ELSEVIER

European Journal of Pharmacology 347 (1998) 57–66

ejp

Pharmacologic restoration of suppressed temperature rhythms in rats by melatonin, melatonin receptor agonist, S20242, or 8-OH-DPAT

Petra Deprés-Brummer, Gérard Metzger, Francis Lévi *

Laboratoire 'Rythmes Biologiques et Chronothérapeutique' (Université Paris XI), Institut du Cancer et d'Immunogénétique, Hôpital Paul Brousse, 14 Avenue Paul Vaillant Couturier, 94807 Villejuif, France

Received 10 September 1997; revised 20 January 1998; accepted 27 January 1998

Abstract

Endogenous circadian rhythms in body temperature and locomotor activity rhythms are suppressed in Sprague–Dawley rats exposed to prolonged continuous light, possibly as a result of a profound alteration of the melatonin secretion rhythm. The ability to restore circadian system function with either exogenous melatonin, or melatonin receptor agonist S20242 (*N*-[2-(7-methoxy napth-1-yl)ethyl] propionamide), or 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), was investigated under these conditions. Seven rats received a daily 6-h intravenous infusion of melatonin (0.01 mg kg^{-1}) for 10 days, which generates a nearly physiological circadian rhythm of urinary 6-sulfatoxy-melatonin, the main urinary metabolite of melatonin. Nevertheless, there was no effect on body temperature or locomotor activity rhythms. Then, 49 rats received daily subcutaneous melatonin (0.01, 1 or 5 mg kg^{-1} day^{-1}), S20242 (1 or 5 mg kg^{-1} day^{-1}) or 8-OH-DPAT (5 mg kg^{-1} day^{-1}) for 30 days. The circadian rhythm in body temperature was restored by subcutaneous melatonin or by S20242 as a function of the dose or by 8-OH-DPAT. The effect started within the first 10 days of treatment and persisted for 1 to 3 weeks following the end of treatment in 8 of 10 rats receiving melatonin, in 9 of 11 rats treated with S20242 and in 1 of 4 rats treated with 8-OH-DPAT. Activity was less susceptible to entrainment than temperature with these drugs, since circadian rhythmicity was restored in only 2 of 6 rats treated with melatonin and in 1 of 4 rats treated with 8-OH-DPAT. These data demonstrate a specific action of subcutaneous melatonin, S20242 or 8-OH-DPAT on temperature rather than on activity rhythms. This differential effect on two major outputs of the suprachiasmatic nucleus further supports the existence of two independent oscillators in this hypothalamic circadian clock, which may be considered as separate pharmacological targets in the circadian system. © 1998 Elsevier Science B.V.

Keywords: Circadian rhythm; Light, continuous; Body temperature; Locomotor activity; Melatonin; Melatonin receptor agonist; 5-HT_{1A} receptor agonist; Desynchronization

1. Introduction

The rhythmicity of biological functions along the 24-h time scale is a feature of normal physiology in mammals. These rhythms are endogenous and coordinated by the suprachiasmatic nucleus, a hypothalamic clock (reviewed in Klein et al., 1991). The regular alternation of light and darkness over 24 h sets the circadian period to precisely 24 h as a result of known effects exerted on the suprachiasmatic nucleus by light (Illnerova and Vanecek, 1979; Klein et al., 1991) and by melatonin, a hormone mostly secreted by the pineal gland during darkness (Lewy et al., 1980; Vanecek et al., 1987; Weaver et al., 1989; Dubocovich et al., 1995; Shibata et al., 1989; MacArthur et al., 1991; Starkey et al., 1995; Cassone et al., 1988).

Exposing Sprague–Dawley rats to constant darkness for up to 3 months only marginally alters the circadian rhythms of body temperature and locomotor activity, two output rhythms of suprachiasmatic nucleus function. Exposure of these animals to continuous light, however, abolishes the circadian rhythm of activity within 4 weeks and that in body temperature within 8 weeks. We hypothetized that continuous light uncouples some of the oscillators which compose the circadian system. Both activity and temperature rhythms were restored within one week of continuous darkness, which suggested a role for melatonin (Deprés-Brummer et al., 1995, 1997). Thus, continuous exposure to light decreased mean plasma melatonin concentration 6-fold and damped but did not abolish its circadian rhythm. Restoration of temperature and activity circadian rhythms

* Corresponding author. Tel.: +33-1-4559-3855; fax: +33-1-4559-3602.

by exposure to continuous darkness was associated with recovery of a 'normal' melatonin rhythm (Deprés-Brummer et al., 1995).

Daily exogenous melatonin was able to synchronize to precisely 24 h the period of free-running circadian locomotor activity rhythm of rats kept in continuous darkness (Redman and Armstrong, 1983). This effect depended upon both melatonin dose and suprachiasmatic nucleus integrity (Cassone et al., 1986). This synchronizing effect of melatonin treatment was controversial in animals with disrupted activity circadian rhythm (Chesworth et al., 1987; Cheung and McCormack, 1982; Thomas and Armstrong, 1988; Phillips and Berger, 1992; Hyde and Underwood, 1995). Together, these data suggested that to obtain a pharmacologic effect of melatonin on the circadian system might require an intermittent delivery schedule. If melatonin rhythmicity is an internal synchronizer as suggested earlier (Armstrong, 1989), a threshold mean level or amplitude could be necessary for expression of such a property. Therefore, we first studied the effect of an intermittent intravenous delivery schedule on the suppressed temperature and activity rhythms of rats kept in prolonged continuous light. The melatonin schedule that was used almost reproduced the physiological rhythm of the urinary excretion of 6-sulfatoxy melatonin, the major metabolite of melatonin, in this model (Deprés-Brummer et al., 1996). Since the synchronizing effect of melatonin had usually been obtained in rats kept in constant darkness with subcutaneous administration, we also tested three dose levels with this route of injection.

As the sensitivity of the suprachiasmatic nucleus to melatonin can be altered by continuous light exposure (Yu et al., 1993), we also examined the effects of a new melatonin receptor agonist (S20242). This substance has higher affinity than melatonin for melatonin receptors, and could therefore be more effective than the natural hormone in the continuous light rat model (Depreux et al., 1994). This drug, like melatonin, synchronized the activity rhythm of rats kept in continuous darkness and increased the amplitude of the temperature rhythm of aged rats (Koster-Van Hoffen et al., 1993).

On the other hand, suprachiasmatic nucleus function can be influenced in vitro by serotonin (5-HT) or 5-HT receptor agonists (Mason, 1986; Meijer and Groos, 1988; Shibata et al., 1992; Tominaga et al., 1992b). In vivo, the circadian activity rhythm of hamsters kept in constant darkness or in constant light can be phase-advanced by 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)(Tominaga et al., 1992a; Edgar et al., 1993;Prosser et al., 1993; Cutrera et al., 1994).

