Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature

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Kräuchi, Kurt, Christian Cajochen, David Möri, Peter Graw, and Anna Wirz-Justice. Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1178–R1188, 1997.—The phase-shifting capacity and thermoregulatory effects of a single oral administration at 18 h of melatonin (5 mg) or S-20098, a melatonin agonist (5 or 100 mg), was investigated in eight healthy young men in a double-blind placebo crossover design. The unmasking conditions of a shortened constant-routine protocol (mini-CR) were used to collect evening phase markers of physiological parameters. In comparison to placebo, all three drug administrations induced an earlier dim-light melatonin onset (DLMO), an earlier increase in distal skin temperature, and an earlier decrease in core body temperature (CBT), heart rate, and proximal skin temperature. This indicates that administration at 18 h of both melatonin and S-20098 (more pronounced with 100 than 5 mg) induced an earlier regulation of the endogenous circadian nocturnal decline in CBT. On the posttreatment day a second mini-CR revealed persistent significantly phase-advanced circadian rhythms as estimated by DLMO, as well as by the midrange crossing time of CBT and heart rate decline. There were no significant differences between the two doses of S-20098. The data suggest that, in addition to immediate thermoregulatory changes, a phase advance of the circadian system had occurred and that the phase advance could still be measured on the posttreatment day.

circadian rhythms; constant-routine protocol; heart rate; rectal temperature; distal and proximal skin temperature; heat loss; heat production; heat content

THE CIRCADIAN RHYTHM of core body temperature (CBT) is generated by a circadian pacemaker localized in the suprachiasmatic nuclei (SCN) (19). In humans, the circadian pacemaker can be entrained and phase shifted by the zeitgeber light (15, 26) and by exogenous melatonin administration (1, 2, 24, 34). In animals, it has been shown that melatonin feeds back to the circadian pacemaker in the SCN, acting on specific melatonin receptors (13) and thus can be defined as a chronobiotic (2). In addition to their effects on the circadian system, light and melatonin also have direct effects on thermoregulation to increase and decrease human CBT, respectively (5, 9). The nocturnal secretion of melatonin partially contributes to the nocturnal decline in CBT and thus, in turn, to the circadian amplitude of CBT (9).

Under normal living conditions the majority of circadian rhythms are differentially "masked" by various behaviors as well as by external conditions (29). This means that endogenous circadian amplitude and phase are not easily measured; to reduce masking, the "constant-routine" protocol (CR) has been developed (15). The CR permits reduction or elimination of postural and other behavioral effects on the parameters measured (15, 29). We have recently investigated mechanisms underlying the circadian regulation of CBT in a CR (23). The time course of the nocturnal decline of CBT commences with reduced heat production and vasodilation at distal skin regions (23). Because CBT reflects ~80% of body heat content (18), a circadian phase shift in CBT is necessarily also a change in the timing of body heat content. Therefore, it is of special interest to study the chronobiotic effect of melatonin in connection with its hypothermic properties.

The two main aims of this study were *1*) to determine, under CR conditions and during subsequent sleep, whether acute melatonin administration at 18 h (when melatonin is not usually secreted), can induce heat loss and/or reduce heat production as measured by changes in heart rate (23) and *2*) whether this single application leads to an immediate phase advance that is still measurable on the posttreatment day. Melatonin was compared with S-20098 [*N*-[2-(7-methoxy-1-naphtalenyl)-ethyl]-acetamide], a potent and specific agonist of melatonin receptors in vitro (33) whose chronobiotic properties in vivo in rats have been shown by entrainment of rest-activity cycles under free-running conditions (7, 25, 28) and phase advance under a light-dark cycle (3). This is the first study of acute administration of S-20098 on the human circadian system, and its comparison with melatonin should be indicative of the strength of its agonistic properties. A first analysis of these data has been presented in abstract form (21).

MATERIALS AND METHODS

Subjects

Eight male students were screened for general medical and psychological health [age: 27 ± 4 yr (SD, range: 23–32); height: 180 ± 6 cm (170–190); weight: 73.3 ± 6.5 kg (66.0–84.4); body mass index (wt/ht^2): 22.66 ± 2.36 kg/m^2 (20.37–27.55); body surface area [wt (kg) 0.444 × ht (cm)] 0.663 × 88.83 (cm/kg): 1.87 ± 0.10 m^2 (1.74–2.00)]. They had no reported sleep disorders (Pittsburgh Sleep Quality Index <5), no extreme phase type (Torsvall-Åkerstedt morning-eveningness questionnaire), no shift work or transmeridian travel within 1 mo of the study, a regular way of life, no medication or drug use, and all were nonsmokers. The experimental protocol was approved by the Human Research Committee of the Department of Medicine, University of Basel. The nature, purpose, and risks of the study were explained to the subjects before they gave their written consent. It was explicitly permitted to break off the experiment at any time. Each subject spent a trial night in the sleep laboratory. All subjects completed the study without any complaints.

Experimental Protocol

The double-blind placebo-controlled study was performed according to a 2 × 4 × 4 partial Latin square design. Each treatment period comprised 1 pretreatment day with placebo, 1 day of treatment [placebo, melatonin 5 mg (Mel), S-20098 5 mg (S5), or S-20098 100 mg (S100) in randomized order], and 1 posttreatment day with placebo. This was repeated for 4 consecutive weeks. The subjects were not informed on which day drug or placebo was given. The experiments were carried out between April and July 1994.

The subjects were asked to restrict their sleep times to between 2300–0700 for 1 wk before and during the entire study. Continuously worn activity monitors verified compliance to this rest-activity cycle (data not shown). To control outdoor light exposure, light logs were collected daily (data not shown). The subjects entered the chronobiology laboratory (temperature 22°C, humidity 60%, light <10 lx) at 1500 on each experimental day for electrode placement. The study consisted of two parts: a shortened CR, followed by nocturnal sleep. In this "mini"-CR protocol, the subjects remained supine and awake in bed from 1600. The rationale for developing a mini-CR was to have estimates of circadian phase under unmasked conditions, without the prolonged full sleep deprivation of the classic 40-h CR, which would have compromised our study of the nocturnal sleep electroencephalogram (EEG). Water (100 ml) together with isocaloric sandwiches (50 kcal, 50% carbohydrate, 25% protein, and 25% fat; foods used: tuna fish, turkey, or cheese, with lettuce on brown bread) were administered at hourly intervals. The subjects were cared for by trained personnel and remained awake until 2300 without information about time of day. Reading, writing, talking, and playing games were allowed during the experiment (providing they were not overstimulating). The pill was administered at 1800. Lights were extinguished at 2300 until wake time at 0700. Sleep EEG was recorded and analyzed in detail together with temperature and heart rate (see companion paper, Ref. 11). Thermometry was carried out continuously from 1600 until ~0715 the next morning.

