

Daily Nighttime Melatonin Reduces Blood Pressure in Male Patients With Essential Hypertension

Frank A.J.L. Scheer, Gert A. Van Montfrans, Eus J.W. van Someren, Gideon Mairuhu, Ruud M. Buijs

Abstract—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 might reduce ambulatory blood pressure in patients with essential hypertension. We conducted a randomized, double-blind, placebo-controlled, crossover trial in 16 men with untreated essential hypertension to investigate the influence of acute (single) and repeated (daily for 3 weeks) oral melatonin (2.5 mg) intake 1 hour before sleep on 24-hour ambulatory blood pressure and actigraphic estimates of sleep quality. Repeated melatonin intake reduced systolic and diastolic blood pressure during sleep by 6 and 4 mm Hg, respectively. The treatment did not affect heart rate. The day–night amplitudes of the rhythms in systolic and diastolic blood pressures 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. Improvements in blood pressure and sleep were statistically unrelated. 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 who would benefit most from melatonin intake. The present study suggests that support of circadian pacemaker function may provide a new strategy in the treatment of essential hypertension. (*Hypertension*. 2004;43:192-197.)

Key Words: blood pressure monitoring ■ circadian rhythm ■ hormones ■ human ■ hypertension

The endogenous circadian pacemaker, located in the suprachiasmatic nucleus (SCN), imposes 24-hour biological rhythms by endocrine and autonomic mechanisms.¹ For example, the circadian rhythm in adrenal cortex activity is regulated via an endocrine and a sympathetic route,^{2,3} that of heart and liver via sympathetic and parasympathetic control,^{4–6} and of the pineal gland via the sympathetic nervous system.⁷ 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.^{8,9} Patients with coronary heart disease, a major complication of chronic hypertension, show a blunted day–night rhythm in vasodilatation¹⁰ and suppressed nighttime melatonin levels.¹¹ 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,¹² 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.¹³ Together, these findings suggest compromised cardiovascular anticipation to the activity period in patients with essential hypertension, possibly leading to increased risk for cardiovascular incidents in the early morning.^{14,15}

Melatonin (N-acetyl-5-methoxy-tryptamine) secretion from the pineal gland is controlled by the SCN.⁷ Melatonin also provides feedback via high-affinity melatonin receptors in the SCN,^{16,17} thus influencing the rhythm of its own production and other circadian rhythms.^{18,19} Nighttime melatonin amplifies circadian rhythms directly via the central pacemaker^{18,19} and is used to improve disturbed day–night rhythms,²⁰ as in dementia,²¹ shift work,²² and blindness.²³ Because the SCN influences the autonomic output to the cardiovascular system,^{4,5,24} restoration of proper functioning of the SCN in patients with hypertension could improve the autonomic regulation of blood pressure (BP). We therefore investigated, in a double-blind, placebo-controlled, crossover study, the effect of single and 3-week daily bedtime melatonin intake on ambulatory BP in patients with essential hypertension.

Received July 9, 2003; first decision, July 28 2003; accepted November 18, 2003.

From Netherlands Institute for Brain Research (F.A.J.L.S., E.J.W.v.S., R.M.B.), the Department of Internal Medicine, Academic Medical Center (G.A.V.M., G.A.), and VU University Medical Center (E.J.W.v.S.), Amsterdam, The Netherlands.

Correspondence to Dr Frank A.J.L. Scheer, Division of Sleep Medicine, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115. E-mail fscheer@rics.bwh.harvard.edu

© 2004 American Heart Association, Inc.

Hypertension is available at <http://www.hypertensionaha.org>

DOI: 10.1161/01.HYP.0000113293.15186.3b

Average Blood Pressure and Heart Rate After 3 Weeks of Placebo or Melatonin

Treatment	Sleeping Period			Waking Period		
	Systolic BP	Diastolic BP	Heart Rate	Systolic BP	Diastolic BP	Heart Rate
Placebo (n=16)	136.2±14.1	86.3±6.6	59.8±6.6	152.8±13.0	96.8±7.8	69.6±7.6
Melatonin (n=16)	130.6±10.0*	82.4±4.0*	61.1±6.5	147.6±12.3	95.4±7.7	71.2±7.8

Plus–minus values are mean±SD.

*Significant difference compared to placebo treatment.