Thus, we examined whether in vivo administration of melatonin, S20242 or 8-OH-DPAT could mimic the synchronizing effects of constant darkness in rats previously exposed to continuous light for a minimum of two months. This latter model may be useful for devising pharmacological treatments against circadian system alterations such as those encountered in depressive or malignant diseases.

2. Materials and methods

2.1. Chemicals

Melatonin was purchased from Sigma (St. Quentin Fallavier, France). S20242, a melatonin receptor agonist (*N*-[2-(7-methoxy napth-1-yl)ethyl] propionamide) was kindly provided by I.R.I.S. (Servier, Courbevoie, France). 8-OH-DPAT was purchased from R.B.I., Bioblock (Illkirch, France).

2.2. Animals and housing

Male Sprague–Dawley rats (Iffa-Credo, St. Germain-sur-L'Arbresle, France), 3-month-old on arrival at the laboratory and weighing 350 ± 25 g, were housed in individual polystyrene cages in a light-tight and temperature-controlled room (23 ± 1°C). Lighting was provided by four 40-W white fluorescent tubes. Mean lighting intensity was 300 lx at cage level (range 200–1000). Food and water were available ad libitum and cages were changed once a week on an irregular schedule.

Intraperitoneal temperature and locomotor activity were monitored every 10 min using an intraperitoneal temperature and activity sensor (TA1OTA-F40, Datasciences, St. Paul, MN, USA). This sensor was placed in the abdominal cavity under ether anaesthesia 1 to 14 weeks prior to the experiment.

Five days before intravenous melatonin administration (experiment 1), the jugular vein was cannulated with a silastic catheter (Vermed, Neuilly-en-Thelle, France) under anaesthesia with sodium thiopental (50 mg kg^{-1}, i.p.). The catheter was pulled subcutaneously through a slit in the skin on top of the skull, where it was fixed with screws and dental cement (Nicolaïdis et al., 1974). The catheter was filled with 0.1 ml viscous polyvinyl pyrrolidone solution (40%) until use.

2.3. Experimental designs

2.3.1. Experiment 1

Seven rats were exposed to continuous light for a median of 16 weeks (range 14–21). The rats received melatonin for 10 days then the control solution (0.25% ethanol–NaCl 0.9%) for another 10 days or the reverse sequence, according to a randomized crossover design. Four rats started with melatonin, three rats started with control solution. Melatonin or control solution was infused for 6 h from 2200 to 0400 and followed by NaCl 0.9% infusion for 18 h from 0400 to 2200. The melatonin dose was 0.01 mg kg^{-1} day^{-1} and the total daily infusion volume was 10 ml. This melatonin delivery scheme and

Table 1
Mean period and amplitude of body temperature and locomotor activity rhythms in 56 rats kept in continuous light (LL)

Result of LL exposure	Variable	No. of rats	Period (h ± min)	Amplitude
Suppressed rhythms	Temperature	42	5.4 ± 6	0.16 ± 0.01°C
	Activity	49	5.3 ± 3	43 ± 1%
Persistent circadian rhythms	Temperature	14	25.6 ± 6	0.27 ± 0.01°C
	Activity	7	25.2 ± 24	45 ± 3%

Number of rats with suppressed or persistent circadian rhythms for each variable, as indicated by spectral analysis of the 21-day time series preceding start of treatment.

dose level had been previously administered to rats exposed to continuous light and had produced a near normal circadian rhythm for urinary 6-sulfatoxy-melatonin, the major metabolite of melatonin (Deprés-Brummer et al., 1996). One rat was switched to continuous darkness following treatment completion.

2.3.2. Experiment 2

Forty-nine rats were exposed to continuous light for a median of 14 weeks (range 14–29). In four successive experiments, all rats received drug or control solutions daily subcutaneously at 1600 for 30 days. The drug was either melatonin, S20242 or 8-OH-DPAT. The control solutions were either ethanol 2% in NaCl 0.9%, or dimethylsulfoxide in water (20 or 50%, depending on the dose of S20242). Appropriate vehicle controls were run in each experiment. The melatonin daily dose was 0.01 mg kg^{-1} for four rats, similar to experiment 1. Nine rats received 1 mg kg^{-1}, in agreement with a previous report (Redman and Armstrong, 1983). Five rats were treated with 5 mg kg^{-1}. The daily dose of S20242, was either 1 mg kg^{-1} (eight rats) or 5 mg kg^{-1} (five rats). The daily dose of 8-OH-DPAT was 5 mg kg^{-1} (four rats), according to previous reports (Tominaga et al., 1992a; Prosser et al., 1993; Cutrera et al., 1994). Treatments were followed by an additional 3-week washout span in continuous light for all rats. Fifteen of these rats were subsequently exposed to continuous darkness for another 2 weeks, as a further control of the effect of these environmental conditions in these animals. Five rats had been previously treated with melatonin (1 mg kg^{-1}), four with S20242 (1 mg kg^{-1}) and six rats had received a control solution.

2.4. Data analysis

Temperature (°C) and activity data (arbitrary units or AU) were analysed using Dataquest III software. Successive seven-day time series (serial section shifted by 3–4 days) from each rat were analyzed with three methods (De Prins and Hecquet, 1992). Power spectrum analysis (Fourier transform) was first applied to the data series in order to detect the main periodicities. Least-square cosine regression was then applied with different test periods, at 5-min intervals, within the range of the dominant period ±1 h.