Data Acquisition

Thermometry. Temperatures were continuously recorded by a computerized system (System Hofstetter, SHS Allschwil) in 2-min intervals. Rectal temperature (T_{re}) as a measure of CBT was registered by a thermocouple (polyoxymethylene probe: 2 mm diameter, copper constantan; accuracy ±0.01°C, Interstar, Cham, Switzerland; Therm, type 5500–3, Ahlborn, Holzkirchen, Germany) inserted 10 cm past the anal sphincter. Skin temperatures were also registered by thermocouples (silver disk: 1 cm diameter, copper constantan, type P 224, Prof. Schwamm, Ahlborn, accuracy ±0.01°C; Therm, type 5500–3, Ahlborn) fixed to the skin with thin air-permeable adhesive surgical tape (Fixomull, Beiersdorf, Hamburg, Germany). Eight sites were taken as follows: midforehead, 1 cm above the navel (stomach), right infraclavicular area, center of the back of the left and right hand (later averaged); middle of left and right foot instep (later averaged); and midthigh on right musculus rectus femoris.

Heart rate. Standard electrocardiogram (ECG) leads were placed on the lateral thorax at approximately the sixth intercostal space and on the manubrium of the sternum. The analog signal was amplified by a Nihon-Khodon 18-channel polygraph. A computerized system (System Hofstetter, SHS Allschwil) digitized this signal and detected heart rate by the length of the R-R interval.

Salivary melatonin. Saliva was collected for 4 min every 30 min and stored at −80°C until determination of melatonin levels by gas chromatography-mass spectrometry. The method involves liquid-liquid extraction, derivatization with pentafluoropropionic anhydride, followed by capillary gas chromatographic separation of the melatonin derivative with a column switching technique and mass spectrometric detection in a negative chemical ionization mode. This method proved to be highly specific and sensitive for determination of very low melatonin levels in biological samples and was therefore successfully applied to the measurement of endogenous concentrations of melatonin in humans after administration of the melatonin agonist S-20098 (E. Mocaër, G. Simonin, P. Got, J. P. Jeanniot, C. Boursler-Neyret, G. Bru, and N. Bromet, unpublished data). The linearity of the method has been assessed over the range of 1–100 pg/ml, with a limit of quantification set at 1 pg/ml. Intra- and interassay precision and accuracy are within 20% limits for all concentrations investigated. The determinations were carried out by Servier Department of Technology.

Data Analysis

Raw data of temperatures and heart rate were inspected visually, and data segments that were affected, e.g., by probe slips or malfunctioning of the temperature sensors or ECG electrodes, were removed. These missing data (<1%) were replaced by a value derived from a linear interpolation procedure. On the basis of theoretical reasons (4) and of the similarities to our earlier study (23), we combined skin temperatures of hands and feet to provide an average for the "distal regions" (T_{dist}), and forehead, thigh, infraclavicular region, and stomach for the "proximal regions" (T_{prox}). A weighted average was calculated for T_{prox} according to Ref. 18 with slight modifications: forehead ×0.093, thigh ×0.347, infraclavicular region ×0.266, and stomach ×0.294.

Analysis of Phase Markers

The full 40-h CR has been developed and validated as a method to determine endogenous amplitude and phase of the CBT (15). A specific statistical procedure determined the phase of the minimum temperature (T_{min}) (8). This is not the only phase marker: under unmasking conditions, a number of additional phase markers of CBT can be measured (e.g., phase of the fitted 24-h component and midrange crossing time, both decline and rise) (32). The mini-CR was designed to measure one of these phase markers, the midrange crossing time of the evening decline in CBT. Figure 1 illustrates the design and analysis of the midrange crossing time of the nocturnal decline in T_{re}, which was used as phase marker for CBT; a similar method was used for heart rate (2-h moving average curves). Due to masking effects of sleep cycles on the time course of T_{re} (20), the endogenous phase of T_{min} cannot be assessed. However, given that the same conditions of sleep were present after each mini-CR, the value of T_{min} in terms of degrees Celsius was assumed to remain masked in a consistent manner (for statistical confirmation of this assumption see RESULTS).

The midrange crossing time of CBT and heart rate for each subject was determined as follows (see Fig. 1): the minimum value (in °C and beats/min) and the maximum value after 18 h (in °C and beats/min) were averaged (= midrange value, °C and beats/min). This value was taken to determine the midrange crossing time for each subject. It can be seen from this example (Fig. 1) that both the T_{min} (°C) and the maximum temperature (T_{max}; °C) values are broad with respect to timing, but that values in degrees Celsius can be reliably estimated (small rate of change at these phases). Thus any changes in the midrange crossing time are considered as a

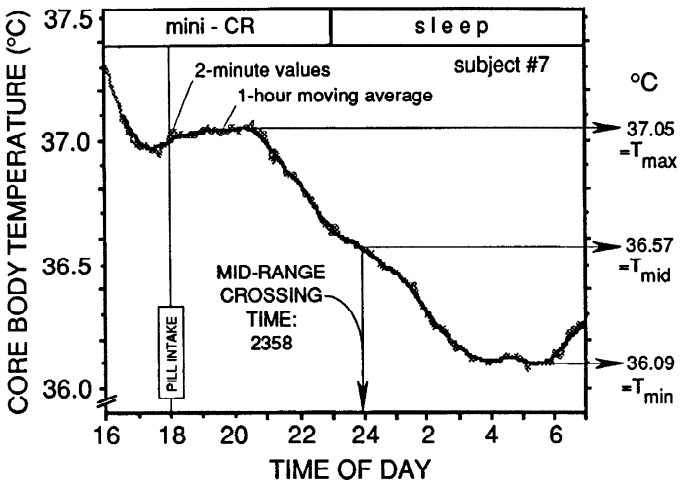

Fig. 1. Design of study together with graphic presentation of method for determining midrange crossing value of rectal temperature (T_{mid}) and, from this, midrange crossing time as a phase marker for circadian core body temperature rhythm (raw data of pooled placebo from *subject no. 7*). Same method was used for heart rate, except that 2-h moving averages were calculated. T_{max}, T_{min}, maximum and minimum temperatures, respectively; mini-CR, shortened constant-routine protocol.

change in the circadian timing. Due to the much more extensive masking effect of sleep on T_{dist} and T_{prox} (see Fig. 3), no midrange crossing times were calculated.

Dim-light melatonin onset (DLMO) time was used as a validated sensitive marker for circadian phase (24). Given the conditions of the CR, there were, in addition to controlling for masking by light, no postural changes that might have affected melatonin secretion (17). The onset of melatonin secretion in saliva was defined as the time when melatonin concentrations exceeded the detection limit of the assay (1 pg/ml) and remained above this limit.

Analysis of the Time Course During the Mini-CR Period

The dynamics of the measured parameters were analyzed to characterize acute effects on the treatment day and to determine any phase-shifting effect on the posttreatment day. If a phase shift had been induced by the treatment, a different time course to placebo should still be present on the posttreatment day.

To reduce short-term fluctuations and the number of time segments, all temperature data were averaged in 30-min blocks for the 7-h mini-CR period. Due to the higher variability and more extensive masking effects on heart rate induced by the protocol (e.g., hourly food intake) these data were averaged in 60-min blocks. Before statistical analysis was carried out, melatonin concentrations were log+1 transformed to achieve a normal distribution and stabilized variances.