Methods

Patients

Sixteen male patients aged 55±8 years (range: 36 to 68) with BMI 26.8±1.7 kg/m² (range: 23.3 to 29.1) with either mild or moderate (140 to 179/90 to 109 mm Hg) untreated, uncomplicated, essential hypertension, according to the WHO classification,²⁵ were included in the study (Table). Five days before and during ambulatory BP measurements, subjects maintained fixed sleep–wake cycles according to their habitual sleep–wake cycles. All procedures were performed with adequate understanding and written consent of the subjects and were approved by the institutional review committee.

Study Design

The study had a balanced, randomized, double-blind, placebo-controlled, crossover design (Figure 1). The effect of acute (1 day) and repeated (once daily for 3 weeks) melatonin on ambulatory BP and heart rate was investigated. Melatonin (2.5-mg controlled-release; 100% dissolved in water in vitro in 60 minutes; Terafarm, Katwijk, The Netherlands) or matching placebo was taken orally 1 hour before bedtime.

Measurements

Four ambulatory BP recordings were conducted (SpaceLabs 90207, Redmond, Wash)²⁶ while using melatonin or placebo (Figure 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 BP was measured every 30 minutes for 32 hours, from 8 hours before bedtime until bedtime the next day. During ambulatory BP recordings, the subjects reported sleep details in a sleep–wake diary and wore an Actiwatch (Cambridge Neurotechnology Ltd, Cambridge, UK), on the non-dominant wrist to assess motor activity at 1-minute intervals for the estimation of sleep quality.

Data Analysis

Sleep BP was defined as the mean BP from the time of falling asleep until the time of awakening, as determined by actigraphy. Awake BP was defined as the mean BP during the remaining portion of the day. Automatic sleep/wake scoring was performed with Actiwatch Sleep Analysis 98 (Cambridge Neurotechnology Ltd, V4.15; sensitivity set at medium) on the actigraphy data between “bedtime” 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 bedtime 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-Wilk *W* Test), nonparametric tests were used as required. Consequently, Altman crossover analysis methods were applied.²⁷ Paired Student *t* tests or Wilcoxon matched pairs tests were applied to test differences between (1) single melatonin versus single placebo period and (2) repeated melatonin versus repeated placebo period. Student *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 sleep BP was tested by linear correlation. Two-tailed *P*<0.05 were considered statistically significant. All group data are presented as mean±SD or mean including 95% CI.

An expanded Methods section can be found in an online supplement available at <http://www.hypertensionaha.org>.

Results

Ambulatory BP

Three weeks of 2.5-mg melatonin 1 hour before bedtime caused a significant reduction of sleep systolic and diastolic BP of 6±10 (95% CI: −1 to −8) and 4±6 (−1 to −6) mm Hg as compared with 3 weeks of placebo (*P*=0.046 and *P*=0.020), without a change in heart rate (*P*=0.23) (Figure 2 and Table). After excluding subject 14 from statistical analysis, there was a trend for a reduction in sleep systolic BP and a significant reduction in sleep diastolic BP (*P*=0.092 and *P*=0.039, respectively). 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 BPs did not decrease significantly when we compared 3-week melatonin with 3-week placebo (−5 and −1 mm Hg; *P*=0.14 and *P*=0.41) (Table). The BP 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 BPs while asleep (*P*=0.89 and *P*=0.86) or awake (*P*=0.20 and *P*=0.80).

24-Hour Rhythm Analysis of Ambulatory BP

For 24-hour rhythm analysis of ambulatory BP, the peaked and skewed cosine analysis was used. Only the data of the patients with a significant fit for the systolic (n=14) and diastolic (n=15) ambulatory BP for the repeated melatonin and placebo treatment were used for further analysis. For patient 13, no significant fit was reached for systolic and diastolic BPs, and for patient 3, no significant fit was reached for systolic BP. Three weeks of melatonin enhanced the

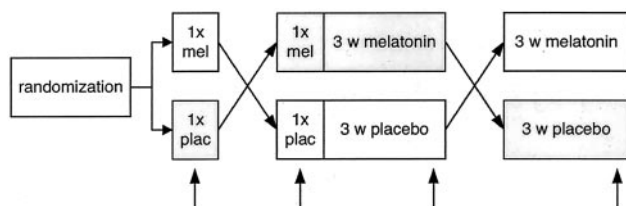


Figure 1. Study design. After randomization, patients started with the two single applications followed by the two 3-week applications. Arrows indicate time of ambulatory measurements.