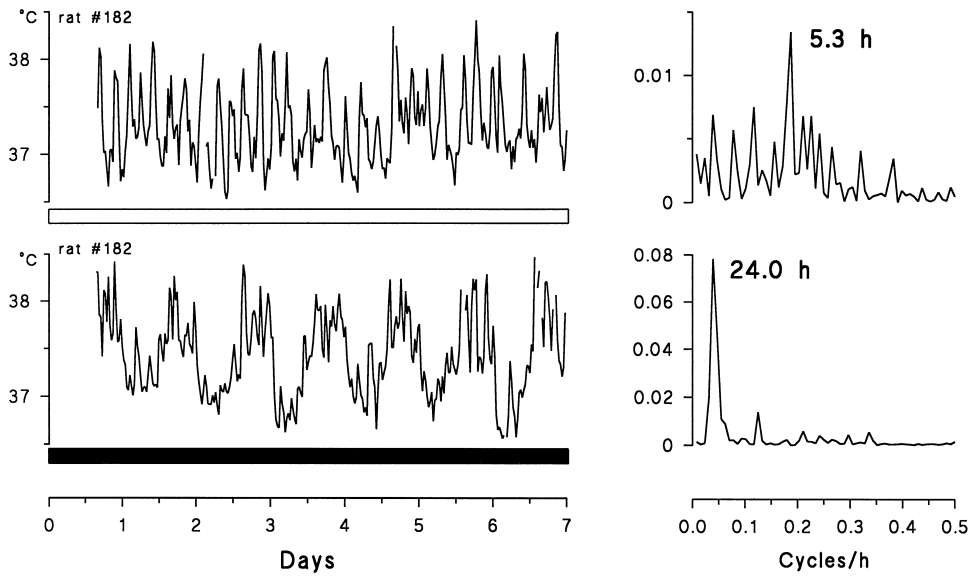

Fig. 1. Restoration of circadian rhythm of rat temperature by exposure to continuous darkness (DD). Seven-day body temperature records of a representative rat maintained first under continuous light (open box at *x*-axis, upper left panel) then switched to continuous darkness (dark box at *x*-axis, lower left panel). Power spectra in the right panels document the dominant period in the corresponding time series (Note increased ordinate scale for the continuous darkness analysis).

Table 2
Effects of continuous darkness on rhythms in body temperature and locomotor activity

Circadian rhythms	Variable	No. of rats	Period (h ± min)	Amplitude
Previously suppressed	Temperature	10	24.3 ± 6	0.50 ± 0.06°C
	Activity	10	24.2 ± 12	50 ± 3%
Previously free-running	Temperature	5	24.0 ± 2	0.49 ± 0.05°C
	Activity	5	24.2 ± 12	46 ± 5%

Continuous light exposure had previously led to circadian system suppression in 10 rats, while 'free running' circadian rhythms persisted in five animals. Mean periods and amplitudes (±S.E.M.) in continuous darkness were similar, irrespective of prior status of the circadian system in continuous light.

The period which corresponded to the highest percent rhythm (highest amplitude) was considered as the dominant one if $P < 0.001$. The mean values of rhythm parameters (±1 S.E.M.) were computed from individual values and compared whenever indicated using a paired Student's *t*-test. Autocorrelation analysis, which calculates and plots the serial correlation within the data at various time lags, was also used as an indicator of the strength of the rhythm. Mean 24-h correlation coefficients (r_{24}) were computed using Action3 software (Ambulatory Monitoring, NY, USA). $r_{24} = 1$, in the case of an exact reproducibility of a rest–activity pattern with a 24-h period from one 24-h span to the next throughout the whole time series. Conversely, $r_{24} = 0$ in the absence of any reproducible 24-h rhythm. Circadian rhythmicity was considered to be restored if a dominant peak in the circadian domain was detected by spectral analysis for at least 3 consecutive weeks during treatment.

Both mean estimated circadian amplitudes and mean r_{24} values were compared between different treatments with paired Student's *t*-test. Dose-dependence was validated with an analysis of variance.

3. Results

3.1. Effect of continuous light exposure on temperature and activity rhythms

Exposure to continuous light resulted in a gradual suppression of circadian rhythms of body temperature in 42 of

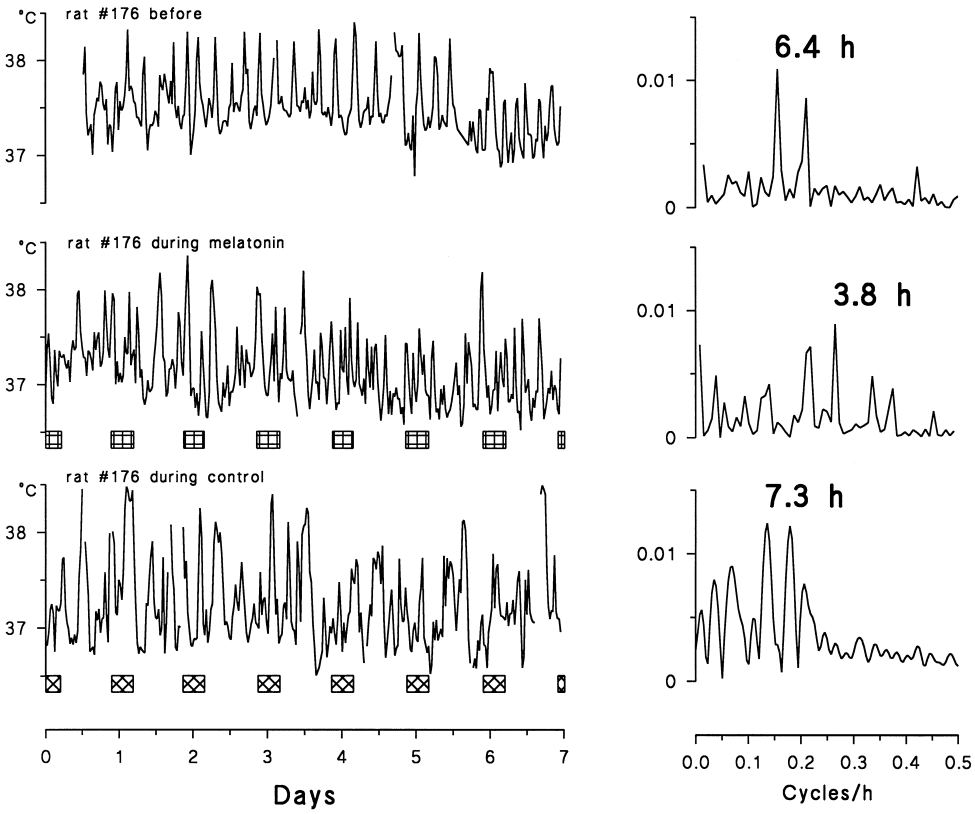

Fig. 2. Seven-day body temperature records of a representative rat kept in continuous light in the absence of any treatment (upper left panel), during intravenous melatonin 6-h infusion (middle left panel) and during control infusion (lower left panel). Power spectra in the right panels document the dominant period in the corresponding time series.

56 rats (75%). The activity rhythm was suppressed in 49 of 56 rats (88%). Dominant ultradian rhythms were validated by spectral analysis. Mean period was ≈5 h for both temperature and activity (Table 1). Amplitudes of the ultradian temperature and activity rhythms were respectively 4- and 2-fold lower than the circadian amplitude of age-matched rats kept with light:dark = 12:12 (0.6°C and 70%). The 24-h autocorrelation coefficients (r_{24}) of the temperature and activity rhythms were negligible (0.02 ± 0.02 and 0.015 ± 0.02, respectively). Transient desynchronization was observed in 42 rats: the circadian activity rhythm was suppressed 2 to 5 weeks earlier than the temperature rhythm.

