Cardiovascular control by the biological clock
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CHAPTER 8

Daily nighttime melatonin reduces blood pressure in patients with essential hypertension

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Submitted

Summary

BACKGROUND
Patients with essential hypertension have disturbed autonomic cardiovascular regulation and circadian pacemaker function. Recently the biological clock was shown to be involved in autonomic cardiovascular regulation. Our objective was to determine whether enhancement of the functioning of the biological clock by repeated nighttime melatonin intake may reduce ambulatory blood pressure in patients with essential hypertension.

METHODS
We conducted a randomized, double-blind, placebo-controlled, crossover trial in sixteen men with untreated essential hypertension to investigate the influence of acute (single) and repeated (3 weeks daily) oral melatonin (2.5 mg) intake 1 hour before sleep on 24-hour ambulatory blood pressure and actigraphic estimates of sleep quality.

FINDINGS
Repeated melatonin intake reduced systolic and diastolic blood pressure during sleep by, respectively, 6 and 4 mm Hg. The treatment did not affect heart rate. The day-night amplitude of the rhythms in both systolic and diastolic blood pressure were increased by 15% and 25%, respectively. A single dose of melatonin had no effect on blood pressure. Repeated (but not acute) melatonin also improved sleep efficiency. Improvements in blood pressure and sleep were statistically unrelated.

INTERPRETATION
In patients with essential hypertension, repeated bedtime melatonin intake significantly reduced nocturnal blood pressure. Future studies in larger patient group should be performed to define the characteristics of the patients that would benefit most from melatonin intake. The present study suggest that support of circadian pacemaker function provides a new strategy in the treatment of essential hypertension.
Introduction
The endogenous circadian pacemaker, located in the suprachiasmatic nucleus (SCN), imposes 24-hour biological rhythms by endocrine and autonomic mechanisms.[45] For example, the circadian rhythm in adrenal cortex activity is regulated via both an endocrine and a sympathetic route,[47, 290] that of heart and liver via both sympathetic and parasympathetic control,[97, 291, 292] and of the pineal gland via the sympathetic nervous system.[164] Thus, the SCN promotes adaptation to the rest and activity periods by regulating - for example - the morning increase in cortisol, heart rate and glucose, and the evening increase in melatonin.

Evidence for disturbed circadian pacemaker function in essential hypertension is accumulating. Patients with hypertension show blunted day-night rhythms in sympathetic and parasympathetic heart tone.[117, 237] Patients with coronary heart disease - a major complication of chronic hypertension - show a blunted day-night rhythm in vasodilation[301] and suppressed nighttime melatonin levels.[40] We demonstrated recently that, in comparison with normotensive subjects, the levels of three important SCN-neurotransmitters are reduced by more than 50% in patients with essential hypertension,[110] corroborating its functional impairment. Furthermore, we provided anatomical support for a changed SCN output to the sympathetic nervous system and to the hypothalamo-pituitary-adrenal axis in patients with essential hypertension.[111] Together, these findings suggest compromised cardiovascular anticipation to the activity period in patients with essential hypertension, possibly leading to increased risk of cardiovascular incidents in the early morning.[236, 318]

Melatonin (N-acetyl-5-methoxy-tryptamine) secretion from the pineal gland is controlled by the SCN.[164] Melatonin also provides feedback via high affinity melatonin receptors in the SCN,[269, 361] thus influencing the rhythm of its own production and other circadian rhythms.[36, 376] Nighttime melatonin amplifies circadian rhythms directly via the central pacemaker,[36, 376] and is used to improve disturbed day-night rhythms,[12] as in dementia,[227] shift work,[299] and blindness.[280] Because the SCN influences the autonomic output to the cardiovascular system,[291, 292, 309] restoration of proper functioning of the SCN in patients with hypertension could improve the autonomic regulation of blood pressure. We therefore investigated, in a double blind, placebo-controlled crossover study, the effect of single and 3-week daily bedtime melatonin intake on ambulatory blood pressure in patients with essential hypertension.