Correlation Analysis

To compare the hypothermic effect of melatonin and S-20098 with functions of heat loss, heat production, and salivary melatonin, correlations were calculated between the reduction of T_{re} in the first 4 h after pill intake (time of maximal hypothermic effect, see Fig. 4*A1*) with the increase in T_{dist}, the increase in salivary melatonin, the decrease in T_{prox}, and heart rate in the same time period (mean areas under the curve of $n = 8$ subjects for each of the 12 treatment days were calculated by the trapezoid method). These calculations were based on individual differences to the preadministration value at 1730–1800 (6 × 30-min blocks). Additionally, the phase relationships between the midrange crossing times of T_{re} and of heart rate were calculated, as well as the relationship between the midrange crossing time of T_{re} and DLMO. For all these comparisons Pearson's product-moment correlations were used.

Statistics

Statistical evaluation of the data were performed by analysis of variance (ANOVA) for repeated measures. Huynh-Feldt (H-F) statistics were used to adjust the covariance matrix for violations of sphericity. H-F's P values were based on corrected degrees of freedom, but the original degrees of freedom are reported. When the F ratio proved significant, Duncan's multiple-range post hoc tests and linear contrasts with H-F statistics were applied to locate significant differences between the means.

RESULTS

Analysis of Phase Markers

The treatment effects were analyzed as follows. Each treatment group on the treatment and posttreatment day was compared *1*) with its pretreatment day, *2*) with placebo administration on the corresponding day, and *3*) to achieve the best estimate of a baseline value, with a "pooled placebo" group. Data from the maximum number of placebo days were pooled (only placebo days without any pretreatment with drug were taken; $n = 6$, Tables 1–3). Calculation of a pooled placebo presupposes homogeneity (i.e., no significant differences). Homogeneity was tested in different ways: *1*) between the 4 successive wk for all pretreatment days, *2*) within the 3 successive experimental days for the placebo treatment, and *3*) within the maximal pool of placebo days ($n = 6$ experimental days). One-way ANOVAs for repeated measures revealed no significant time effect within the 4 successive wk or the 3 successive experimental days. Additionally, post hoc tests of the data in Tables 1–3 showed no significant differences between the 6 placebo days. In summary, no carryover effects could be found either in a daily or in a weekly timespan. Therefore, a pooled placebo group can be declared as a better estimate for a relative value than one of its constituents alone; fluctuations, e.g., in the preadministration values between the groups, are found by chance.

CBT

The midrange crossing time of T_{re} decline is summarized in Table 1 for all 12 experimental days. Two-way ANOVA for the midrange crossing times of T_{re} showed a significant main effect of factor day and a significant interaction (Table 1, legend). It is important to document that the values in degrees Celsius of T_{min}, T_{max}, and midrange temperature (see Fig. 1) did not significantly differ with respect to group and days [2-way ANOVAs for repeated measures, main effects (day, treatment group): NS; interaction: NS; data not shown]. Thus the values used to calculate phase markers were similarly masked under all treatment conditions.

The first result is that, in comparison to the pretreatment day, Mel and S100 induced an earlier midrange crossing time on the treatment day, and S5 showed a

Table 1. *Midrange crossing time of core body temperature decline*

Group	Pretreatment Day	Treatment Day	Posttreatment Day
Placebo	23:17 ± 20	23:21 ± 18	23:12 ± 18
Melatonin (5 mg)	23:53 ± 16	22:01 ± 32[a,b,c]	22:40 ± 19[a,b]
S-20098 (5 mg)	23:19 ± 18	22:20 ± 13[a,d,e]	22:56 ± 18
S-20098 (100 mg)	24:00 ± 27	21:50 ± 19[a,b,c]	22:32 ± 21[a,b]
Pooled placebo	23:30 ± 11		

Values are means ± SE in h ± min. Two-way analysis of variance (ANOVA) for repeated measures: day $F(2,14) = 15.77$, $P < 0.001$; group $F(3,21) = 1.98$, NS; day × group $F(6,42) = 2.62$, $P < 0.04$. [a]Significantly different ($P < 0.05$) vs. pooled placebo; vs. [b]pretreatment day; vs. [c]corresponding placebo. [d]Trend ($P < 0.1$) vs. corresponding placebo; [e]vs. pretreatment day.

tendency to an earlier midrange crossing time (Table 1). Second, a comparison with the placebo group on the treatment day showed again that Mel and S100 induced an earlier midrange crossing time (Table 1); S5 again showed a tendency to be earlier. And third, for all drug groups, the midrange crossing time occurred significantly earlier (phase shift = $\Delta\phi$) than that for the pooled placebo (Table 1; linear contrasts; Mel: $\Delta\phi$ = +89 min; S5: $\Delta\phi$ = +70 min; S100: $\Delta\phi$ = +100 min). Although the mean values show a tendency to a dose-dependent effect of S-20098, the three drug treatment groups did not differ significantly in midrange crossing times.

On the posttreatment day the midrange crossing times of Mel and S100 occurred significantly earlier than on the pretreatment day (Table 1). A comparison with the placebo group alone on the posttreatment day revealed no significant differences between the midrange crossing times. However, a comparison with the pooled placebo group was significant, and the midrange crossing time still occurred earlier, 1 day after administration of Mel and S100 (Table 1; linear contrasts; Mel: $\Delta\phi$ = +50 min; S5: $\Delta\phi$ = +35 min, NS; S100: $\Delta\phi$ = +58 min). Again, no significant dose dependency between S5 and S100 could be found.

Heart Rate

The midrange crossing time of heart rate decline is summarized for all experimental days in Table 2. Two-way ANOVA for repeated measures revealed a significant main effect of factor day and a significant interaction (Table 2). On the basis of the finding that no significant differences between placebo treatments were found (the same analyses as for CBT were calculated, see *CBT*), treatment effects were analyzed as described for CBT. The maximum and minimum value of heart rate, as well as the midrange crossing values (beats/min) did not significantly differ with respect to group and days [2-way ANOVAs for repeated measures, main effects (day, treatment group): NS; interaction: NS; data not shown].

In comparison to the pretreatment day, the midrange crossing time of all drug groups occurred significantly earlier (Table 2). This result was confirmed by comparison with the placebo group on the treatment day (Table 2) or with the pooled placebo group (Table 2; linear contrasts; Mel: $\Delta\phi$ = +79 min; S5: $\Delta\phi$ = +66 min; S100: $\Delta\phi$ = +56 min). S100 did not significantly differ from S5.

On the posttreatment day, the midrange crossing times of Mel still occurred earlier than on the pretreatment day, and S100 showed a tendency to occur earlier (Table 2). A comparison between the groups on the posttreatment day revealed no significant differences. The comparison with pooled placebo revealed a significant earlier midrange crossing time for S100 (Table 2; linear contrasts; Mel: $\Delta\phi$ = +33 min, NS; S5: $\Delta\phi$ = +4 min, NS; S100: $\Delta\phi$ = +37 min, $P < 0.05$). Again, no significant dose dependency between S5 and S100 could be found.