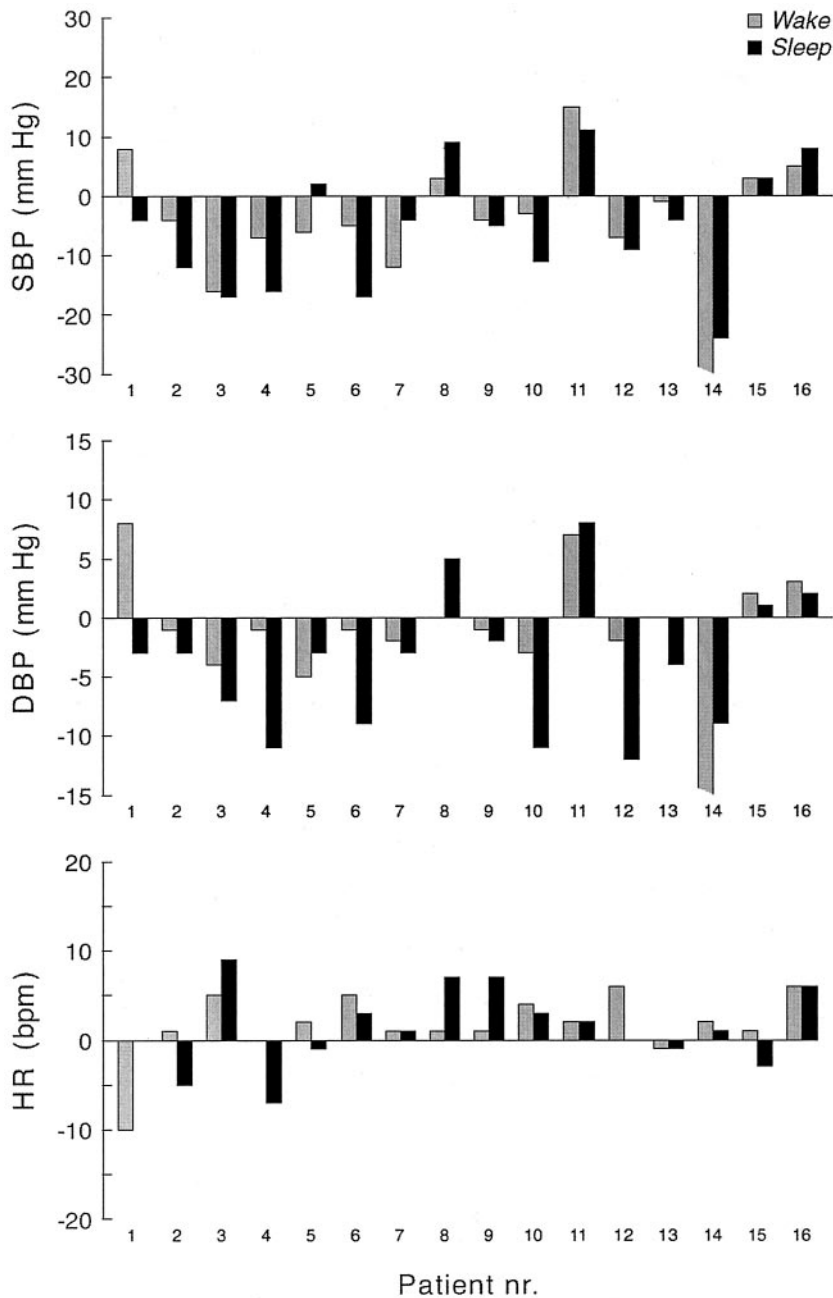


Figure 2. Effect of repeated melatonin on individual sleep and wake BPs and heart rate. Zero values indicate the levels assessed after placebo. The effect of repeated melatonin for patient 14 was -53 and -27 mm Hg for waking systolic and diastolic BPs, respectively.

day–night rhythm amplitude of systolic and diastolic BPs by 15% ($P=0.031$) and 25% ($P=0.029$), respectively, as compared with 3 weeks of placebo (Figure 4). The calculated minimum and mean diastolic BPs decreased by 5 ± 7 and 4 ± 6 mm Hg ($P=0.008$ and $P=0.023$), respectively, comparing 3-week melatonin with 3-week placebo. The decrease in minimum and mean systolic BPs 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 BPs (-2 ± 12 mm Hg; $P=0.50$; and -1 ± 5 mm Hg; $P=0.49$), respectively, or on time of the minimum BP.