Persistent circadian temperature rhythms were observed in 14 of 56 rats (25%), with a lengthened period of 25.6 h and a halved amplitude, as compared to age-matched rats kept with light:dark = 12:12. The mean autocorrelation coefficient at 24 h (±S.E.M.) was 0.23 ± 0.05. Persistent circadian activity rhythms were only observed in seven rats (12%), with a period of 25.2 h, a mean amplitude of 45% and $r_{24} = 0.25 \pm 0.07$ (Table 1). Thus, permanent desynchronization, i.e., a circadian temperature rhythm coexisting with an ultradian activity rhythm, was observed in seven rats.

3.2. Effect of continuous darkness exposure

A total of 15 rats were switched from continuous light to continuous darkness. Ten of these previously displayed ultradian rhythms while in constant light. A circadian rhythm was restored within one week of continuous darkness, for temperature in nine rats and for activity in seven animals (Fig. 1). All rats displayed dominant circadian rhythms for both temperature and activity after 10 days of exposure to constant darkness. The mean period was close to 24 h for both variables (Table 2). Temperature amplitude increased gradually to 0.5°C after 10 days in continuous darkness, which approached the value observed for

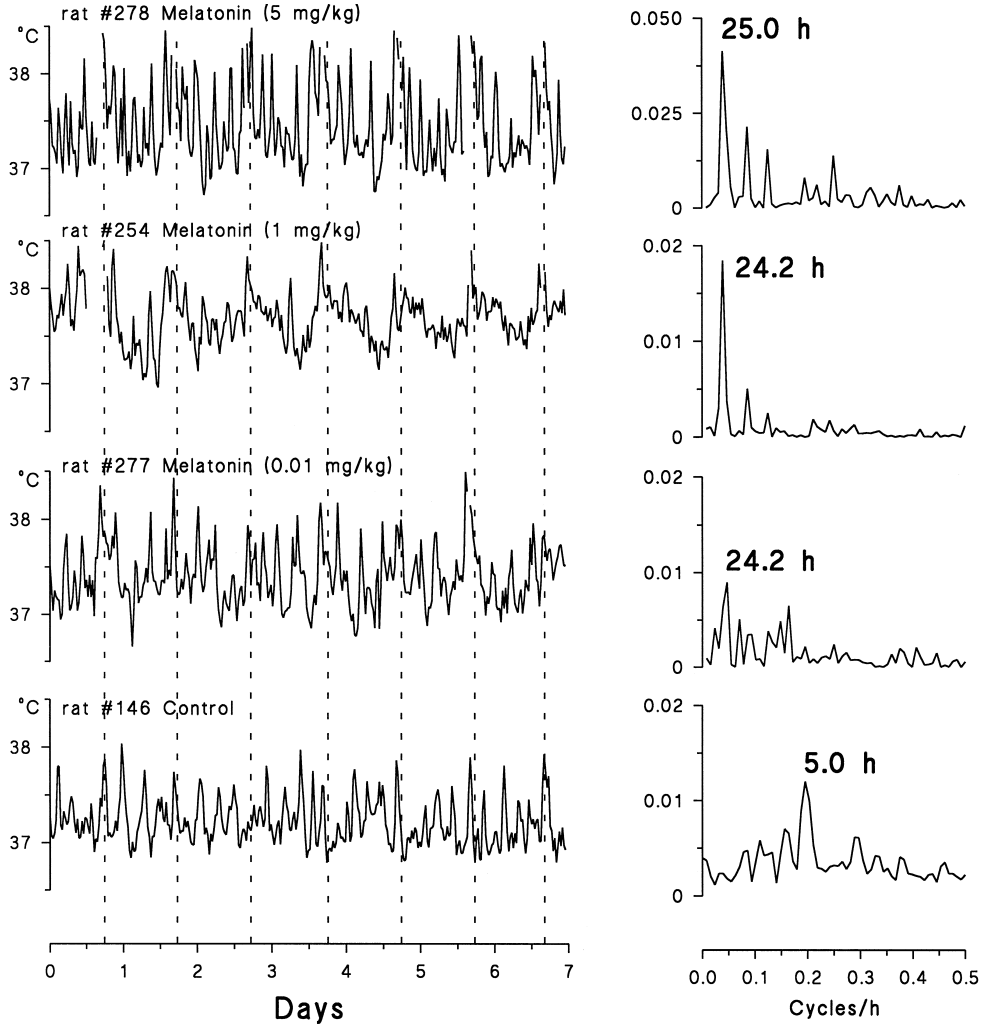

Fig. 3. Seven-day body temperature records of four representative rats kept in continuous light and treated with a daily subcutaneous administration of a control solution (ethanol 2%), or melatonin at one of three dose levels: 0.01, 1 or 5 mg kg^{-1} day^{-1}. Power spectra in the right panels document the dominant period in the corresponding time series (note increased ordinate scale for the 5 mg kg^{-1} dose of MLT).

rats kept in light:dark = 12:12. The increase in activity amplitude was minor (from 43% in continuous light to 50% in continuous darkness), thus it was not restored as compared to age-matched rats exposed to light:dark = 12:12. The mean r_{24} ($\pm$S.E.M.) of the temperature and activity rhythms increased to 0.45 ± 0.2 and 0.29 ± 0.1, respectively.

Five rats with persistent circadian rhythms in continuous light were also switched to constant darkness. The mean period was shortened to $\sim$ 24 h for both temperature and activity (Table 2). The mean amplitude of temperature rhythm increased gradually by 20% in the first and second weeks in continuous darkness, while that in activity rhythm was not modified.

3.3. Six-hour intravenous infusion of melatonin

No consistent 24 h change in temperature or activity was produced by this melatonin infusion schedule. Mean temperature and mean activity were similar during melatonin and control infusions. Neither amplitude nor autocorrelation-coefficient r_{24} were modified during either infusion. Fig. 2 displays the temperature of an animal before catheter implantation and during infusion of melatonin or control solution.

3.4. Subcutaneous administration effects on suppressed temperature and activity rhythms

3.4.1. Control solution

Circadian rhythmicity was not consistently restored for 3 weeks or more in any of the nine control rats. Three rats, treated with 50% dimethylsulfoxide control solution, transiently displayed a dominant circadian temperature rhythm for the first treatment week. Nevertheless, neither circadian amplitude nor autocorrelation-coefficient r_{24} varied.

