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# Melatonin-Induced Decrease of Body Temperature in Women: A Threshold Event

**Key Words**
Clinical neuroendocrinology
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**Abstract**
Whether the biological effect of melatonin in humans is directly related to the circulating levels of the hormone, has not heretofore been investigated. In this study, we investigated whether previously described hypothermic melatonin properties are dose related. The nocturnal decline of the body temperature (BT) observed in 16 early follicular phase women, following placebo administration at 18.00 h, was compared with that observed during the preceding or following night, after melatonin suppression with the $\beta_1$-adrenergic antagonist atenolol (100 mg). In 6 subjects (37.5%) with lower nocturnal melatonin levels ($p < 0.05$) atenolol induced a complete melatonin suppression and an attenuation of the nocturnal BT decline ($p < 0.02$), whereas in the remaining 10 subjects (62.5%) atenolol induced an incomplete melatonin suppression with no modification of the nocturnal BT decline. During a 3rd night, 2 of the 6 subjects with complete and 6 of the 10 subjects with incomplete melatonin suppression blindly received atenolol plus melatonin (1 mg at 19.30 h and 0.75 mg at 21.00 and 23.00 h). Exogenous melatonin restored the full expression of the nocturnal BT decline in the 2 subjects with complete melatonin suppression, but did not modify the BT decline in the 6 subjects with atenolol-induced incomplete melatonin suppression. Our data show that markedly, but not completely attenuated nocturnal melatonin levels are sufficient to exert maximal thermoregulatory effects, indicating rather a threshold than a dose-response effect of melatonin action on human BT.

## Introduction

Melatonin, selectively secreted at night following $\beta_1$-adrenergic activation of pinealocytes [1–6], is entrained to the 24-hour rhythm by the environmental photoperiod [1–13]. The melatonin secretion can be acutely inhibited by nighttime exposure to bright-light stimuli [13–16] or by $\beta_1$-adrenergic antagonist administration [1–6, 17]. Although quantitative differences in circulating melatonin have frequently prompted theories on the role exerted by the hormone on various human pathophysiological conditions [for reviews see refs. 1, 18, 19], a direct relationship between the levels and the effects of the hormone is still unproven. In previous studies, we have reported that melatonin, by decreasing the body temperature (BT), generates about 40% of the circadian BT rhythm amplitude and that this effect is maximal within physiological levels [17]. In this study we show that the melatonin effect on BT is not dose related, and levels slightly above daytime baseline values are capable to fully express melatonin action.

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## Materials and Methods

Sixteen cycling women, 20–41 years of age, within 15% of their ideal body weight, and entrained to a normal sleep-wake cycle, gave their written informed consent to participate in the study, previously approved by the local ethical committee. The subjects were studied during the early follicular phase of their menstrual cycle, on 2 consecutive days. The study, preceded by an accommodation night, was performed in a room kept at an ambient temperature of 20–22 °C and with a strictly controlled light-dark cycle (lights off 23.00–070.00 h), as previously described [17]. An intravenous catheter was placed in an antecubital vein of the nondominant arm, and blood samples (1.5 ml) were collected every 20 min for 48 h. The subjects received standardized meals at 07.00, 12.00, and 17.00 h and were allowed to sleep only from 23.00 to 07.00 h. Wakefulness between 07.00 and 23.00 h was maintained.

On 2 consecutive days, each subject received at 18.00 h randomly and in a double-blind manner one pill of placebo or the peripheral $\beta_1$-adrenergic antagonist atenolol [17] at a dose of 100 mg. Eight of the subjects blindly received on a third separate night atenolol 100 mg at 18.00 h plus melatonin. Melatonin was administered using three divided doses, 1 mg at 19.30 h and 0.75 mg at 21.00 and 23.00 h, in order to achieve relatively constant pharmacological melatonin levels, as previously described [17, 20].

The BT was recorded at 10-min intervals with a probe (CorTemp®) radiotransmitter device; Human Technologies, St. Petersburg, Fla., USA) inserted deeply into the vagina. The temperature data were smoothed using a three-point moving average and then analyzed at 20-min intervals.