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; $P=0.036$). Also, the time from falling asleep until last awakening (over which sleep BP was determined) increased significantly (from 6.5 hours to 6.7 hours; $P=0.037$) by repeated melatonin. 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 BP. 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 BP in male patients with untreated uncomplicated essential hypertension by 6 and

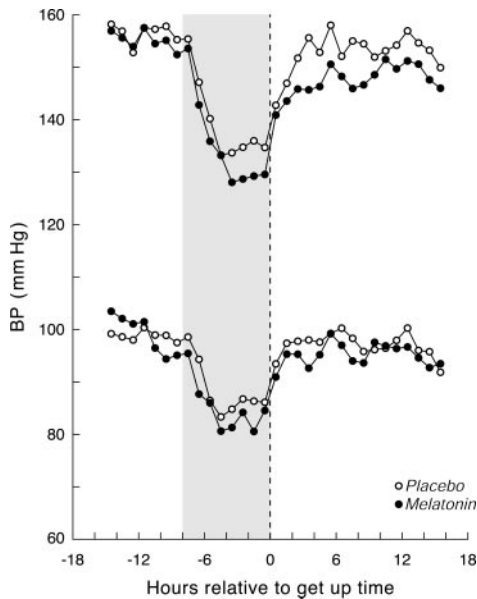


Figure 3. Hourly means of ambulatory BP after repeated melatonin and repeated placebo. The average systolic and diastolic ambulatory BPs of all patients relative to get-up time ($n=16$). The light gray background indicates the average period in bed (7.1 hours ± 44 minutes). The vertical line indicates the get-up time.

4 mm Hg for systolic and diastolic BPs, respectively. A reduction of ≈ 6 mm Hg over day and night, which reached significance during sleep, when BP levels are more stable, is a meaningful reduction, because this is close to that reached by most regular anti-hypertensive medication and because a decrease of as little as 2 to 3 mm Hg systolic BP has clear clinical relevance.²⁸ Furthermore, a reduction of sleep BP by melatonin is important because we are asleep for approximately one third of our lives, and because nighttime BP seems to better-predict cardiovascular risk than does daytime BP.²⁹ Moreover, as Figure 3 illustrates, bedtime melatonin might also be beneficial in reducing the BP in the morning, a period when the BP elevation may be involved in the increased risk for cardiovascular incidents at that time.^{14,15}

This is the first double-blind crossover study to investigate the effect of repeated melatonin intake on 24-hour BP rhythm in untreated hypertensive patients. A strong reduction of BP through intranasal melatonin (2 mg) in patients with hypertension was reported by Birau et al.³⁰ However, the time of BP measurement was unclear and the time of melatonin application (1:00 PM) would have been disruptive for the endogenous melatonin rhythm.²⁰ Lusardi et al investigated the effect of 4 weeks of 5-mg oral melatonin at night (10:30 PM) in hypertensive patients treated by nifedipine, which resulted in an unexplained increase in 24-hour mean BP.³¹ The present study was conducted with untreated patients with hypertension, thereby preventing possible unknown interactions of melatonin with antihypertensive drugs. Future experiments are required to elucidate potential interactions between melatonin and the different classes of antihypertensive medicine in BP regulation.

Melatonin acts via high-affinity G-protein coupled receptors. In mammals, two receptor subtypes are distinguished:

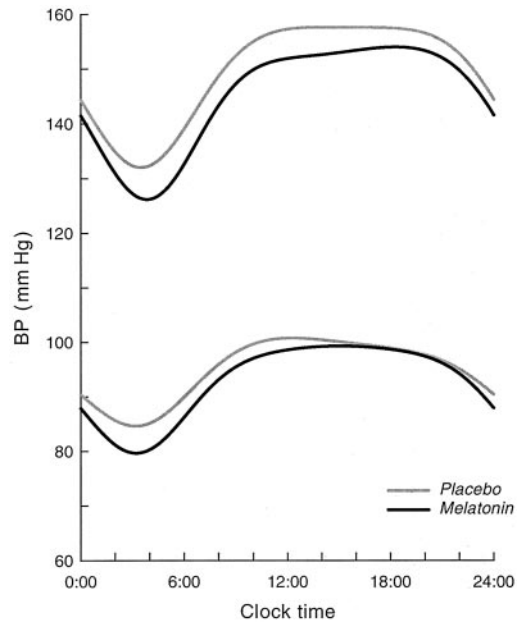


Figure 4. Twenty-four hour fit of systolic BP and diastolic BP rhythm. The average fit for ambulatory BP for the repeated melatonin and placebo treatment relative to clock time. The 24-hour rhythm in BP was fitted by standard cyclic regression models: cosine, cosine with second harmonic, skewed cosine, peaked cosine, and skewed and peaked cosine analysis.⁴² Of these, the latter best-fit the data as indicated by the Akaike Information Criterion (AIC)⁴³ and was used for subsequent analysis. Circular statistics were used for the analysis of differences in bathypphase (time of minimum) between the placebo and melatonin condition in BP (Mardia-Watson-Wheeler χ^2 test).⁴² Lines indicate mean values.

Mella and Mel1b. In the human, Mella is mainly found in the SCN and to a lesser extent in the pituitary and cerebral vasculature,^{16,17,32} whereas Mel1b is present in the retina.³³ Outside the central nervous system, melatonin binding has been demonstrated in several peripheral tissues, including blood vessels.³⁴ In the present study, only repeated, and not single, nighttime melatonin intake reduced BP, whereas heart rate was unaffected. The mode of action therefore seemed different from that of directly acting vasodilator drugs that cause a rapid lowering of BP accompanied by a baroreflex-mediated increase in heart rate. Human experimental data further suggest that an effect of melatonin on BP is mediated via the autonomic nervous system.³⁵

Because the SCN has autonomic projections to the different divisions of the cardiovascular system, ie, heart^{4,5} and kidney,²⁴ and because melatonin receptors are present in peripheral organs that display rhythmic clock gene expression,^{34,36} we suggest that the reduction in sleep BP by repeated nocturnal melatonin intake is mediated via the amplification of the circadian output of SCN to,^{16,17,19–23} and/or its clock genes in,³⁶ the cardiovascular system. Repeated melatonin intake is required to improve disturbed circadian rhythmicity.^{19–23} Similarly, only repeated melatonin intake was effective in the present study to lower sleep BP. The involvement of circadian pacemakers is further supported by the observed increase in 24-hour BP rhythm amplitude and the improved sleep quality by repeated, and

not by single, melatonin intake. Furthermore, because reduced sleep leads to reduced memory function, lower growth hormone levels, and increased cortisol levels at night, all characteristics also of older age,³⁷ the improved sleep quality by repeated melatonin treatment might be of additional clinical benefit in comparison to regular anti-hypertensive drugs.

The observed disturbances of the SCN in patients with essential hypertension^{8–13} and the capacity of melatonin to improve disturbed circadian rhythmicity^{20–23} fit in with a role of the circadian pacemaker in BP 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 BP.

Single melatonin intake can lower BP, 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.^{16,17,38} However, daytime melatonin intake results in sleepiness and hypothermia during the day³⁹ and should thus be avoided. On the contrary, repeated nighttime melatonin supports the endogenous melatonin rhythm, improving circadian rhythmicity.^{19–23}

Three strengths in the design of the present study are the balanced randomized double-blind crossover design, the comparison of 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 BP reduction even in a limited number of patient indicates the value of melatonin as a BP-lowering agent and warrants further studies in a larger patient group including women to define the characteristics of the patients who would benefit most from nighttime melatonin application. Although sleep was not assessed by polysomnography, actigraphy allows objective estimation of changes in sleep variables without affecting sleep.⁴⁰ We used 2.5-mg oral melatonin to ensure plasma melatonin levels at or above endogenous nighttime levels for 4 to 8 hours after intake, thus during most of the sleeping period.⁴¹ Melatonin (1 to 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 inquiring about the presence or absence of headache, insomnia, hyperactivity, irritability, nausea, sleeping limbs, dizziness, constipation, shaky hands, stomach cramp, drowsiness, sweating, hunger, weakness, and sore eyes.

Perspective

The present study showed that repeated bedtime melatonin substantially reduced sleep systolic and diastolic BPs in patients with essential hypertension. 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.

Acknowledgments

The authors thank the subjects who participated in the study, Henk Stoffels for preparing the illustrations, Michel Hofman for statistical advice, and Wilma Verweij for revision of the manuscript. The present study was supported in part by Institut de Recherches Internationales Servier Grant NLD 609.