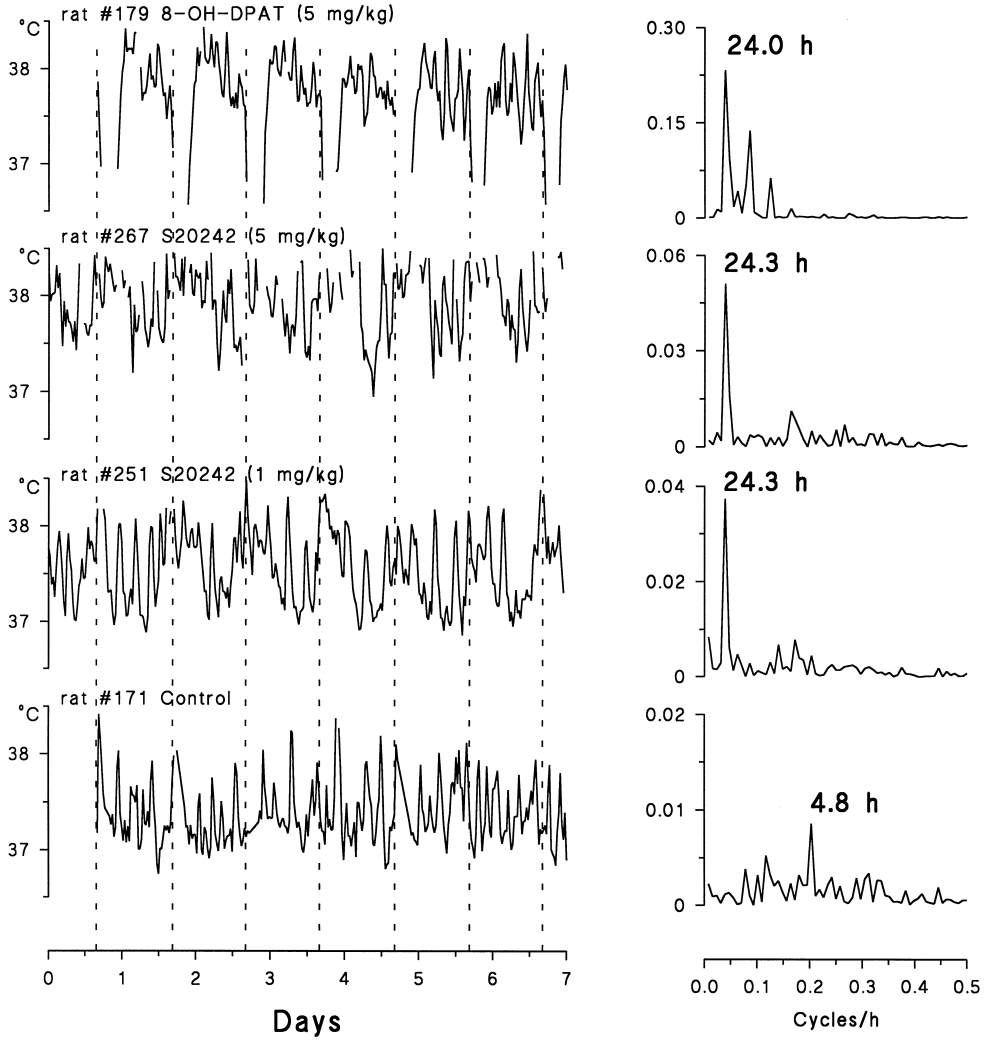

Fig. 4. Seven-day body temperature records of four representative rats kept in continuous light and treated with daily subcutaneous administrations of a control solution (DMSO 20%) or S20242 at a dose of 1 mg kg^{-1} or 5 mg kg^{-1}, or 8-OH-DPAT. Power spectra in the right panels document the dominant period in the corresponding time series (note changes in ordinate scales).

3.4.2. Subcutaneous melatonin administrations

Daily melatonin administration restored a sustained circadian rhythm of body temperature in 1 of 3 rats treated with 0.01 mg kg^{-1}, in three of six animals receiving 1 mg kg^{-1} and in 4 of 4 rats injected with 5 mg kg^{-1} day^{-1} (Fig. 3). During treatment, the period was 24.1 h ± 2 min.

Mean circadian amplitude increased significantly as a function of melatonin dose (Fig. 5). Similar results were obtained for the mean autocorrelation coefficients (data not shown). This circadian rhythm persisted for 2 weeks after the last injection in two rats (5 mg kg^{-1}) and for 3 weeks or more in six rats (0.01 mg kg^{-1}, one rat; 1 mg kg^{-1}, three rats; 5 mg kg^{-1}: two rats). However, its period was lengthened significantly to 25.5 h ± 28 min ($P < 0.001$).

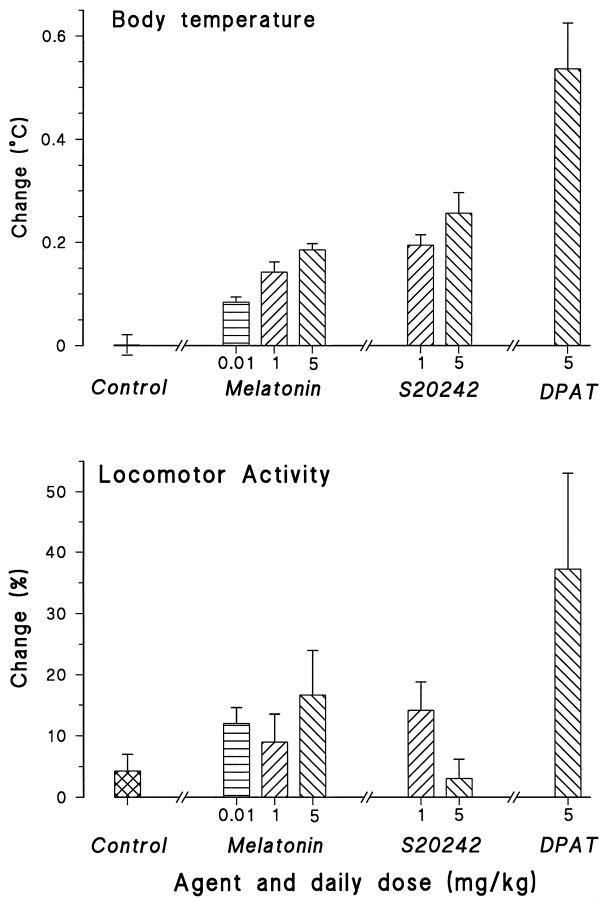

Fig. 5. Effects of melatonin, melatonin receptor agonist, S20242, and 5-HT_{1A} receptor agonist, 8-OH-DPAT (DPAT), on the circadian amplitude of rhythms in body temperature (upper panel) and locomotor activity (lower panel). Controls received either DMSO in water (20 or 50%) or ethanol 2% in NaCl 0.9%. All agents were administered subcutaneously daily for 30 days to rats with suppressed circadian rhythms for body temperature and locomotor activity as a result of continuous light exposure. The figure illustrates mean ± S.E.M. of change in individual circadian amplitude according to the dose of the agent. Daily vehicle administration did not restore any rhythmicity. Conversely, mean circadian amplitude of body temperature increased with melatonin and S20242 and in a dose-dependent manner ($P < 0.01$ and $P = 0.06$, respectively) as well as with 8-OH-DPAT. The rest–activity cycle was restored with 8-OH-DPAT but was minimally influenced by melatonin or S20242, at any dose level tested.