Methods

Patients
Sixteen male patients with untreated, uncomplicated, essential hypertension were included in the study (Table). Women were excluded to prevent possible interference of
the menstrual cycle and oral anticonceptives on cardiovascular and circadian regulation. Patients were considered hypertensive, with a daytime ambulatory blood pressure between 140-179 mm Hg systolic or between 90-109 mm Hg diastolic. Secondary hypertension was excluded by medical history and routine laboratory tests. Five patients had never received antihypertensive medication; in the others antihypertensive treatment was stopped at least three weeks before participation. Their mean (±SD) age was 55±8 (range 36-68) years and mean BMI was 26.8±1.7 (23.3-29.1) kg/m². Mean self-reported habitual waking and bedtimes were 06:38 a.m.±55 minutes and 11:29 p.m.±26 minutes, respectively. The subjects did not travel across time zones or participate in shift work for at least 6 weeks before and during the study. Five days before and during ambulatory blood pressure measurements, subjects maintained fixed sleep-wake cycles according to their habitual sleep-wake cycle. All procedures were carried out with adequate understanding and written consent of the subjects and were approved by the Ethics Committee of the Academic Medical Center of the University of Amsterdam.

Study Design
The study had a balanced, randomized, double blind, placebo-controlled, crossover design (Fig. 1). The effect of acute (1 day) and repeated (once daily for 3 weeks) melatonin on ambulatory blood pressure and heart rate was investigated. Melatonin (2.5 mg controlled-release (100% dissolved in 60 minutes); Terafarm, Katwijk, The Netherlands) or matching placebo was taken orally 1 hour before bedtime. On assessment days, patients abstained from heavy physical exercise, daytime napping, alcohol and nicotine consumption and coffee was restricted to two consumptions per day during breakfast or lunch. Meal times were restricted to the following periods: breakfast 1 to 1.5 hours after waking; lunch 11 to 10 hours before bedtime; and dinner 5 to 4 hours before bedtime.

![Fig. 1 Study design.](image)
After randomization, patients started with the two single applications followed by the two 3 week applications. Arrows, time of ambulatory measurements.
Measurements

Four ambulatory blood pressure recordings were conducted (SpaceLabs 90207, Redmond, WA, USA) [241] while taking melatonin or placebo (Fig. 1). During the first two recordings, 1 week apart, the acute placebo-controlled effect of melatonin was studied. During the last two recordings, 3 weeks apart, the repeated placebo-controlled melatonin effect was investigated. Ambulatory blood pressure was measured every half hour for 32 hours, from 8 hours before bedtime until bedtime the next day. In addition, non-invasive finger arterial blood pressure recordings were performed by Finometer (TNO-Biomedical Instruments, Amsterdam, The Netherlands) [35], to estimate changes in total peripheral resistance, stroke volume, and cardiac output, as possible explanations for changes in blood pressure. These recordings were performed before and at the end of both 3-week periods with melatonin or placebo. The three recordings were performed in the morning and at least 10 hours after melatonin or placebo intake in a quiet room during at least 10 minutes in supine position followed by at least 5 minutes standing. During all ambulatory blood pressure recordings, the subjects reported sleep details in a sleep-wake diary and wore an Actiwatch (Cambridge Neurotechnology Ltd, Cambridge, UK), a piezo-electric accelerometer, on the non-dominant wrist to assess motor activity at 1 min intervals for the estimation of sleep quality.

Data analysis

Sleep blood pressure was defined as the mean blood pressure from the time of falling asleep until the time of awakening, as determined by actigraphy. Awake blood pressure was defined as the mean blood pressure during the remaining portion of the day. Continuous Finometer data were analyzed by Beatscope software (TNO-Biomedical Instruments, Amsterdam, The Netherlands) [35]. Periods of 5 min with a stable blood pressure signal were used for both supine and standing periods. To accurately determine the sleeping period and estimate sleep quality, automatic sleep/wake scoring was performed with Actiwatch Sleep Analysis 98 (Cambridge Neurotechnology Ltd, V4.15), with sensitivity set to medium, on the actigraphy data between “bed time” and “get up time” derived from the sleep-wake diaries. Three sleep variables were objectively computed by this analysis. ‘Sleep latency’ was the calculated time between bed time and sleep onset. The time asleep was termed ‘actual sleep time’. The percentage of time asleep while in bed was defined as ‘sleep efficiency’.