Melatonin, expressed as picomoles per liter, was measured without extraction in each serum sample by radioimmunoassay, using a sheep polyclonal antibody (Guildhay Antisera, Guildford, UK) [17, 20]. Intra- and inter-assay coefficients of variation were 5 and 8%, respectively, and the assay sensitivity was 28 pmol.

The nocturnal melatonin peak was defined by the onset and offset of nocturnal melatonin secretion, identified as the first or last point 2 SD above mean daytime values. The mean nighttime or daytime melatonin levels and the corresponding BT values were calculated accordingly. Mesor (24-hour mean), amplitude (difference between mesor and nadir), and acrophase (time of maximal cosine value) of the circadian BT rhythm were evaluated by single-cosinor analysis. Polynomial regression analyses of atenolol-induced melatonin inhibition as a function of melatonin levels and of net descent of mean nighttime BT as a function of mean nocturnal melatonin levels, were performed using the least squares method.

Statistical analysis of the results was performed using Student's t test for paired or unpaired data. Numerical results are expressed as mean values ± SE.

## Results

The atenolol-induced melatonin inhibition was inversely related ($r = 0.832$; $p < 0.0001$) to the mean nocturnal melatonin values (fig. 1) and was complete (100%) only in 6 of the 16 (37.5%) subjects. Those on placebo had lower mean nocturnal melatonin values: 112.1 ± 11.8 vs. 212.8 ± 93.4 pmol/l ($p < 0.05$; fig. 2). The daytime melatonin values were below the assay sensitivity in both groups of subjects.

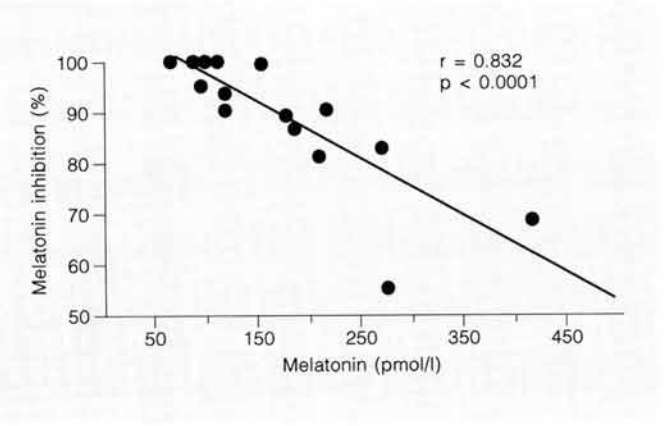


**Fig. 1.** Linear regression analysis between mean nocturnal melatonin levels, defined by the onset and offset of the nocturnal melatonin peak after the placebo night, and percent melatonin inhibition induced by the administration of the $\beta_1$-adrenergic antagonist atenolol (100 mg at 18.00 h). Atenolol-induced melatonin inhibition was inversely related to the nocturnal levels of the hormone, and complete (100%) only in subjects with lower nocturnal melatonin values.

The complete nocturnal melatonin suppression, as evidenced by net above daytime melatonin values of 1.26 ± 1.5 vs. 85.6 ± 11.5 pmol/l ($p < 0.001$) after the placebo night, was associated with a previously published [17] 40% reduction of the circadian BT rhythm amplitude, as a result of a reduced nocturnal BT decline (–0.222 ± 0.06 vs. –0.418 ± 0.06 °C, $p < 0.02$; fig. 2) and with no modification of the acrophase of the circadian BT rhythm.

The incomplete nocturnal melatonin suppression, evidenced by net above daytime melatonin values of 43.5 ± 15.0 vs. 182.3 ± 29.7 pmol/l ($p < 0.001$) after the placebo night, was not associated with any modification of nocturnal BT descent (–0.34 ± 0.02 vs. –0.37 ± 0.04 °C; fig. 2), circadian BT rhythm amplitude (0.43 ± 0.04 vs. 0.42 ± 0.02 °C), or acrophase (16.35 ± 0.4 vs. 16.01 ± 0.5 h).