DLMO

The DLMO time is summarized for all experimental days in Table 3. Two-way ANOVA for repeated measures revealed a significant main effect of factor day and a significant interaction (Table 3, legend). On the treatment day, DLMO time of Mel showed no variance (all values >1 pg/ml 30 min after Mel administration), resulting in a lower residual variance. To validate the results of the two-way ANOVA, separate one-way ANOVAs for repeated measures were calculated for pretreatment day, treatment day, and posttreatment day without Mel. Comparable results were found as described in Table 3 for the two-way ANOVA (data not shown). On the basis of the finding that no significant differences between placebo treatments were found (the same analyses as for CBT were calculated, see above), treatment effects were analyzed as described for CBT.

Table 2. *Midrange crossing time of heart rate decline*

Group	Pretreatment Day	Treatment Day	Posttreatment Day
Placebo	22:26 ± 08	22:40 ± 16	22:08 ± 16
Melatonin (5 mg)	22:54 ± 14	21:17 ± 26[a,b,c]	22:03 ± 17[b]
S-20098 (5 mg)	22:41 ± 25	21:30 ± 22[a,b,c]	22:32 ± 14
S-20098 (100 mg)	22:49 ± 15	21:40 ± 25[a,b,c]	22:00 ± 12[a,d]
Pooled placebo	22:36 ± 08		

Values are means ± SE in h ± min. Two-way ANOVA for repeated measures: day $F(2,14) = 6.988$, $P < 0.025$; group $F(3,21) = 1.735$, NS; day × group $F(6,42) = 2.820$, $P < 0.025$. [a]Significantly different ($P < 0.05$) vs. pooled placebo; [b]vs. pretreatment day; [c]vs. corresponding placebo. [d]Trend ($P < 0.1$) vs. pretreatment day.

Table 3. *Dim light melatonin onset*

Group	Pretreatment Day	Treatment Day	Posttreatment Day
Placebo	21:07 ± 14	21:04 ± 09	21:11 ± 05
Melatonin (5 mg)	21:15 ± 13	18:30 ± 0[a,b,c]	20:26 ± 17[a,b,c]
S-20098 (5 mg)	21:15 ± 14	20:07 ± 20[a,b,c]	20:49 + 13
S-20098 (100 mg)	21:07 ± 11	19:41 ± 10[a,b,c]	20:37 ± 13[a,b,c]
Pooled placebo	21:10 ± 06		

Values are means ± SE in h:min ± min. Two-way ANOVA for repeated measures: day $F(2,14) = 82.4$, $P < 0.0001$; group $F(3,21) = 15.08$, $P < 0.0005$; day × group $F(6,42) = 12.1$, $P < 0.0001$. [a]Significantly different ($P < 0.05$) vs. pooled placebo; [b]vs. pretreatment day; [c]vs. corresponding placebo.

In comparison to the pretreatment day, all three drugs induced an earlier DLMO time on the treatment day (Table 3). This was confirmed by significant comparisons with the placebo group on the treatment day (Table 3) or by comparisons with the pooled placebo group (Table 3; linear contrasts; Mel: $\Delta\phi = +160$ min; S5: $\Delta\phi = +63$ min; S100: $\Delta\phi = +89$ min). S100 did not significantly differ from S5.

On the posttreatment day, DLMO time of Mel and S100 still occurred significantly earlier than on the pretreatment day (Table 3). These results were confirmed by comparisons with the placebo group on the posttreatment day (Table 3) or by comparisons with the pooled placebo group (Table 3; linear contrasts; Mel: $\Delta\phi = +44$ min; S5: $\Delta\phi = +21$ min, NS; S100: $\Delta\phi = +33$ min). Again, no significant dose dependency between S5 and S100 could be found.

Dynamics of CBT, Heart Rate, Skin Temperatures, and Salivary Melatonin During the Mini-CR Period

On the basis of the analyses of the midrange crossing time of CBT and of heart rate decline, the pooled placebo group was used as the most stable relative value. The raw data means of the time course of CBT, skin temperatures, and heart rate are represented for the entire course of the experiment from the moment of lying down at 1600 (Figs. 2 and 3). All parameters revealed clearly that at least 2 h are required to recover from this postural change and manifest the true endogenous values characteristic of the CR. The acute effects of Mel and S-20098 during the mini-CR from 1800–2300 (see below) partially continue into the sleep period; some changes are still present in the second mini-CR 24 h later. It is important to note that there are no postural changes at sleep onset (subjects are supine throughout), thus there is no apparent masking on CBT decline at sleep onset. Heart rate, in contrast, rose slightly during the preparations for sleep (a plateau when averaged into 1-h blocks) and dropped immediately after lights off to a value that is a linear extrapolation from the decline initiated during the mini-CR.

To show the drug treatment effect during the mini-CR more clearly, the time course of the temperatures was adjusted relative to the preadministration values at 1730–1800 taken as zero (Fig. 4). For a similar adjustment of heart rate, the preadministration value at 1700–1800 was taken. Separate ANOVAs of the preadministration values showed no significant differences between the groups in any parameter.

CBT. A first analysis of the 7-h mini-CR period on the treatment day (14 × 30-min blocks) showed a significant main effect for time and a significant interaction (Table 4). Post hoc tests revealed that Mel, S5, and S100 all reduced T_{re} below the preadministration value earlier than pooled placebo (Fig. 4*AI*). A direct comparison with pooled placebo showed that mean T_{re} between 1800–2300 was significantly lower in all three drug conditions, whereas Mel and S100 showed a larger decrease in T_{re} than S5 (linear contrasts).

On the posttreatment day, two-way ANOVA for the mini-CR period showed a significant main effect for time and a significant interaction (Table 4). Post hoc tests with the preadministration value at 1730–1800 revealed that Mel, S5, and S100 all reduced T_{re} earlier than pooled placebo (Fig. 4*BI*). A direct comparison with pooled placebo showed that mean T_{re} between 1800–2300 was significantly reduced in all three drug conditions to a comparable level (linear contrasts).

Heart rate. Analysis of the 7-h mini-CR period on the treatment day (7 × 60-min blocks) revealed a significant main effect for time and a significant interaction (Table 4). Post hoc tests revealed that Mel, S5, and S100 reduced heart rate below this preadministration