Statistics

Because not all variables were normally distributed (Shapiro’s-Wilk’s W Test), non-parametric tests were used as required. Consequently, Altman’s crossover analysis methods were applied [7]. Paired student’s t-tests or Wilcoxon matched pairs tests were applied to test differences between (1) single melatonin versus single placebo period, and (2) re-
peated melatonin versus repeated placebo period. Student's t-tests for independent variables or Mann-Whitney U tests were applied for all dependent variables to test a carry-over effect for (1) single treatment periods, and (2) repeated treatment periods. Correlation between a change in sleep quality and a change in blood pressure was tested by linear correlation. The 24-hour rhythm in blood pressure was fitted by standard cyclic regression models: cosine, cosine with second harmonic, skewed cosine, peaked cosine, and skewed and peaked cosine analysis[23]. Of these, the latter best fitted the data as indicated by the Akaike's Information Criterion (AIC)[75] and was used for subsequent analysis. Two-tailed p values lower than 0.05 were considered to indicate statistical significance. All group data are presented as means ± standard deviation (SD) or means including 95% confidence interval (95% CI).

Results

**Ambulatory blood pressure**

Three weeks of 2.5 mg melatonin 1 hour before bedtime caused a significant reduction of sleep systolic and diastolic blood pressure of 6±10 (95% CI, -1 to -8) and 4±6 (-1 to -6) mm Hg as compared to 3 weeks placebo (p=0.046 and p=0.020), without a change in heart rate (p=0.23) (Fig. 2 and Table). There was no period effect (p=0.29 and p=0.76, for SBP and DBP) and no treatment-period interaction (p=0.18 and p=0.27, for SBP and DBP). Awake systolic and diastolic blood pressure did not decrease significantly when we compared 3 weeks melatonin with 3 weeks placebo (-5 and -1 mm Hg; p=0.14 and p=0.41) (Table). The blood pressure rhythms after repeated melatonin and placebo relative to get up time are shown in figure 3, illustrating the main effect at night and in the early morning hours. Acute melatonin application had no effect on systolic and diastolic blood pressure, whilst asleep (p=0.89 and p=0.86) or awake (p=0.20 and p=0.80).

**Table**  Average Blood Pressure and Heart Rate after 3 Weeks Placebo or Melatonin*

<table>
<thead>
<tr>
<th></th>
<th>Sleeping period</th>
<th>Waking period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic BP</td>
<td>Diastolic BP</td>
</tr>
<tr>
<td>Placebo</td>
<td>136.2±14.1</td>
<td>86.3±6.6</td>
</tr>
<tr>
<td>Melatonin</td>
<td>130.6±10.0</td>
<td>82.4±4.0</td>
</tr>
</tbody>
</table>

* Plus-minus values are means ± SD. BP indicates blood pressure.
† Significant difference compared to placebo treatment.
Fig. 2 Effect of repeated melatonin on individual sleep and wake blood pressure and heart rate. Zero values indicate the levels assessed after placebo. The effect of repeated melatonin of patient 14 was -53 and -27 mm Hg, for waking systolic and diastolic blood pressure, respectively. Dark bars, sleep; light bars, wake.
Fig. 3 Hourly means of ambulatory blood pressure after repeated melatonin and repeated placebo. The average systolic and diastolic ambulatory blood pressure of all patients is depicted relative to get up time (n = 16). The light gray background indicates the average period in bed (7.1 hours±44 minutes). The vertical line indicates the get up time. Open circles, placebo; closed circles, melatonin.