References

1. Buijs RM, Kalsbeek A. Hypothalamic integration of central and peripheral clocks. *Nat Rev Neurosci*. 2001;2:521–526.
2. Buijs RM, Wortel J, Van Heerikhuizen JJ, Feenstra MGP, Ter Horst GJ, Romijn HJ, Kalsbeek A. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *Eur J Neurosci*. 1999;11:1535–1544.
3. Scheer FAJL, Buijs RM. Light affects morning salivary cortisol in humans. *J Clin Endocrinol Metab*. 1999;84:3395–3398.
4. Scheer FAJL, van Doornen LJP, Buijs RM. Light and diurnal cycle affect human heart rate: possible role for the circadian pacemaker. *J Biol Rhythms*. 1999;14:202–212.
5. Scheer FAJL, Ter Horst GJ, Van der Vliet J, Buijs RM. Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *Am J Physiol*. 2001;280:H1391–H1399.
6. Fleur SEL, Kalsbeek A, Wortel J, Buijs RM. Polysynaptic neural pathways between the hypothalamus, including the suprachiasmatic nucleus, and the liver. *Brain Res*. 2000;871:50–56.
7. Klein DC, Weller JL. Rapid light-induced decrease in pineal serotonin N-acetyltransferase activity. *Science*. 1972;177:532–533.
8. Guzzetti S, Dassi S, Pecis M, Casati R, Masu AM, Longoni P, Tinelli M, Cerutti S, Pagani M, Malliani A. Altered pattern of circadian neuronal control of heart period in mild hypertension. *J Hypertens*. 1991;9:831–838.
9. Nakano Y, Oshima T, Ozono R, Higashi Y, Sasaki S, Matsumoto T, Matsuura H, Chayama K, Kambe M. Non-dipper phenomenon in essential hypertension is related to blunted nocturnal rise and fall of sympatho-vagal nervous activity and progress in retinopathy. *Auton Neurosci*. 2001;88:181–186.
10. Shaw JA, Chin-Dusting JP, Kingwell BA, Dart AM. Diurnal Variation in Endothelium-Dependent Vasodilatation Is Not Apparent in Coronary Artery Disease. *Circulation*. 2001;103:806–812.
11. Brugger P, Marktl W, Herold M. Impaired secretion of melatonin in coronary heart disease. *Lancet*. 1995;345:1408.
12. Goncharuk VD, Heerikhuizen JV, Dai JP, Swaab DF, Buijs RM. Neuropeptide changes in the suprachiasmatic nucleus in primary hypertension indicate functional impairment of the biological clock. *J Comp Neurol*. 2001;431:320–330.
13. Goncharuk VD, Van Heerikhuizen J, Swaab DF, Buijs RM. Paraventricular nucleus of the human hypothalamus in primary hypertension: activation of corticotropin-releasing hormone neurons. *J Comp Neurol*. 2002;443:321–331.
14. Muller JE, Tofler GH, Stone PH. Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation*. 1989;79:733–743.
15. Stergiou GS, Vemmos KN, Pliarchopoulou KM, Synetos AG, Roussias LG, Moutakalakis TD. Parallel morning and evening surge in stroke onset, blood pressure, and physical activity. *Stroke*. 2002;33:1480–1486.
16. Reppert SM, Weaver DR, Rivkees SA, Stopa EG. Putative melatonin receptors in a human biological clock. *Science*. 1988;242:78–83.
17. Weaver DR, Reppert SM. The Mela1a melatonin receptor gene is expressed in human suprachiasmatic nuclei. *Neuroreport*. 1996;8:109–112.
18. Zaidan R, Geoffriau M, Brun J, Taillard J, Bureau C, Chazot G, Claustrat B. Melatonin is able to influence its secretion in humans: description of a phase-response curve. *Neuroendocrinology*. 1994;60:105–112.
19. Bothorel B, Barassin S, Saboureaux M, Perreau S, Vivien-Roels B, Malan A, Pevet P. Exogenous melatonin increases the amplitude of the pineal melatonin secretion by a direct action on the circadian clock in the rat. *Eur J Neurosci*. 2002;16:1–10.
20. Arendt J, Bojowski C, Folkard S, Franey C, Marks V, Minors D, Waterhouse J, Wever RA, Wildgruber C, Wright J. Some effects of melatonin and the control of its secretion in humans. *Ciba Found Symp*. 1985;117:266–283.