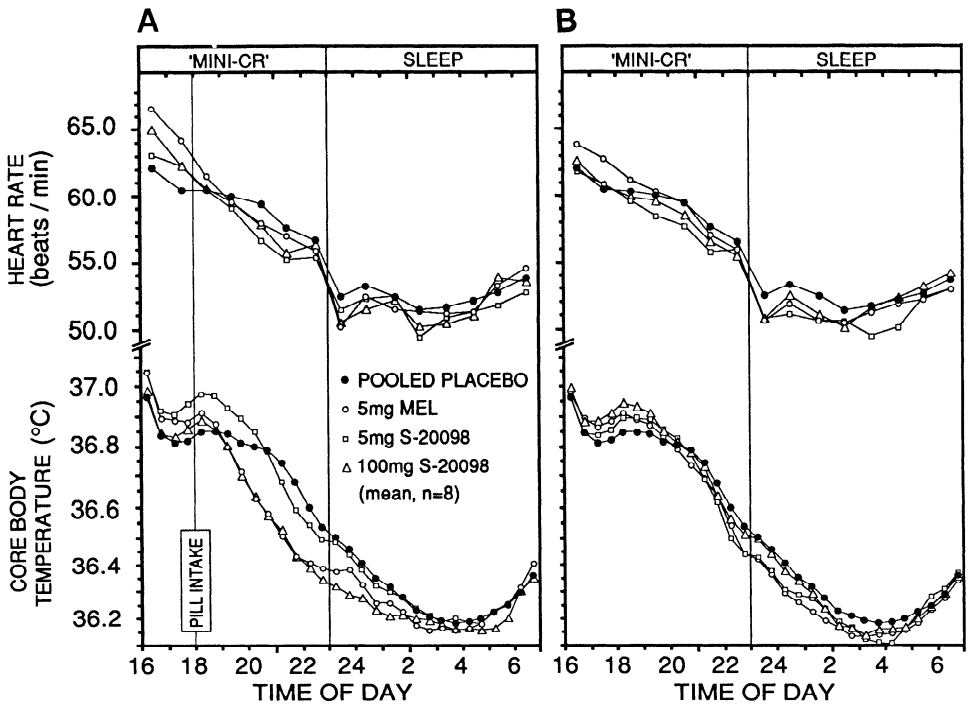

Fig. 2. Time course of raw data values for core body temperature (*bottom*) and heart rate (*top*) on treatment day (*A*) and posttreatment day (*B*) throughout entire observation period. Vertical lines separate baseline/adaptation 1600–1800 from effects of pill intake (1800–2300), as well as time of lights off (2300–0700). Data points are mean values of 30-min blocks. Pooled placebo, mean of all 6 placebo days without any pretreatment with drug (for further details see MATERIALS AND METHODS and RESULTS). Mini-CR, shortened constant-routine protocol; Mel, melatonin.

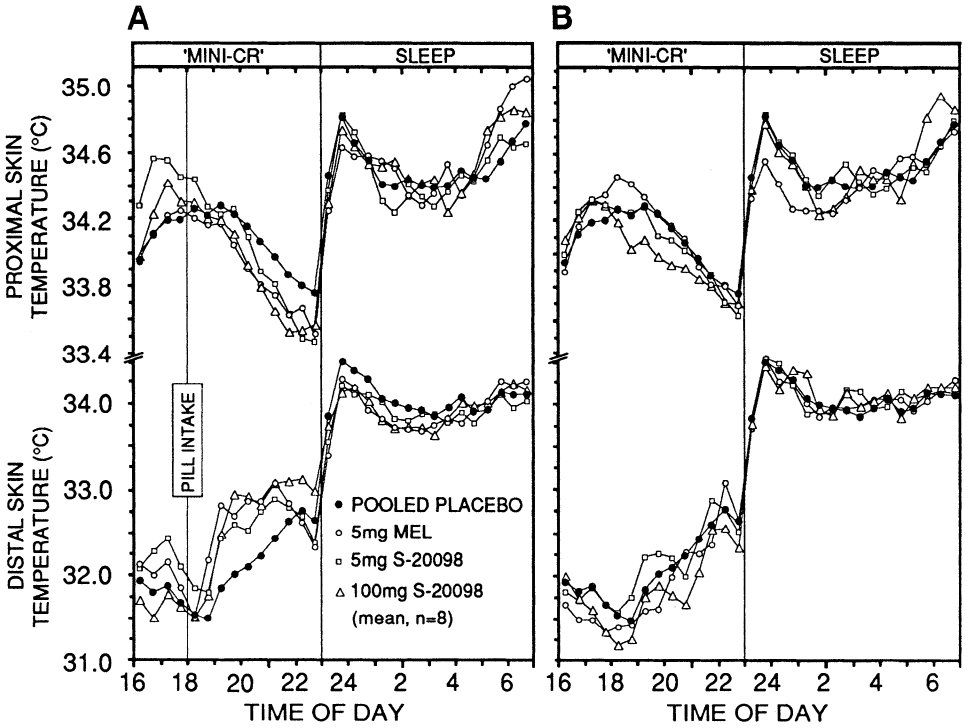

Fig. 3. Time course of distal skin temperature (*bottom*) and proximal temperature (*top*) on treatment day (*A*) and posttreatment day (*B*) throughout entire observation period (for further details see Fig. 2).

value earlier than placebo (Fig. 4*AII*). A direct comparison with pooled placebo showed that mean heart rate between 1800 and 2300 was significantly lower in all three drug conditions, whereas Mel showed a more pronounced reduction in heart rate than S5 and S100 (linear contrasts).

On the posttreatment day, two-way ANOVA for the mini-CR period showed only a significant main effect for time (Table 4, Fig. 4*BII*).

T_{dist}. On the treatment day, a two-way ANOVA for the mini-CR period (14 × 30-min blocks) showed a significant main effect for time and a significant interaction (Table 4). Post hoc tests revealed that Mel, S5, and S100 increased T_{dist} above the preadministration value earlier than pooled placebo (Fig. 4*AIII*). A direct comparison with pooled placebo showed that mean T_{dist} between 1800 and 2300 was significantly higher after administration of Mel and S100, and the increase in T_{dist} induced by S100 was significantly larger than after Mel and S5 (linear contrasts).

On the posttreatment day, a two-way ANOVA for the mini-CR period showed a significant main effect for time. The factor group and the interaction term were not significant (Table 4, Fig. 4*BIII*).

T_{prox}. On the treatment day, a two-way ANOVA for the mini-CR period (14 × 30-min blocks) indicated a significant main effect for time and a significant interaction (Table 4). Post hoc tests with the preadministration value at 1730–1800 revealed that Mel, S5, and S100 reduced T_{prox} earlier than pooled placebo (Fig. 4*AIV*). A direct comparison with pooled placebo showed that mean T_{prox} between 1800 and 2300 was significantly reduced in all three drug conditions to a comparable level (linear contrasts).

On the posttreatment day, a two-way ANOVA for the mini-CR showed a significant main effect for time. The factor group and the interaction term were not significant (Table 4, Fig. 4*BIV*).

Salivary melatonin. The raw data for salivary melatonin levels are presented separately for pretreatment, treatment, and posttreatment days with the corresponding placebo group (Fig. 5).

On the pretreatment day, a two-way-ANOVA indicated a significant main effect for time and no significant interaction or main effect for treatment group (Fig. 5*A*, see legend).

On the treatment day, significant main effects (time, group) and a significant interaction were found (Fig. 5*B*, see legend). Post hoc comparisons with the preadministration value at 1800 revealed that Mel increased salivary melatonin to a maximum level in the first sample after administration at 1830. Mel declined exponentially, but was still high at sleep onset (7 × placebo levels). S5 and S100 increased salivary melatonin earlier than placebo (Fig. 5*B*). A direct comparison with placebo showed that mean salivary melatonin between 1800 and 2300 was significantly higher in all three drug conditions, and each group significantly differed from the other (linear contrasts).

On the posttreatment day, a two-way ANOVA indicated a significant main effect for time and a significant interaction (Fig. 5*C*, see legend). Post hoc comparisons with the preadministration value at 1800 revealed that Mel, S5, and S100 increased salivary melatonin earlier than placebo. A direct comparison with placebo showed that mean salivary melatonin between 1800 and 2300 was significantly higher 1 day after administration of Mel and S100, and S5 did not statistically differ from S100 (linear contrasts). Mel showed a significantly higher mean salivary melatonin between 1800 and 2300 than S5 and S100 (linear contrasts).