**24-Hour rhythm analysis of ambulatory blood pressure**

For 24-hour rhythm analysis of ambulatory blood pressure, the peaked and skewed cosine analysis was used. For patient 13 no significant fit was reached for systolic and diastolic blood pressure and for patient 3 not for systolic blood pressure, and these data were excluded from further analysis. Three-weeks melatonin enhanced the day-night rhythm amplitude of systolic and diastolic blood pressure by 15% (p=0.031) and 25% (p=0.029), respectively, as compared to 3 weeks placebo (Fig. 4). The calculated minimum and mean diastolic blood pressure decreased by 5±7 and 4±6 mm Hg (p=0.008 and p=0.023), respectively, comparing 3 weeks melatonin with 3 weeks placebo. The
decrease in minimum and mean systolic blood pressure was not significant (-6±15 mm Hg; p=0.13 and -5±12 mm Hg; p=0.19). There were no effects of repeated melatonin on maximum systolic and diastolic blood pressure (-2±12 mm Hg; p=0.50 and -1±5 mm Hg; p=0.49), respectively, or on time of the minimum blood pressure.

*Finger arterial blood pressure recordings*

There was no effect of repeated melatonin on blood pressure, heart rate, stroke volume, cardiac output, or total peripheral resistance during either supine resting conditions or standing during daytime as measured by Finometer.

![Graph showing blood pressure rhythm](image)

**Fig. 4** Twenty-four hour fit of systolic and diastolic blood pressure rhythm. The average peaked and skewed cosine fit for systolic (n = 14) and diastolic (n = 15) ambulatory blood pressure of all patients with a significant fit is depicted relative to clock time. Circular statistics were used for the analysis of differences in bathyphase (time of minimum) between the placebo and melatonin condition in blood pressure (Mardia-Watson-Wheeler Chi² test) [23].
Sleep-wake rhythm
Repeated melatonin significantly increased sleep efficiency (from 80% to 85%; p=0.017) and actual sleep time (from 5.6 hours to 6.1 hours; p=0.013) and significantly reduced sleep latency (from 33 to 22 minutes; p=0.036). There was no correlation between the effect of melatonin on any of the sleep variables and the effect of melatonin on sleep systolic and sleeping diastolic blood pressure. Acute melatonin application had no significant effect on sleep efficiency (p=0.39), actual sleep time (p=0.18), or sleep latency (p=0.35).

Discussion
We found that repeated but not single bedtime melatonin intake significantly reduced sleep blood pressure in male patients with untreated uncomplicated essential hypertension, by 6 and 4 mm Hg for systolic and diastolic blood pressure, respectively. A reduction of about 6 mm Hg over day and night, which reached significance during sleep, when blood pressure levels are most stable, is a meaningful reduction, since a drop of as little as 2-3 mm Hg systolic blood pressure has great clinical relevance.[315] Furthermore, a reduction of sleep blood pressure by melatonin is important since we are asleep for approximately one third of our life and since nighttime blood pressure seems to better predict cardiovascular risk than daytime blood pressure.[314] Moreover, as figure 3 illustrates, bedtime melatonin might be beneficial in reducing the blood pressure also in the morning, a period when the blood pressure elevation may participate in the increased risk for cardiovascular incidents at that time.[236, 318]

This is the first double-blind crossover study to investigate the effect of repeated melatonin intake on 24-hour blood pressure rhythm in untreated hypertensive patients. A strong reduction of blood pressure through intranasal melatonin (2 mg) in patients with hypertension was reported by Birau and coworkers.[26] However, the time of blood pressure measurement was unclear and the time of melatonin application (01:00 p.m.) would have been disruptive for the endogenous melatonin rhythm.[12] Lusardi et al. investigated the effect of 4 weeks 5 mg oral melatonin at night (10:30 p.m.) in hypertensive patients treated by nifedipine, which resulted in an unexplained increase in 24-hour mean blood pressure.[197]

