unclear from previous studies whether melatonin has a significant biological influence on GH secretion, we chose an alternative physiological stimulus, exercise, to determine whether there might be a physiological relationship between melatonin and GH secretion.

Subjects and methods

Seven male medical students volunteered to take part in the study. All subjects were healthy, taking no co-existent medication and accustomed to regular exercise. None had a significant previous medical history. Ethical approval was obtained from Lambeth and Southwark

Ethical Committee and all subjects gave full and informed written consent. Prior to each study, a full cardiovascular examination was performed. Maximum oxygen uptake ($\text{VO}_{2\text{max}}$) and workload were determined during an incremental workload test to exhaustion by friction-braked mechanical bicycle ergometry (Tunturi, Ergometer (W), Pispansri, Finland) using the CPX-MAX measurement chart (Medical Graphics, London, UK) for the analysis of expired O_2 and CO_2 content. $\text{VO}_{2\text{max}}$ was determined as a plateau measurement where VO_2 differed by <2 ml/kg per min for two consecutive steps and the respiratory exchange ratio was greater than 1.5. Exercise was commenced at 20 Watts (W) and increased by 20 W every 1 min until exhaustion. Pulse rate and blood pressure were monitored throughout exercise. Maximum workload corresponded to that achieved at exhaustion. (These data are shown in Table 1.)

Each subject was then studied on two occasions, 3 weeks apart. The duration of each study was 180 min and began at 1400 h. The subjects were allowed a light breakfast (0800 h) and then fasted. Thirty minutes before each study commenced, a 20 gauge intravenous cannula was inserted into a forearm vein under local anaesthetic (1% lignocaine solution). The subjects received either melatonin (5 mg orally) or identical placebo in random order at the onset of the study (-60 min). They were rested supine for 60 min prior to performing a period of exercise. They were then exercised on the bicycle ergometer for 8 min at an intensity corresponding to 70% of the maximum workload achieved at $\text{VO}_{2\text{max}}$. Each subject remained recumbent after exercise and was awake throughout. Blood was sampled at 15-min intervals until completion of the study. Blood was collected and centrifuged at 2400 r.p.m. at 4°C for 10 min. The plasma was separated and stored at 20°C until assay.

Assays

Serum GH was measured by a two site immunoradiometric assay (NETRIA; St Bartholomews, London, UK; lower limit of detection for assay (limit) = 0.2 mU/l, coefficient of variation (C.V.) = 3%). IGF-binding proteins-1 and -3 (IGFBP-1 and IGFBP-3) were measured by coated-tube immunoradiometric assay (DSL-7200,

DSL-6600 respectively, TX, USA; limits = 0.012 and 0.17 nmol/l, C.V. = 6% and 4% respectively) and serum insulin was measured by double-antibody RIA (PH Sönksen, St Thomas', London, UK) (limit = 4 mU/l, C.V. = 6%) (12). Plasma glucose was measured using the Clandon analyser (YSI, Yellow Springs, OH, USA; C.V. = 2%). Non-esterified fatty acids (NEFA) were measured using the Acetyl CoA Synthase-Acetyl CoA Oxidase method (Wako Chemicals, Neuss, Germany; limit = 0.02 nmol/l, C.V. = 4%). Samples for vasopressin assay were collected directly onto ice prior to centrifuging and levels were then measured by RIA on plasma extracts prepared using SepPak cartridges (limit = 0.1 pg/ml, C.V. = 7.7%) (13). Melatonin samples were assayed by direct RIA using sheep antibody to melatonin (limit = 5 pg/ml, C.V. = 3%) (14).

Statistical analysis

Since variance between groups was different, statistical analysis was performed by Fisher's *F* test. Comparison tests for non-normally distributed continuous data were made using Wilcoxon's matched pairs signed-rank test for difference in medians. Areas under the curve (AUC) relative to baseline (0 min) were calculated using the trapezoidal method. Peak hormone responses were calculated as maximal post-exercise level minus baseline (0 min). A *P* value of <0.05 was selected as indicative of a significant difference. The results are presented as means $\pm$ S.E.M.