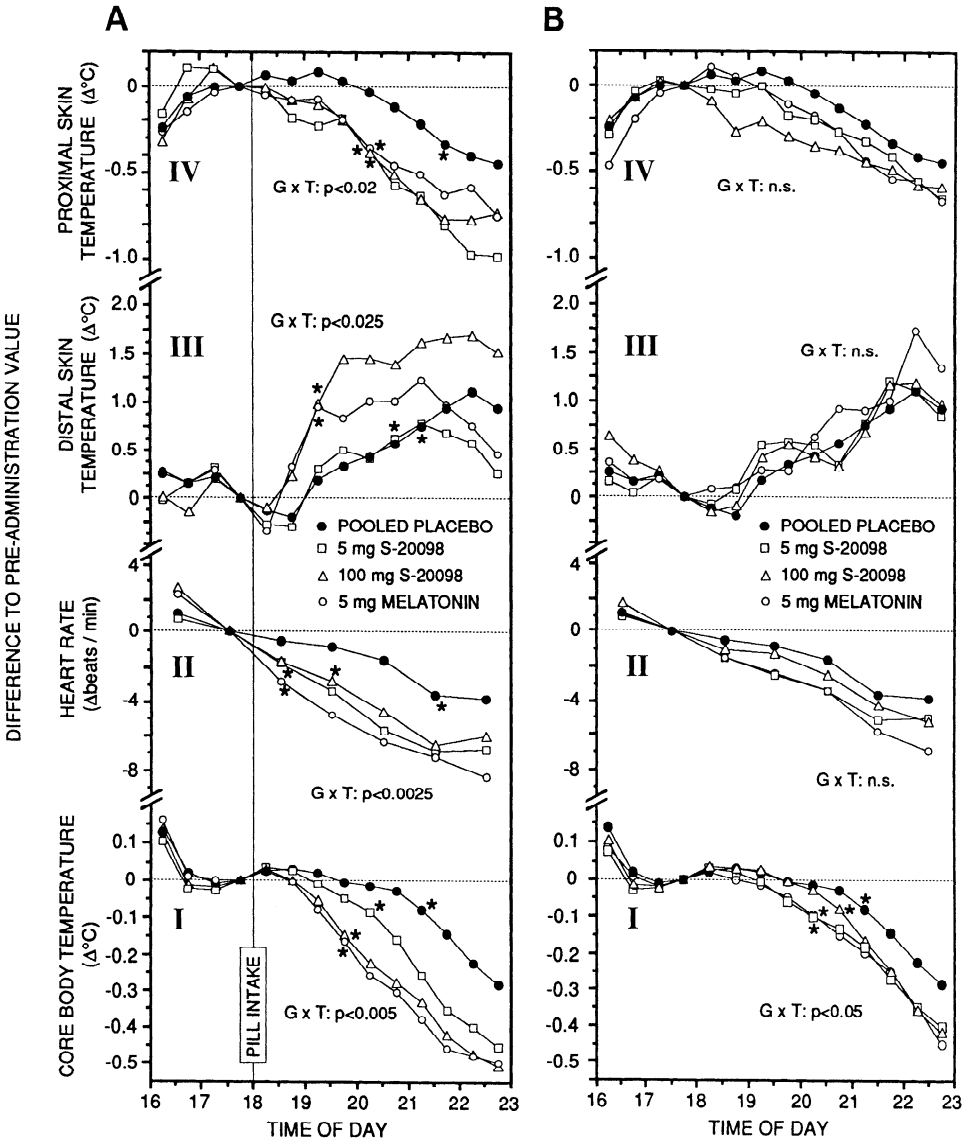

Fig. 4. Time course of core body temperature (*I*), heart rate (*II*), distal skin temperature (*III*), and proximal skin temperature (*IV*) adjusted to preadministration values during mini-CR period (1600–2300) on treatment day (*A*) and posttreatment day (*B*). Preadministration value of 1730–1800 (= zero line; heart rate: 1700–1800) was subtracted from raw data presented in Figs. 2 and 3. *First appearance of a value significantly different from preadministration value [$P < 0.05$, Duncan's multiple-range test; only calculated when interaction group × time (G × T) was $P < 0.05$, Table 4; for further details see MATERIALS AND METHODS and Figs. 2 and 3].

Table 4. *Two-way ANOVA for repeated measures*

Parameter	Factor	Treatment Day	Posttreatment Day
T_{rect}	Group	$F(3,21) = 5.04, P < 0.009$	$F(3,21) = 2.77$, NS
	Time	$F(13,91) = 48.89, P < 0.0001$	$F(13,91) = 39.74, P < 0.0001$
	G×T	$F(39,273) = 4.03, P < 0.004$	$F(39,273) = 1.74, P < 0.05$
Heart rate	Group	$F(3,21) = 2.81$, NS	$F(3,21) = 1.22$, NS
	Time	$F(6,42) = 45.27, P < 0.0001$	$F(6,42) = 23.12, P < 0.0001$
	G×T	$F(18,126) = 2.58, P < 0.002$	$F(18,126) = 0.77$, NS
T_{dist}	Group	$F(3,21) = 2.77$, NS	$F(3,21) = 0.24$, NS
	Time	$F(13,91) = 9.50, P < 0.0006$	$F(13,91) = 7.52, P < 0.0008$
	G×T	$F(39,273) = 2.53, P < 0.025$	$F(39,273) = 0.86$, NS
T_{prox}	Group	$F(3,21) = 1.48$, NS	$F(3,21) = 1.29$, NS
	Time	$F(13,91) = 22.76, P < 0.0006$	$F(13,91) = 14.24, P < 0.0001$
	G×T	$F(39,273) = 2.35, P < 0.015$	$F(39,273) = 1.25$, NS

Statistical analyses of the time course of rectal temperature (T_{rect}), heart rate, distal and proximal skin temperature (T_{dist}, T_{prox}) on treatment and posttreatment day during the mini-constant-routine period (1600–2300). G × T, group × time. Group (4 levels): pooled placebo, 5 mg S-20098, 100 mg S-20098, 5 mg melatonin. Time (14 levels): 14 × 30-min blocks. Heart rate (7 levels): 7 × 60-min blocks.