Melatonin acts via high-affinity G-protein coupled receptors. In mammals, two receptor subtypes are distinguished: Mel1a and Mel1b. In the human, Mel1a is mainly found in the SCN and to a lesser extent in the pituitary and cerebral vasculature,[269, 288, 361] while Mel1b is present in the retina.[268] Outside the central nervous system, melatonin binding has been demonstrated in several peripheral tissues, including blood vessels.[88] In the present study, only repeated and not single nighttime melatonin intake reduced blood pressure, while heart rate was unaffected. The mode of action therefore seemed
different from that of directly acting vasodilator drugs that cause a rapid lowering of blood pressure accompanied by a baroreflex-mediated increase in heart rate. Human experimental data further suggest that an effect of melatonin on blood pressure is mediated via the autonomic nervous system.[239]

Since the SCN has autonomic projections to the different divisions of the cardiovascular system, i.e. heart,[291, 292] and kidney,[309] and melatonin receptors are present in peripheral organs that display rhythmic clock gene expression,[88, 209] we suggest that the reduction in sleep blood pressure by repeated nocturnal melatonin intake is mediated via the amplification of the circadian output of SCN to,[12, 36, 227, 269, 280, 299, 361] and/or its clock genes in,[209] the cardiovascular system. Repeated melatonin intake is required to improve disturbed circadian rhythmicity.[12, 36, 227, 280, 299] Similarly, only repeated melatonin intake was effective in the present study to lower sleep blood pressure. The involvement of circadian pacemakers is further supported by the observed increase in 24-hour blood pressure rhythm amplitude and the improved sleep quality by repeated, and not by single melatonin intake.

The observed disturbances of the SCN in patients with essential hypertension[40, 110, 111, 117, 237, 301] and the capacity of melatonin to improve disturbed circadian rhythmicity[12, 227, 280, 299] fit in with a role of the circadian pacemaker in blood pressure reduction through repeated melatonin intake. Amplification of the circadian rhythmicity of the SCN in patients with essential hypertension could thus influence their autonomic regulation of heart and/or vasculature, resulting in lower nocturnal blood pressure.

Single melatonin intake can lower blood pressure, but only when melatonin is taken during the day, when general SCN neuronal activity is high and endogenous melatonin levels are low. This effect could be mediated via an immediate inhibition of SCN-neuronal activity inducing a state resembling nocturnal SCN-output.[269, 340, 361] However, daytime melatonin intake results in sleepiness and hypothermia during the day,[55] and should thus be avoided. On the contrary, repeated nighttime melatonin supports the endogenous melatonin rhythm, improving circadian rhythmicity.[12, 36, 227, 280, 299]

Three strengths in the design of the present study are: the balanced randomized double-blind crossover design, the comparison of both acute and repeated effects in the same patients, and the application of melatonin just before sleep, which supports the endogenous melatonin rhythm. The small number of male patients studied is a limitation. However, the fact that we could demonstrate a significant blood pressure reduction even in a small patient population indicates the value of melatonin as a blood pressure lowering agent and warrants further studies in a larger patient group including women to define the characteristics of the patients that would benefit most from nighttime melatonin application. Although sleep was not assessed by polysomnography, actigraphy allows objective estimation of changes in the sleep variables without affecting sleep.[175]
We used 2.5 mg oral melatonin to ensure plasma melatonin levels at or above endogenous nighttime levels for 4 up to 8 hours after intake, thus during most of the sleeping period.[5] Melatonin (1-5 mg) has been widely used as a nutritional supplement in the United States for several years, without any serious adverse side effects being reported. Also in the present study, 3 weeks of melatonin had no adverse effects on the subjects' general health as determined through a questionnaire.

In conclusion, we found that repeated bedtime melatonin substantially reduced sleep systolic and diastolic blood pressure. Melatonin taken at night could thus be a gentle alternative or supplement to regular antihypertensive medication. This warrants future studies on the long-term contribution of melatonin as hypotensive treatment.