No relationship was observed between degree of the nocturnal BT decline and detectable melatonin levels. In 2 subjects with atenolol-induced complete melatonin suppression, exogenous melatonin restored the full expression of the nocturnal BT decrease, whereas in 6 subjects with atenolol-induced incomplete melatonin suppression, exogenous melatonin did not modify the degree of the BT decline after the placebo or the atenolol night (fig. 3).

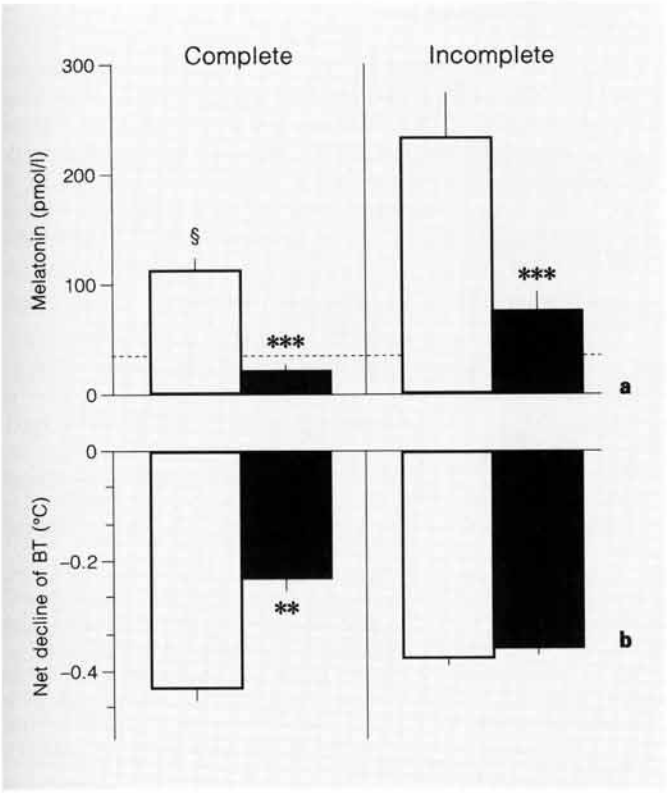


**Fig. 2.** Mean (±SE) nocturnal melatonin values (**a**) and net decline of nighttime BT values (**b**) in 16 early follicular phase women after administration (at 18.00 h) of placebo (open columns) or the $\beta_1$-adrenergic antagonist atenolol (dark columns). After atenolol administration, the melatonin inhibition was complete in 6 and incomplete in 10 subjects. Complete melatonin inhibition was associated with a significant reduction in the nocturnal BT decline which was not evident in subjects with incomplete melatonin inhibition. The horizontal dotted line in **a** represents the mean (± 3 SD) sensitivity of the melatonin assay. § $p < 0.05$ vs. placebo (incomplete melatonin inhibition); ** $p < 0.02$, *** $p < 0.001$ vs. placebo.