Mean temperature was not affected by melatonin. None of the rats displayed hypothermia following injection. The mean activity level was not modified with either 0.01 or 1 mg kg^{-1} day^{-1} of melatonin. However, mean activity was significantly reduced by 2 ± 0.3 arbitrary units (16%) in rats receiving a daily dose of 5 mg kg^{-1} of melatonin ($P < 0.02$), as early as during the first treatment week.

The circadian rhythm of activity was restored only in two rats treated with 1 mg kg^{-1} day^{-1}, with a period of 24.1 h ± 3 min and a doubled circadian amplitude (15% before and 34% during treatment). In addition, five rats displayed a dominant circadian rhythm of activity for 1 to 2.5 weeks during treatment. The mean estimated circadian amplitude increased significantly by 12 ± 3%, irrespective of melatonin dose ($P < 0.01$) (Fig. 5). The mean autocorrelation coefficient r_{24} increased significantly as a function of dose, to 0.06, 0.18 or 0.21 in rats treated with, respectively, 0.01, 1 or 5 mg kg^{-1} of melatonin ($P < 0.04$).

3.4.3. Subcutaneous administrations of S20242

Neither mean temperature nor mean activity was modified by S20242 injections. A daily injection of S20242 restored the circadian rhythm for temperature in 11 out of 11 rats, irrespective of dose (Fig. 4). During treatment, the mean period was 24.2 h ± 2 min. Circadian amplitude increased according to dose ($P = 0.06$; Fig. 5). The mean autocorrelation coefficient r_{24} increased significantly to 0.25, irrespective of dose. Furthermore, the temperature rhythm remained in the circadian periodic domain for 1 to 3 weeks following S20242 withdrawal in 9 of 11 rats. Its period was however lengthened to 25.4 h ± 16 min. Although activity transiently displayed a circadian dominant period in five rats (for less than 3 consecutive weeks during or after treatment), this rhythm was not consistently restored in any animal.

3.4.4. Subcutaneous administrations of 8-OH-DPAT

Daily injection of 8-OH-DPAT induced acute hypothermia (Fig. 4). Temperature dropped to a mean nadir of 34.7°C ± 0.6°C within 2 h (range 1 to 3 h) and recovered 14 ± 1 h later. However, the mean temperature was similar before, during or after treatment. Consequently, the circadian rhythm for temperature was restored in all rats (period = 23.8 h ± 12 min), and persisted in 1 of 4 rats for 3 weeks following treatment withdrawal with a longer period (24.7 h). In this animal, the circadian activity rhythm was restored with a period of 24.2 h, and two additional rats had dominant ≈ 12 h rhythms during the entire treatment regimen.

The mean circadian amplitude for both temperature and activity increased, respectively, by 0.54 ± 0.1°C ($P < 0.02$) and 37 ± 16% ($P = 0.1$; Fig. 5). The mean autocorrelation coefficients r_{24} of temperature and activity increased to 0.6 and 0.56, respectively ($P < 0.02$).

3.5. Effects of daily administration on persistent circadian temperature and activity rhythms

The 12 rats with persistent circadian rhythms received a daily injection of melatonin (one rat: 0.01 mg kg^{-1}; three rats: 1 mg kg^{-1}; one rat: 5 mg kg^{-1}) or S20242 (two rats: 1 mg kg^{-1}) or control solution (five rats). In rats receiving melatonin or S20242, the mean period of temperature rhythm was shortened significantly to 24.3 h ± 12 min ($P < 0.02$) during treatment, then lengthened to its previous values (25.3 h ± 10 min), following treatment withdrawal. Neither circadian amplitude nor autocorrelation coefficient r_{24} was modified. No change in period or amplitude of rhythm was observed in rats receiving a control solution.

4. Discussion

Prolonged exposure of rats to continuous light resulted in a gradual suppression of circadian rhythms for body temperature and locomotor activity in 75% and 88% of the animals respectively. The suppression was preceded by a transient uncoupling of temperature and activity rhythms, the latter rhythm being suppressed 2 to 5 weeks before that of temperature. Subsequent exposure to continuous darkness for 10 days restored both circadian rhythms in all the rats exposed to these conditions. These results were very similar to those reported earlier (Deprés-Brummer et al., 1995, 1997). We further examined whether treatment with exogenous melatonin, the melatonin receptor agonist S20242 or the 5-HT_{1A} receptor agonist, 8-OH-DPAT, could alleviate the functional suppression of the circadian system induced by continuous light exposure.

Melatonin plasma clearance and distribution volume were doubled in rats kept under continuous light as compared to those kept with light:dark = 12:12 (Deprés-Brummer et al., 1996). This might explain the failure of a chronomodulated delivery scheme to restore circadian rhythms of temperature or activity, probably as a result of melatonin accumulation (Deprés-Brummer and Lévi, unpublished observations). These results led us to devise a daily intermittent infusion scheme. Exogenous melatonin, at a dose of 0.01 mg kg^{-1} day^{-1}, administered intravenously for 6 h every 24 h, produced a nearly physiological rhythm of urinary 6-sulfatoxymelatonin in rats exposed to prolonged continuous light (Deprés-Brummer et al., 1996). The present study however failed to demonstrate any effect of this delivery schedule on suppressed temperature or activity rhythms throughout a 10-day treatment. As a possible explanation, continuous light exposure could alter melatonin responsiveness. Thus, continuous light exposure for 2 or 3 days suppressed the circadian rhythm for both electrical activity in the suprachiasmatic nucleus and responsiveness to melatonin, increased the overall responsiveness of the suprachiasmatic nucleus to melatonin and increased the density of melatonin binding sites in the suprachiasmatic nuclei of Syrian hamsters or guinea pigs (Gauer et al., 1992; Poon and Pang, 1992; Yu et al., 1993). Nevertheless, these findings imply that low doses of melatonin should be effective on circadian function in animals kept in continuous light. In addition, it could be necessary to make exogenous melatonin administration coincide with the sensitive phase of the endogenous melatonin rhythm in order to restore circadian function with this agent, a requirement similar to that needed for the melatonin effect on gonadal regression in hamsters (Bartness, 1993). However, pinealectomy did not alter the ability of daily melatonin treatment to synchronize activity rhythms of rats kept in continuous darkness, thus the pharmacologic effects of melatonin on the circadian system are likely to be independent of the endogenous melatonin rhythm (Warren et al., 1993).