Results

The subjects were all male, aged 20–21 years with a body mass index (BMI) of 23.9 ± 0.9 kg/m² (Table 1). During incremental workload testing by bicycle ergometry they achieved a maximum pulse rate (bpm) of 193 ± 2.1 /min. This was $96.7 \pm 1.0\%$ of the predicted maximal bpm, calculated by subtracting the patient's age (in years) from the number 220. A $\text{VO}_{2\text{max}}$ of 56.3 ± 5.0 ml/min per kg was achieved with a maximum workload of 285.7 ± 11.1 W.

Baseline levels of melatonin were not detected since the lower limit of detection of the assay is 5 pg/ml.

Table 1 Subject characteristics.

Subject no.	Age (years)	Height (cm)	BMI (kg/m ²)	Baseline blood pressure (mmHg)	Expected max. pulse rate (bpm)	Max. pulse rate achieved (bpm)	$\text{VO}_{2\text{max}}$ (ml/min)	Workload achieved (W)
1	20	179.5	26.9	130/70	200	192	3650	280
2	21	172.5	26.3	130/80	199	184	3892	280
3	20	179	20.3	95/55	200	184	3593	240
4	21	167	22.1	110/60	199	199	4582	320
5	20	187.5	22.9	140/80	200	194	5749	320
6	20	188.5	23.9	120/80	200	199	5091	320
7	21	185	25.2	125/70	199	199	3564	240

Following oral ingestion of melatonin, levels reached a mean peak value of 1638 ± 61.9 pg/ml 30 min after ingestion.

The GH secretion profiles are shown in Fig. 1. Basal levels of GH (AUC 0–60 min) were not significantly different between the melatonin and placebo groups ($P=0.68$). Exercise led to an increase in GH levels in both melatonin ($P<0.0001$) and placebo groups ($P<0.001$). The increase in GH levels following exercise (AUC 0–120 min) was significantly greater with melatonin compared with placebo ($P<0.01$) (Fig. 1). Peak GH response was also significantly greater in the melatonin group (22.8 ± 5.34 vs 9.5 ± 3.5 mU/L, $P<0.01$). Mean GH levels peaked at 30 min post-exercise in the melatonin group compared with 15 min in the placebo group.

Basal IGFBP-1 concentration tended to be lower in the melatonin group but this was not significant ($P=0.09$) (Fig. 1). There was a significant increase in peak IGFBP-1 concentration following exercise in the melatonin group ($P<0.0001$) but no increase was seen in the placebo group ($P<0.67$) (Fig. 2). The increase in

IGFBP-1 with melatonin compared with placebo as measured by AUC did not reach levels of significance ($P=0.07$). No significant difference between melatonin and placebo groups was seen in terms of serum insulin ($P=0.46$), NEFA ($P=0.53$), glucose ($P=0.70$), vasopressin ($P=0.16$) or IGFBP-3 concentration ($P=0.60$) (Table 2). Vasopressin concentration at baseline was 0.90 ± 0.26 and 0.99 ± 0.21 and peak post-exercise vasopressin concentration was 1.4 ± 0.4 and 2.0 ± 0.3 pmol/l in the melatonin and placebo groups respectively. Exercise led to an increase in vasopressin concentration at 15 min compared with baseline in all subjects.

Discussion

Oral melatonin administration greatly increased the exercise-induced GH response in our subjects compared with placebo, with mean peak GH responses increased by 72%. There are many factors that can influence the GH response to exercise. Opioid, serotonergic and dopaminergic control may increase its secretion (3,

Figure 1 Mean GH and IGFBP-1 concentration relative to the onset of exercise (0 min) following melatonin (■) and placebo (◆) administration. * $P<0.05$ compared with placebo.

Figure 2 Peak increase in GH and IGFBP-1 concentration from baseline (0 min) following melatonin and placebo administration. * $P < 0.01$, ** $P < 0.05$ compared with placebo.