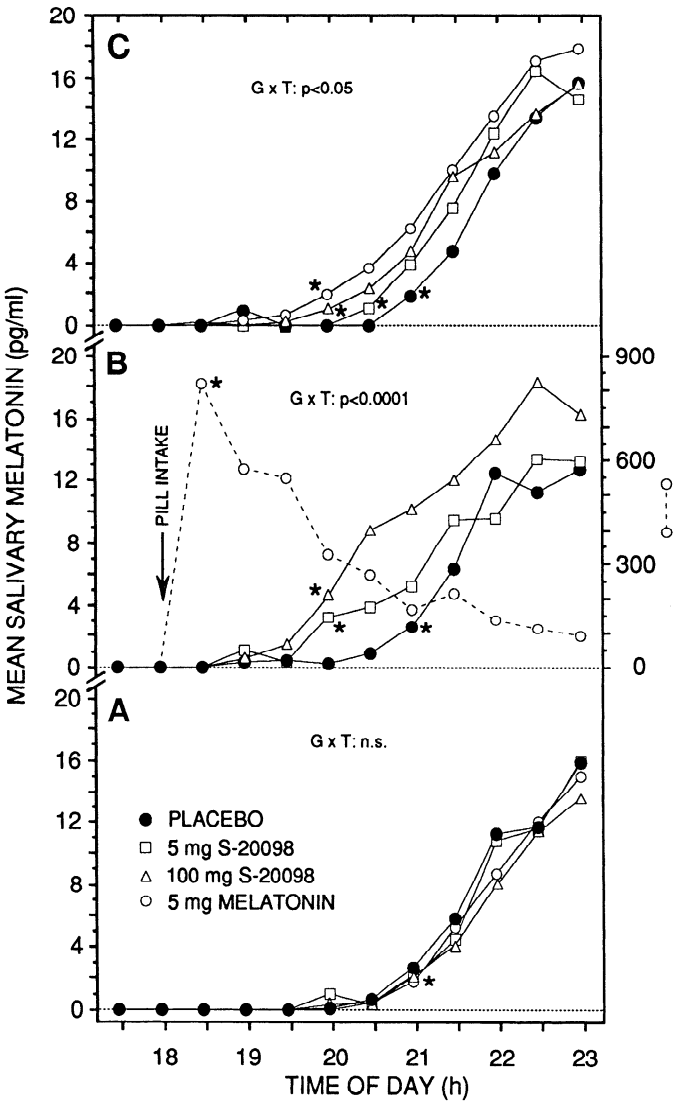

Fig. 5. Time course of salivary melatonin during the pretreatment (*A*), treatment (*B*), and posttreatment day (*C*) throughout mini-CR period. Data points are mean values of 8 men at 30-min intervals. Two-way ANOVA for repeated measures (based on log − 1-transformed data) is as follows. For pretreatment day, group: $F(3,21) = 0.12$, NS; time: $F(11,77) = 130.5, P < 0.0001$; G × T: $F(33,231) = 0.26$, NS. For treatment day, group: $F(3,21) = 125.6, P < 0.0001$; time: $F(11,77) = 98.7, P < 0.0001$; G × T: $F(33,231) = 34.6, P < 0.0001$. For posttreatment day, group: $F(3,21) = 2.98$, NS; time: $F(11,77) = 122.5$, $P < 0.0001$; G × T: $F(33,231) = 2.22, P < 0.04$. *First appearance of a value significantly increased ($P < 0.05$) above preadministration value (Duncan's multiple-range test; only calculated when interaction G × T or factor time was $P < 0.05$; for further details see MATERIALS AND METHODS and RESULTS).

Relationship Between T_{dist}, T_{prox}, Heart Rate, Salivary Melatonin, and T_{re}

The acute effect of Mel, S5, and S100 on different measures of temperature, heart rate, and salivary melatonin in the first 4 h after drug intake were calculated with the values integrated from 1800 to 2200 (see MATERIALS AND METHODS). The decrease in T_{prox} and heart rate, as well as the increase in T_{dist}, showed a significant correlation with the decrease in T_{re} (all $n = 12$ observations per subject; T_{prox} vs. T_{re}: $r = 0.784, P < 0.003$; heart rate vs. T_{re}: $r = 0.867, P < 0.0005$; T_{dist} vs. T_{re}: $r = -0.640, P < 0.025$). The increase in salivary melatonin and the decrease in T_{re} was also correlated (without values of group Mel on the treatment day; $n = 11, r = 0.764, P < 0.007$). There are significant intercorrelations between DLMO time, midrange crossing times of T_{re}, and midrange crossing times of heart rate (heart rate vs. T_{re}: $r = 0.915, P < 0.0001, n = 12$; DLMO vs. T_{re}: $r = 0.892, P < 0.0002, n = 11$; DLMO vs. heart rate: $r = 0.805, P < 0.002, n = 11$).

Masking Effects Induced by Lights Off and Sleep Onset

Lights off and sleep onset at the end of the mini-CR induced pronounced masking effects on the circadian rhythm of T_{prox} and T_{dist}; however, no such masking effect was found on the circadian rhythms of T_{re} and heart rate. This was tested by comparing the differences in the 2 h before and after lights off (placebo group, this study) with the differences of the corresponding time points during a 35-h CR (constant wakefulness) in our earlier study in a similar group of healthy young men (23). Due to slight activation of the subjects by preparatory activities for the night (controlling of EEG electrodes, urine collection, etc.), heart rate showed increased values ~40 min before lights off followed by an immediate decrease at lights off.

To compare the circadian time course, masking effects were smoothed by 2-h moving averages before statistical analysis. The results with sleep ($n = 8$, this experiment) vs. without sleep ($n = 7$, data from Ref. 23) are T_{prox}: $+0.77 \pm 0.08$ vs. -0.18 ± 0.05°C, $P < 0.0001$; T_{dist}: $+1.64 \pm 0.27$ vs. $+0.16 \pm 0.13$°C, $P < 0.0005$; T_{re}: -0.19 ± 0.02 vs. -0.21 ± 0.02°C, NS; heart rate: -2.48 ± 0.42 vs. -2.09 ± 0.61 beats/min, NS (2-tailed Student's *t*-test). Both T_{prox} and T_{dist} increased concomitantly with lights out (Fig. 3, *A* and *B*). Detailed analyses of the relationship between sleep parameters and T_{prox}, T_{dist}, T_{re}, and heart rate, as well as the influence of Mel administration, are reported elsewhere (12).

DISCUSSION

This study shows that a single administration of melatonin (5 mg) and the melatonin agonist S-20098 (5 and 100 mg) at 1800 has significant effects on the nocturnal regulation of CBT decline and on circadian phase position.

On the first day of administration, these two effects were indistinguishable. All three drug conditions immediately induced an earlier midrange crossing time of CBT and heart rate decline. The phase marker analysis was supplemented by the analysis of the dynamics of CBT, heart rate, and T_{dist} and T_{prox} during the mini-CR period. In comparison with placebo, CBT, heart rate, and T_{prox} showed an earlier evening decline in all drug conditions, and T_{dist} showed an earlier increase. The thermoregulatory effects were most pronounced after S100 and Mel.

On the posttreatment day, the "pure" phase advance of the circadian system could still be significantly

detected for CBT (Mel: $\Delta\phi = +50$ min; S100: $\Delta\phi = +58$ min) and heart rate (S100: $\Delta\phi = +34$ min). This analysis could, at least partially, be confirmed by analysis of the dynamics of these variables during the mini-CR period. The time course of skin temperatures and heart rate, which show high variability, did not retain their significant differences on the posttreatment day, although a similar trend to that of the treatment day persisted (see Fig. 4*B*). The phase-advance capacity of the circadian system was most pronounced after Mel and S100, although S5 did not differ statistically.

The analysis of DLMO time and of the dynamics of evening salivary melatonin secretion corresponded well with the physiological data. DLMO showed an earlier increase not only after Mel administration (masking effect) but also after S-20098 at both doses.