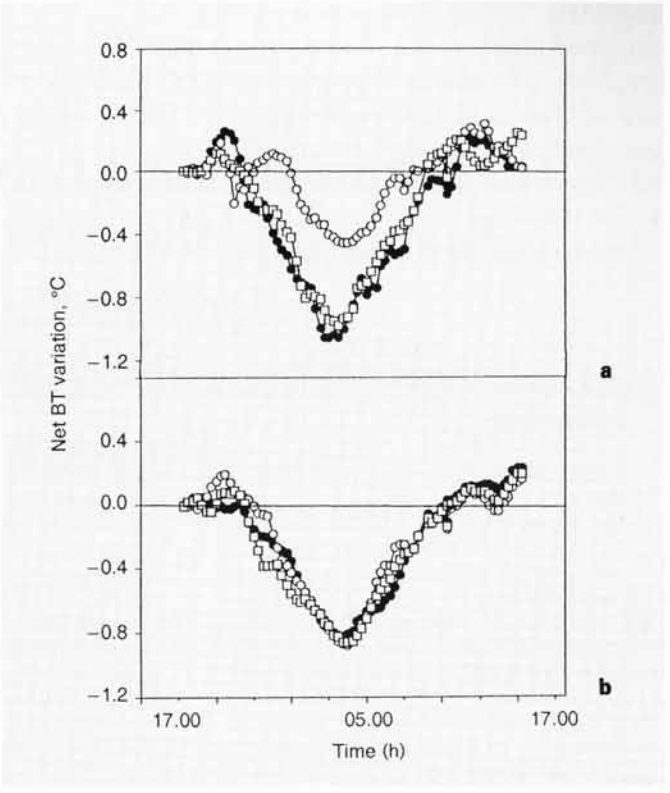


**Fig. 3.** Circadian variations of BT from baseline (net) after administration (18.00 h) of placebo (●) the $\beta_1$-adrenergic antagonist atenolol (○), or atenolol plus melatonin (1 mg at 19.30 and 0.75 mg at 21.00 and 23.00 h; □) in 2 subjects with atenolol-induced complete (**a**) or incomplete (**b**) melatonin inhibition. Complete melatonin suppression was associated with a marked reduction of the nocturnal BT decline, reversible by melatonin administration. By contrast, incomplete melatonin suppression was not associated with any modification of BT which was not influenced by exogenous melatonin, too.

## Discussion

The nighttime melatonin secretion can be blocked either by $\beta_1$-adrenergic antagonists or by bright light [2–17]. In contrast to our previous experience, according to which bright light (>3,000 lx) induced a complete melatonin suppression in all subjects investigated [21], we show in this work that the administration of atenolol, at the commonly used dose of 100 mg [2–5, 22, 23], is capable to completely suppress melatonin secretion only in a subset of subjects (40%) with lower circulating levels of the hormone.

The complete melatonin suppression was associated with the already reported [17, 21, 24] 40–50% reduction of the nocturnal BT decline. The incomplete melatonin suppression, by contrast, was not associated with any attenuation of the nocturnal BT descent, the degree of which was not related to the levels of circulating melatonin, and not enhanced by the simultaneous administration of the hormone. Taken together, these data seem to indicate that just detectable levels of circulating melatonin are capable to exert a maximal thermoregulatory effect and they suggest rather a threshold than a dose-response effect of the melatonin action on human BT.

Such a phenomenon may have relevant clinical implications, when applied to biological functions other than BT. Low concentrations of melatonin, observed during daytime, in situations such as anorexia nervosa [25] or amenorrhea [26, 27] may have greater clinical implications than quantitative modifications of melatonin during

nighttime. Furthermore, as a consequence of such a threshold phenomenon, inconsistent or misleading results might be obtained in experimental trials where the administration of $\beta_1$-adrenergic antagonists fails to induce a complete melatonin withdrawal [22, 23, 28].

These novel insights on the physiology of melatonin action might be critical when interpreting melatonin data or setting experimental trials.