15, 16). Changes in fuel substrates, particularly glucose and free fatty acids, may have similar effects. However, since our subjects were studied twice under identical circumstances, with no differences seen in NEFA or glucose concentrations, it is unlikely that any of these factors played a role in our observed differential GH responses. The effect of melatonin to increase exercise-induced GH secretion in our subjects would therefore

Table 2 Mean peak concentration of serum insulin, NEFA, glucose, vasopressin and IGFBP-3 following exercise after melatonin or placebo administration.

	Melatonin	Placebo	P
Insulin (mU/l)	20.1 ± 3.1	16.9 ± 2.2	0.46
NEFA (nmol/l)	0.7 ± 0.2	0.6 ± 0.1	0.53
Glucose (mmol/l)	5.5 ± 0.2	5.6 ± 0.2	0.7
Vasopressin (pmol/l)	1.4 ± 0.4	2.0 ± 0.3	0.16
IGFBP-3 (nmol/l)	95.9 ± 3.6	89.4 ± 3.1	0.6

suggest that it has an effect on central hypothalamic regulation through either GHRH or somatostatin release. Enhanced GH secretion following melatonin administration has been previously shown by Valcavi *et al.* (5) who studied the GH response to GHRH. It was postulated then that the increased GH response was facilitated through an inhibition of endogenous somatostatin release since the melatonin effect was abolished following the administration of pyridostigmine.

It is known that central α -1 and α -2 adrenergic stimulation during exercise leads to increased GHRH release, a response which can be abolished by the administration of somatostatin (17) or reduced by adrenergic antagonists such as phentolamine (18, 19). Peripheral catecholamines, however, do not appear to influence GH release during exercise since there is a lack of any GH response to infused adrenaline (20). Hypothalamic cholinergic tone is, however, stimulatory to the exercise-induced GH response (21, 22) probably through the inhibition of somatostatin release. Since the hormones GHRH and somatostatin are responsible for the stimulation and inhibition of GH release respectively it is likely that the GH response to exercise in our subjects is mediated through the balance of hypothalamic function.

To clearly observe the independent effects of melatonin, our studies were performed early in the afternoon when endogenous melatonin is at its lowest. A period of short-duration exercise at submaximal intensity was chosen as an appropriate exercise schedule to avoid the maximum secretion of GH. Increased GH concentration has previously been observed following exercise of similarly short duration (23, 24). Our results might be considered pharmacological rather than physiological but they are consistent with findings from related studies outlined above. We deliberately chose a low melatonin dose to minimise pharmacological effects.

Resting levels of IGFBP-1 were different for melatonin-treated and control subjects and this makes the interpretation of change during exercise more difficult to assess. However, the increase in peak IGFBP-1 concentration following melatonin ingestion is clear. Given the limited duration of exercise chosen for this study, it was thought unlikely that IGFBP-1 concentration would change (no effect was seen following 30 min of moderate exercise in a previous study (25)). IGFBP-1 concentration has, however, been shown to increase in response to exercise of longer duration or intensity (26, 27). The production of IGFBP-1 by the liver is thought to be activated when circulating concentrations of metabolic substrates are low (28) and is also known to fluctuate dramatically according to insulin concentration (29, 30). In our patient group, however, exercise workload was matched and there were no observed differences in levels of plasma glucose, NEFA or serum insulin. Thus, the mechanism by which IGFBP-1 concentration was increased following melatonin administration remains unclear.

We did not detect any significant post-exercise change in IGFBP-3 concentration in the melatonin or placebo groups. This finding is consistent with the work of Galt *et al.* (31) who studied a group of healthy women but not Schwarz *et al.* (32) who reported an increase in IGFBP-3 concentration following short duration exercise in a group of young men.

In summary, we have shown that melatonin increases the GH response to a short period of exercise and it appears likely that this is through a hypothalamic mechanism. These findings provide further evidence for the importance of melatonin in the regulation of the endocrine system.

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