There are at least three explanations possible for the effect of S-20098 on DLMO. First, S-20098 could act on unknown nonmelatoninergic receptors in the pineal gland that might activate the synthesis of melatonin. Second, S-20098 could influence salivary melatonin levels via changes of the salivary compartment (e.g., changes in elimination by the liver, changes in secretion rate from the plasma into saliva). And third, S-20098 may act directly on melatonin receptors in the SCN and phase advance the circadian clock and, thereby, the synthesis of melatonin in the pineal gland, advancing DLMO. This latter explanation would fit the animal data (3, 25, 28). The first suggestion is not favored, because S-20098 is a very specific and selective melatonin receptor agonist (33). There is no evidence for the second suggestion.

The consistent pattern found using three independent circadian phase markers strengthens the conclusion that a single administration of 5 mg melatonin or 100 mg S-20098 at 18 h is capable of phase advancing the human circadian system. The phase marker analysis is confirmed and extended by analyses of the time course of the measured variables during the mini-CR period. Our results confirm previous findings carried out under less controlled (masked) conditions that a single administration of melatonin (0.05–5 mg) in the late afternoon can phase advance the circadian system (16, 34).

Between 0800 and 1500 the subjects were not kept in the laboratory, but were instructed to remain indoors as much as possible and not to exercise. Analysis of the light logs and activity monitors revealed no correlation of outdoor light exposure and motor activity with circadian phase (data not shown; for methods, see Ref. 23).

We have previously determined under the unmasking conditions of a 35-h CR that the endogenous nocturnal decline in CBT is initiated by a prior increase in skin temperature at the distal regions, concomitant with a reduction in T_{prox} and heart rate (which is correlated with heat production; Ref. 23). In this mini-CR over a shorter period, the same temporal relationships were found. The significant correlations between changes in skin temperatures and heart rate with CBT during the first 4 h after drug intake suggest a functional relationship. In comparison with placebo, all drug administrations induced an earlier increase in skin temperature at the distal regions parallel with an earlier nocturnal decline in T_{prox} and heart rate. As a consequence, CBT also declined earlier. Therefore, it can be assumed that melatonin and the agonist S-20098 given at 1800 induce an earlier regulation of the endogenous nocturnal heat loss and reduced heat production, resulting in an earlier decline in CBT.

This study confirms and extends the results of Cagnacci et al. (10), who showed that, in aged women, 100 mg of melatonin administration at 0800 increases T_{dist} between 0800 and 2000 and decreases CBT. However, no time course was given and no measure of heat production (e.g., heart rate) was carried out. Therefore, no conclusions about the temporal relationship between heat loss and heat production could be made. Additionally, the dose used in that study was quite large, precluding comparison with our study. In other studies, a wide range of melatonin doses have been used (0.05–100 mg; e.g., Refs. 10, 30, 34). It is therefore possible that different receptors are involved in the effects observed with higher doses.

The phenomenon of masking is crucial in measurement of pure phase shifts. The development of the CR for human rhythm studies has permitted control of major external masking effects (induced by, e.g., light, sound, temperature) and keeps internal masking effects either at a reduced level (e.g., activity) or relatively constant (e.g., food intake) (15). However, the disadvantage of a long-lasting 40-h CR is that it replaces one form of masking (sleep) by another (sleep deprivation) (29). A 40-h CR also precludes measuring direct effects of drug administration on sleep. We developed the mini-CR protocol to exclude this sleep-deprivation effect as well as to reduce expense (both time, money, and the probability of recruiting volunteers), but forfeited the accurate determination of the phase of the CBT minimum (this "classical" circadian phase marker is replaced with another valid one).

The mini-CR starts at 1600, ~3 h before the usual circadian maximum of CBT (23). The first 2 h allow for adaptation to the orthostatic changes that occur after lying down, which include an increase in skin temperature, most pronounced on stomach, thigh, and feet (data not presented), and a decrease in CBT (Fig. 2). Heart rate shows an immediate decrease after lying down (22) (data not shown). Because of the length of time necessary for this adaptation, we now routinely commence the mini-CR at 1500 to extend baseline to a minimum of 3 h. After this adaptation, the late afternoon maximum of CBT and heart rate and the descending limb of both curves can be measured accurately under unmasked conditions as in a classical CR. The evening midrange crossing time of the declining portion of the T_{re} and heart rate curves, as well DLMO time, are reliable measures of circadian phase. Moreover, the midrange crossing time of heart rate and DLMO time was significantly correlated with the midrange crossing time of CBT, indicating a good internal validation of the

method. The midrange crossing times of CBT and heart rate, as well as the DLMO time, were stable under placebo over 3 successive days and over 4 successive wk. Therefore, the nonsignificant differences between the placebo days can be interpreted as differences by chance induced by the group randomization.

In addition, we have been able to show that the circadian decline in CBT and heart rate does not differ significantly with or without sleep, provided that the subjects have been lying down for many hours. This postural control is an important point because sleep usually occurs around the midrange crossing time (32). In contrast, lights off and sleep onset markedly increased (and thus masked) the circadian time course of T_{dist} and T_{prox}. However, it is not known whether the muscular relaxation or the reduction of light intensity from 10 to 0 lx is responsible for this marked increase in skin temperatures. No treatment group differed in the minimum, maximum, and midrange crossing values of CBT and heart rate, indicating that masking, if it occurred, remained constant across the treatment groups. Therefore, the phase shifts found in this study cannot be dismissed as a result of masking effects on the parameters themselves.

The parallel increase of T_{dist} and T_{prox} after lying down and at the beginning of sleep contrast clearly with their changes related to the circadian regulation of heat loss. It is known that distal regions of the body are the major sites for vasomotor heat loss (4). These regions are rich in arteriovenous anastomoses, which adjust blood flow through the skin and, therefore, play a central role in temperature regulation. It is generally assumed that arteriovenous anastomoses are not present in the skin of the thorax or abdomen, proximal regions with only minor thermoregulatory function (4). Recent studies have shown that vasodilation of arteriovenous anastomoses in distal skin regions may result in passive blood flow reduction in proximal regions lacking arteriovenous anastomoses (6). It is conceivable that regulation of circadian heat loss may be affected by vasodilation of arteriovenous anastomoses in distal regions via a direct hormonal action of endogenous melatonin on vascular melatonin receptors, which, in turn, changes blood flow and skin temperature in the proximal regions in an opposite direction. Vascular melatonin receptors with a specific function in heat dissipation have already been described in rats (31), but not yet in humans.

Several lines of evidence, when taken together, indicate that melatonin is involved in thermogenic processes (5). In animals, exogenous melatonin induces bradycardia and hypotension (14). In addition, a direct modulatory action on oxygen-supplying organs making up the cardiovascular and respiratory systems has been suggested (27). It is likely that melatonin affects several physiological systems (peripheral and central) that, in conjunction, regulate heat generation and dissipation. Recently, we have found that melatonin administered at 1300 increases T_{dist} and reduces T_{prox} and CBT without modifying heart rate (22). At this time of day, melatonin is not expected to phase advance the circadian system. If anything, it might phase delay (24, 30, 34). Thus our data favor the interpretation that melatonin and S-20098 can phase advance the circadian regulation of CBT via the SCN only when administered in the few hours before melatonin onset in the evening (24, 34).

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