The results we now obtained with daily subcutaneous administration of melatonin, S20242 or 8-OH-DPAT support this latter concept. Thus, the temperature circadian rhythm was restored with these drugs, given subcutaneously either within the first 10 days of treatment or not at all, which mimicked the effect of continuous darkness on this variable. In addition, subcutaneous melatonin restored suppressed temperature rhythms as a function of dose whether based on number of rats with a circadian rhythm or on mean circadian amplitude. No hypothermia was induced by melatonin administration in rats, as opposed to humans (Deacon et al., 1994). In rats, in contrast to humans, high nocturnal activity levels and body temperature coincide with high melatonin secretion. In the present study, melatonin or S20242 injections coincided with the maximum of the restored body temperature rhythm.

Subcutaneously administered melatonin restored the suppressed rhythms, while intravenous infusion had no such effect. A similar discrepancy between modes of administration was reported for Syrian hamsters kept in continuous darkness. Both melatonin and control solution injected subcutaneously 2 h before the start of activity produced a phase advance, while a subcutaneous infusion was ineffective (Hastings et al., 1992). Although arousing stimuli may exert potent effects on the circadian activity rhythm in rodents kept in continuous light or darkness (Cutrera et al., 1994), no such effect was observed in any of the nine control rats from our study. Furthermore, the dose–response relationship for melatonin activity rules out a possible role of animal handling. The fact that no synchronization was achieved with the lower dose of 0.01 mg kg^{-1}, whether melatonin was given daily as a 6-h i.v. infusion or as a subcutaneous injection supports dose dependence rather than schedule-dependence of the activity of melatonin in the continuous light exposure rat model.

The melatonin receptor agonist, S20242, restored the circadian rhythm for temperature in all the rats exposed to constant light, at either dose level, yet the mean amplitude

was highest in animals receiving the higher dose. The higher affinity of S20242 for melatonin receptors might thus result in increased potency for circadian synchronization as well as a possibly increased specificity for body temperature.

A 3°C drop in body temperature was elicited with 8-OH-DPAT within 1 h of its administration, confirming previous reports (Hjorth, 1992). Consequently, this agent restored a circadian rhythm for body temperature in all rats.

In rats with persistent free-running circadian rhythms despite continuous light, daily melatonin or S20242 administration shortened the period of temperature rhythm by nearly 1 h in comparison with that of the control rats. Similarly, daily evening administration of melatonin was reported to set to 24 h the period of the free-running sleep/wake rhythm in blind men (Arendt et al., 1988; Tzischinsky et al., 1992).

Locomotor activity was less susceptible than body temperature to circadian synchronization with any of the agents tested. Only two rats treated with melatonin (1 mg kg^{-1}) and one animal receiving 8-OH-DPAT displayed circadian activity rhythms.

The restoration of circadian rhythms in body temperature usually persisted for 1 to 3 weeks following melatonin, S20242 or 8-OH-DPAT withdrawal, yet with an increased period length. Thus, the synchronizing effect of these substances most likely resulted from their effects on function of the circadian clock. Furthermore, the transient loss of synchronization between temperature and activity rhythms in rats exposed to continuous light and the rather specific activity of melatonin or 5-HT_{1A} receptor agonists for body temperature rhythm support the existence of at least two distinct functional oscillators in the circadian clock (Moore, 1996). These oscillators may constitute separate pharmacological targets to be considered in the treatment of circadian system disorders.

Acknowledgements

This work was supported by the Association pour la Recherche sur le Temps Biologique et la Chronothérapie. We are grateful to Dr. C. Larue-Achagiotis, S. van den Heiligenberg and E. François for helpful technical assistance. We are indebted to Pr. Y. Touitou, Dr. P. Delagrange and Dr. B. Guardiola-Lemaître for constructive discussions. We thank M. Lévi for editing this manuscript.

References

Arendt, J., Aldhous, M., Wright, J., 1988. Synchronisation of a disturbed sleep–wake cycle in a blind man by melatonin treatment. Lancet (April 2) 772–773.

Armstrong, S.M., 1989. Melatonin, the internal zeitgeber of mammals? In: Reiter, R.J. (Ed.), Pineal Research Reviews. Alan R. Liss, New York, pp. 157–202.

Bartness, T.J., 1993. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception and the photoperiodic control of seasonal responses. J. Pineal Res. 15, 161–190.

Cassone, V.M., Chesworth, M.J., Armstrong, S.M., 1986. Entrainment of rat circadian rhythms by daily injection of melatonin depends upon the hypothalamic suprachiasmatic nuclei. Physiol. Behav. 36, 1111–1121.

Cassone, V.M., Roberts, M.H., Moore, R.Y., 1988. Effects of melatonin on 2-deoxy-[1-^{14}C]glucose uptake within rat suprachiasmatic nucleus. Am. J. Physiol. 255, R332–R337.

Chesworth, M.J., Cassone, V.M., Armstrong, S.M., 1987. Effects of daily melatonin injections on activity rhythms of rats in constant light. Am. J. Physiol. 253, R101–R107.

Cheung, P.W., McCormack, C.E., 1982. Failure of pinealectomy or melatonin to alter the circadian activity rhythm of the rat. Am. J. Physiol. 242, R261–R264.

Cutrera, R.A., Ouarour, A., Pevet, P., 1994. Effects of the 5-HT1a receptor agonist 8-OH-DPAT and other non-photic stimuli on the circadian rhythm of wheel-running activity in hamsters under different constant conditions. Neurosci. Lett. 172, 27–30.

Deacon, S., English, J., Arendt, J., 1994. Acute phase-shifting effects of melatonin associated with suppression of core body temperature in humans. Neurosci. Lett. 178, 32–34.

Deprés-Brummer, P., Lévi, F., Metzger, G., Touitou, Y., 1995. Light-induced suppression of the rat circadian system. Am. J. Physiol. 268, R1111–R1116.

Deprés-Brummer, P., Metzger, G., Morin, D., Urien, S., Touitou, Y., Tillement, J.P., Claustrat, B., Levi, F., 1996. Pharmacokinetically guided melatonin scheduling in rats with circadian system suppression. Eur. J. Pharmacol. 312, 171–178.

Deprés-Brummer, P., Bourin, P., Pagès, N., Metzger, G., Lévi, F., 1997. Persistent lymphocyte rhythms despite suppressed circadian outputs in rats. Am. J. Physiol. 273, in press.

Depreux, P., Lesieur, D., Mansour, H.A., Morgan, P., Howell, H.E., Renard, P., Caignard, D.H., Pfeiffer, B., Delagrange, P., Guardiola, B., Yous, S., Demarque, A., Adam, G., Andrieux, J., 1994. Synthesis and structure–activity relationships of novel napthalenic and biosteric related amidic derivates as melatonin receptor ligands. J. Med. Chem. 37, 3231–3239.