21. Mishima K, Okawa M, Hozumi S, Hishikawa Y. Supplementary administration of artificial bright light and melatonin as potent treatment for disorganized circadian rest-activity and dysfunctional autonomic and neuroendocrine systems in institutionalized demented elderly persons. *Chronobiol Int*. 2000;17:419–432.
22. Sharkey KM, Eastman CI. Melatonin phase shifts human circadian rhythms in a placebo-controlled simulated night-work study. *Am J Physiol*. 2002;282:R454–463.
23. Sack RL, Brandes RW, Kendall AR, Lewy AJ. Entrainment of free-running circadian rhythms by melatonin in blind people. *N Engl J Med*. 2000;343:1070–1077.
24. Sly DJ, Colvill L, McKinley MJ, Oldfield BJ. Identification of neural projections from the forebrain to the kidney, using the virus pseudorabies. *J Auton Nerv Syst*. 1999;77:73–82.
25. Guidelines Subcommittee. 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. *J Hypertens*. 1999;17:151–183.
26. O'Brien E, Beevers G, Lip GY. ABC of hypertension. Blood pressure measurement. Part III-automated sphygmomanometry: ambulatory blood pressure measurement. *Br Med J*. 2001;322:1110–1114.
27. Altman DG. *Crossover trials*, vol 1. London: Chapman and Hall; 1991.
28. Staessen JA, Wang JG, Thijs L. Cardiovascular protection and blood pressure reduction: a meta-analysis. *Lancet*. 2001;358:1305–1315.
29. Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, de Leeuw PW, Mancia G, Nachev C, Palatini P, Parati G, Tuomilehto J, Webster J. Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. Systolic Hypertension in Europe Trial Investigators. *JAMA*. 1999;282:539–546.
30. Birau N, Peterssen U, Meyer C, Gottschalk J. Hypotensive effect of melatonin in essential hypertension. *IRCS Med Sci*. 1981;9:906.
31. Lusardi P, Piazza E, Fogari R. Cardiovascular effects of melatonin in hypertensive patients well controlled by nifedipine: a 24-hour study. *Br J Clin Pharmacol*. 2000;49:423–427.
32. Savaskan E, Olivieri G, Brydon L, Jockers R, Krauchi K, Wirz-Justice A, Muller-Spahn F. Cerebrovascular melatonin MT1-receptor alterations in patients with Alzheimer's disease. *Neurosci Lett*. 2001;308:9–12.
33. Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. *Proc Natl Acad Sci U S A*. 1995;92:8734–8738.
34. Ekmekcioglu C, Haslmayer P, Philipp C, Mehrabi MR, Glogar HD, Grimm M, Thalhammer T, Markt W. 24h variation in the expression of the mt, melatonin receptor subtype in coronary arteries derived from patients with coronary heart disease. *Chronobiol Int*. 2001;18:973–985.
35. Nishiyama K, Yasue H, Moriyama Y, Tsunoda R, Ogawa H, Yoshimura M, Kugiyama K. Acute effects of melatonin administration on cardiovascular autonomic regulation in healthy men. *Am Heart J*. 2001;141:E9.
36. McNamara P, Seo S, Rudic RD, Sehgal A, Chakravarti D, FitzGerald GA. Regulation of clock and mop4 by nuclear hormone receptors in the vasculature. a humoral mechanism to reset a peripheral clock. *Cell*. 2001;105:877–889.
37. Van Cauter E, Leproult R, Plat L. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. *JAMA*. 2000;284:861–868.
38. van den Top M, Buijs RM, Ruijter JM, Delagrang P, Spanswick D, Hermes ML. Melatonin generates an outward potassium current in rat suprachiasmatic nucleus neurons in vitro independent of their circadian rhythm. *Neuroscience*. 2001;107:99–108.
39. Cagnacci A, Elliott JA, Yen SS. Melatonin: a major regulator of the circadian rhythm of core temperature in humans. *J Clin Endocrinol Metab*. 1992;75:447–452.
40. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. *Sleep Med*. 2001;2:389–396.
41. Aldhous M, Franey C, Wright J, Arendt J. Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br J Clin Pharmacol*. 1985;19:517–521.
42. Batschelet E. *Circular Statistics in Biology*. London: Academic Press; 1981.
43. de Leeuw J. Introduction to Akaike (1973) information theory and an extension of the maximum likelihood principle. In: Kotz S, Johnson NL, eds. *Breakthroughs in statistics*, vol 1. London: Springer-Verlag; 1992: 599–609.