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## References


1 Arendt J: Melatonin. Clin Endocrinol 1988;29: 205–209.
2 Cruickshank JM, Dwyer GN, Cameron MM, McAinsh J: β-Adrenoreceptor-blocking agents and the blood-brain barrier. Clin Sci 1980;59 (suppl):453–455.
3 Cowen PJ, Fraser S, Sammons R, Green AR: Atenolol reduces plasma melatonin concentration in man. Br J Clin Pharmacol 1983;18:579–581.
4 Cowen PJ, Bevan JS, Gosden B, Elliott SA: Treatment with β-adrenoceptor blockers reduces plasma melatonin concentration. Br J Clin Pharmacol 1985;19:258–260.
5 Arendt J, Bojkowski C, Franey C, Wright J, Marks V: Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: Abolition of the urinary 24-hour rhythm with atenolol. J Clin Endocrinol Metab 1985;60:1166–1173.
6 Krause DN, Dubocovich ML: Regulatory sites in the melatonin system of mammals. Trends Neurosci 1990;13:467–470.
7 Moore RY: Organization and function of a central nervous system circadian oscillator: The suprachiasmatic nucleus. Fed Proc 1983;42: 2783–2789.
8 Turek FW: Circadian neural rhythm in mammals. Annu Rev Physiol 1985;47:49–64.
9 Ralph MR, Foster RG, Davis FC, Menaker M: Transplanted suprachiasmatic nucleus determines circadian period. Science 1990;247: 975–978.
10 Takahashi JS, DeCoursey PJ, Bauman L, Menaker M: Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature 1984;308: 186–188.
11 Rusak B, Robertson HA, Wisden W, Hunt SP: Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus. Science 1990;248:1237–1240.
12 Reppert SM, Weaver DR, Rivkees SA, Stopa EG: Putative melatonin receptors in a human biological clock. Science 1988;242:78–81.
13 Lynch HJ, Jimertson DC, Ozaki Y, Post RM, Bunney WE, Wurtman RJ: Entrainment of rhythmic melatonin secretion in man to a 12-hour phase shift in the light/dark cycle. Life Sci 1978;23:1557–1564.
14 Garrick NA, Tamarkin L, Taylor PL, Markey SP, Murphy DL: Light and propranolol suppress the nocturnal elevation of serotonin in the cerebrospinal fluid of rhesus monkey. Science 1983;221:474–476.
15 Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP: Light suppresses melatonin secretion in humans. Science 1980;210:1267–1269.
16 McIntyre IM, Norman TR, Burrows GD, Armstrong SM: Quantal melatonin suppression by exposure to low intensity light in man. Life Sci 1989;45:327–332.
17 Cagnacci A, Elliott JA, Yen SSC: Melatonin: A major regulator of the circadian rhythm of core temperature in humans. J Clin Endocrinol Metab 1992;75:447–452.
18 Brzezinski A, Wurtman RJ: The pineal gland: Its possible roles in human reproduction. Obstet Gynecol Surv 1988;43:197–207.
19 Reiter RJ: Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr Rev 1991;12:151–180.
20 Cagnacci A, Elliott JA, Yen SSC: Amplification of pulsatile LH secretion by exogenous melatonin in women. J Clin Endocrinol Metab 1991;73:210–212.
21 Cagnacci A, Soldani R, Yen SSC: The effect of light on core body temperature is mediated by melatonin in women. J Clin Endocrinol Metab 1993;76:1036–1038.
22 Brismar K, Mogensen L, Wetterberg L: Depressed melatonin secretion in patients with nightmares due to β-adrenoceptor blocking drugs. Acta Med Scand 1988;221:155–158.
23 Rosenthal NE, Jacobsen FM, Sack DA, Arendt J, James SP, Parry BL, Wehr TA: Atenolol in seasonal affective disorders: A test of the melatonin hypothesis. Am J Psychiatry 1988;145: 52–56.
24 Strassman RJ, Qualls CR, Lisansky EJ, Peake GT: Elevated rectal temperature produced by all-night bright light is reversed by melatonin infusion in men. J Appl Physiol 1991;71:2178–2182.
25 Tortosa F, Puig-Domingo M, Peinado M-A, Oriola J, Webb SM, de Leiva A: Enhanced circadian rhythm of melatonin in anorexia nervosa. Acta Endocrinol (Copenh) 1989;120:574–578.
26 Brzezinski A, Lynch HJ, Seibel MM, Deng MH, Nader TM, Wurtman RJ: The circadian rhythm of plasma melatonin during the normal menstrual cycle and in amenorrheic women. J Clin Endocrinol Metab 1988;66:891–895.
27 Laughlin GA, Loucks AB, Yen SSC: Marked augmentation of nocturnal melatonin secretion in amenorrheic athletes, but not in cycling athletes: Unalterded by opioidergic or dopaminergic blockade. J Clin Endocrinol Metab 1991; 73:1321–1326.
28 Demitrack MA, Lewy AJ, Reus VI: Pineal-adrenal interactions: The effect of acute pharmacological blockade of nocturnal melatonin secretion. Psychiatry Res 1989;32:183–189.