De Prins, J., Hecquet, B., 1992. Data processing in chronobiological studies. In: Biological Rhythms in Clinical and Laboratory Medicine. Springer-Verlag, Berlin (All.), pp. 90–113.

Dubocovich, M.L., Benloucif, S., Masana, M.I., 1995. Melatonin receptors in the mammalian suprachiasmatic nucleus. Behav. Brain Res. 73, 141–147.

Edgar, D.M., Miller, J.D., Prosser, R.A., Dean, R.R., Dement, W.C., 1993. Serotonin and the mammalian circadian system: II. Phase shifting rat behavioral rhythms with serotonergic receptor agonists. J. Biol. Rhythms 8, 17–31.

Gauer, F., Masson-Pévet, M., Pévet, P., 1992. Pinealectomy and constant illumination increase the density of melatonin binding sites in the pars tuberalis of rodents. Brain Res. 575, 32–38.

Hastings, M.H., Mead, S.M., Vindlacheruvu, R.R., Ebling, F.J.P., Maywood, E.S., Grosse, J., 1992. Non-photic phase shifting of the circadian activity rhythm of Syrian hamsters: the relative potency of arousal and melatonin. Brain Res. 591, 20–26.

Hjorth, S., 1992. (−)-Penbutolol as a blocker of central 5-HT1a receptor mediated responses. Eur. J. Pharmacol. 222, 121–127.

Hyde, L.L., Underwood, H., 1995. Daily melatonin infusions entrain the locomotor activity of pinealectomized lizards. Physiol. Behav. 58, 943–951.

Illnerova, H., Vanecek, J., 1979. Response of rat pineal serotonin *N*-acetyltransferase to one minute light pulses at different times on the night. Brain Res. 167, 431–434.

Klein, D.C., Moore, R.Y., Reppert, S.M., 1991. Suprachiasmatic Nucleus, The Mind's Clock. Oxford Univ. Press, Oxford, p. 467.

Koster-Van Hoffen, G.C., Mirmiran, M., Bos, N.P.A., Witting, W., Delagrange, P., Guardiola-Lemaitre, B., 1993. Effects of a novel melatonin analog on circadian rhythms of body temperature activity in young, middle-aged and old rats. Neurobiol. Aging 14, 565–569.

Lewy, A.J., Wehr, T.A., Goodwin, F.K., 1980. Light suppresses melatonin secretion in humans. Science 210, 1267–1269.

Mason, R., 1986. Circadian variation in sensitivity of supachiasmatic and lateral geniculate neurones to 5-hydroxytryptamine in the rat. J. Physiol. 377, 1–13.

MacArthur, A.J., Gillette, M.U., Prosser, R.A., 1991. Melatonin directly resets the rat suprachiasmatic nucleus in vitro. Brain Res. 565, 158–161.

Meijer, J.H., Groos, G.A., 1988. Responsiveness of suprachiasmatic and ventral lateral geniculate neurons to serotonin and imipramine: a microiontophoretic study in normal and imipramine-treated rats. Brain Res. Bull. 20, 89–96.

Moore, R.Y., 1996. Entrainment pathways and the functional organization of the circadian system. Prog. Brain Res. 111, 103–119.

Nicolaïdis, S., Rowland, N., Meile, M.J., Marfaing-Jallat, P., Pesez, A., 1974. A flexible technique for long term infusions in unrestrained rats. Pharmacol. Biochem. Behav. 2, 131–136.

Phillips, N.H., Berger, R.J., 1992. Melatonin infusions restore sleep suppressed by continuous bright light in pigeons. Neurosci. Lett. 145, 217–220.

Poon, A.M.S., Pang, S.F., 1992. Constant light exposure increases 2-^{125}I-iodo melatonin binding sites in the guinea pig spleen. Neurosci. Lett. 146, 41–44.

Prosser, R.A., Dean, R.R., Edgar, D.M., Heller, H.C., Miller, J.D., 1993. Serotonin and the mammalian circadian system: I. In vitro phase shifts by serotonergic agonists and antagonists. J. Biol. Rhythms 8, 1–16.

Redman, J.R., Armstrong, S.M., 1983. Free-running activity rhythms in the rat: entrainment by melatonin. Science 219, 1089–1091.

Shibata, S., Cassone, V.M., Moore, R.Y., 1989. Effects of melatonin on neuronal activity in the rat suprachiasmatic nucleus in vitro. Neurosci. Lett. 97, 140–144.

Shibata, S., Tsuneyoshi, A., Hamada, T., Tominaga, K., Watanabe, S., 1992. Phase-resetting effect of 8-OH-DPAT, a serotonin-1a receptor agonist, on the circadian rhythm of firing rate in the rat suprachiasmatic nuclei in vitro. Brain Res. 582, 353–356.

Starkey, S.J., Walker, M.P., Beresford, I.J.M., Hagan, R.M., 1995. Modulation of the rat suprachiasmatic circadian clock by melatonin in vitro. NeuroReport 6, 1947–1951.

Thomas, E.M.V., Armstrong, S.M., 1988. Melatonin administration entrains female rat activity rhythms in constant darkness but not in constant light. Am. J. Physiol. 255, R237–R242.

Tominaga, K., Shibata, S., Ueki, S., Watanabe, S., 1992a. Effects of 5-HT_{1A} receptor agonists on the circadian rhythm of wheel-running activity in hamsters. Eur. J. Pharmacol. 214, 79–84.

Tominaga, K., Shibata, S., Ueki, S., Watanabe, S., 1992b. Effects of inhibitory and excitatory drugs on the metabolic rhythm of the hamster suprachiasmatic nucleus in vitro. Eur. J. Pharmacol. 217, 79–84.

Tzischinsky, O., Pal, U., Epstein, R., Dagan, Y., Lavie, P., 1992. The importance of timing in melatonin administration in a blind man. J. Pineal Res. 12, 105–108.

Vanecek, J., Pavlik, A., Illnerova, H., 1987. Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res. 435, 359–362.

Warren, W.S., Hodges, D.B., Cassone, V.M., 1993. Pinealectomized rats entrain and phase-shift to melatonin injections in a dose dependent manner. J. Biol. Rhythms 8, 233–245.

Weaver, D.R., Rivkees, S.A., Reppert, S.M., 1989. Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. J. Neurosci. 9, 2581–2590.

Yu, G., Rusak, B., Piggins, H.D., 1993. Regulation of melatonin sensitivity and firing-rate rhythms of hamster suprachiasmatic nucleus neurons: constant light effects. Brain Res. 602, 191–199.