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Pharmacokinetically guided melatonin scheduling in rats with circadian system suppression

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Abstract

To obtain a pharmacologic effect of melatonin in rats kept under prolonged continuous light exposure, conditions known to produce functional suppression of the circadian system, mimicking of the physiologic 24-h pattern of melatonin secretion, a hormonal signal of darkness exposure may be needed. The delivery scheme for melatonin was established in rats in the present studies. First, the plasma pharmacokinetics of [^{3}H]melatonin were determined in rats kept under continuous light and in rats synchronized by exposure to alternating 12 h light and 12 h darkness (LD 12 : 12) in the early light span. The pharmacokinetics of total radioactivity were similar in both groups. Further quantitation of melatonin by thin-layer chromatography revealed differences dependent on light conditions. The mean plasma clearance and steady-state distribution volume were ~ twice as low with continuous light as with LD 12 : 12. Plasma protein binding of melatonin was ~ 33%, irrespective of group or sampling time. These pharmacokinetic parameters were used to devise a 24-h periodic delivery schedule consisting of a 6-h constant infusion of exogenous melatonin, followed by an 18-h melatonin-free interval. In a second study, the melatonin 24-h pattern was estimated from the measurement of 2-h fractions of urinary 6-sulfatoxymelatonin excretion for 4 days. 6 unrestrained rats kept under continuous light received melatonin for 2 days from 22:00 to 04:00 h through an indwelling jugular catheter, connected to a reservoir from a programmable pump. Only the administration of low doses (0.01 mg/kg/day) resulted in both a circadian pattern for 6-sulfatoxymelatonin excretion and normal physiological values during the infusion-free intervals. The resynchronizing efficacy of this schedule should be tested in rats with functional suppression of the circadian system.

Keywords: Pharmacokinetics; Melatonin; Continuous light; Circadian rhythm; (Intravenous); 6-Sulfatoxymelatonin

1. Introduction

Daily administration of melatonin entrains the circadian locomotor activity rhythm of rats in constant darkness when the start of running wheel activity coincides with the time of injection (Redman and Armstrong, 1983). This entraining effect was observed with doses ranging from 0.01 to 1 mg/kg and was prevented by lesions of the suprachiasmatic nucleus (Cassone et al., 1986a,b). Moreover, daily melatonin injections alter the rate and direction of responses to phase shifts of the light-dark cycle (Armstrong et al., 1986).

Prolonged exposure of male Sprague-Dawley rats to continuous light induced complete functional suppression of body temperature and locomotor activity circadian rhythms. This was preceded by a transient loss of coupling between the two functions. After 10 weeks of continuous light exposure of the rats, body temperature and activity exhibited dominant ultradian rhythms (with a period, $\tau =$ 4–6 h). Continuous darkness exposure restored both of these circadian rhythms within 7 days. In rats maintained for 10 weeks under continuous light, mean plasma mela-

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tonin decreased 6-fold and its circadian rhythm was strongly dampened, yet not abolished. Restoration of temperature and activity circadian rhythms in continuous darkness was associated with normalization of melatonin rhythm (Deprés-Brummer et al., 1995a).

With continuous light, the entraining effect of melatonin on locomotor activity rhythm was less obvious than in rats kept in continuous darkness. Daily melatonin injection can induce a low-amplitude 24-h rhythm (partial synchronization) in male Long-Evans or Sprague-Dawley rats but not in female rats, with a previously disrupted circadian activity rhythm (Chesworth et al., 1987; Thomas and Armstrong, 1988; Deprés-Brummer et al., 1995b).

This lack of a clear synchronizing effect of melatonin could result from a suboptimal administration schedule. Melatonin has a rather short elimination half-life (20–35 min). Continuous i.v. administration could be required for an adequate pharmacologic efficacy (Kopin et al., 1961; Gibbs and Vriend, 1981). However, continuous subcutaneous (s.c.) administration using silastic implants failed to synchronize disrupted activity rhythms in female Long-Evans rats (Cheung and McCormack, 1982). In contrast, in pigeons, daily intravenous (i.v.) melatonin infusion from 7 to 19 h for 10 consecutive days restored sleep suppressed by continuous bright light (Phillips and Berger, 1992). Furthermore, daily 12-h melatonin infusions entrained the locomotor activity rhythm of previously pinealectomized lizards with altered circadian activity rhythm (Hyde and Underwood, 1995). Although the somnogenic effects of melatonin are not necessarily mediated by the same mechanisms as its circadian effects, these experiments suggest that melatonin may act at several outputs of the circadian system in addition to affecting the circadian clock itself. Therefore, a pharmacologic effect of melatonin on the circadian system might require an intermittent chronomodulated delivery schedule. The present study assessed the pharmacokinetics of exogenous melatonin in rats with synchronized or suppressed circadian rhythms in order to design a delivery schedule which would yield a 'target' circadian rhythm of the plasma melatonin concentration. This target concentration rhythm was defined as that observed in rats after a switch from continuous light to continuous darkness. Both pharmacokinetics and pharmacodynamics of a drug may be significantly altered by a change in circadian dosing time (Bruguerolle, 1984). To our knowledge, the effects of circadian rhythm suppression on drug pharmacokinetics have not yet been explored. This information is, however, essential for drugs to be given under these conditions.

2. Materials and methods

2.1. Animals and housing

Male Sprague-Dawley rats (Iffa-Credo, St. Germain-sur-L'Arbresle, France), mean ± S.D. weight 350 ± 25 g and 3 months old on arrival at the laboratory were housed in individual polystyrene cages in two adjacent light-tight and temperature-controlled rooms (23 ± 1°C). Lighting was provided by four 40-W fluorescent tubes mounted in ceiling fixtures. Mean lighting intensity was 300 lux at cage level (range 200–1000). Food and water were available ad libitum, cage changes occurred once a week on an irregular schedule.

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

1–3 days prior to melatonin administration, the jugular vein was cannulated with a silastic catheter (Vermed, Neuilly-en-Thelle, France) under anaesthesia with sodium thiopental (50 mg/kg i.p.). The catheter was pulled s.c. to a slit in the skin on the top of the skull where it was fixed with screws and dental cement (Nicolaidis et al., 1974). The catheter was filled with 0.1 ml viscous polyvinyl pyrrolidone solution (40%) until use. In order to avoid catheter obstruction and hemoconcentration, the catheter was rinsed first with an injection of 0.1 ml heparin (500 IU/ml) and then with 0.4 ml NaCl 0.9% after each blood sampling.

2.2. Experimental designs

2.2.1. Experiment 1

Upon arrival, the rats were randomly allocated to one of two groups. One group (7 rats) was synchronized with an alternation of 12 h of light (L) and 12 h of darkness (D) (LD 12:12, L from 8 to 20 h) and the second group (6 rats) was exposed to constant light for 14 weeks. Temperature and activity were monitored in 3 rats of each group 1 week prior to melatonin administration. 24 h after catheterization, [^{3}H]melatonin was administered as a bolus i.v. injection between 09:00 and 12:00 h, immediately followed by rinsing with 0.4 ml NaCl 0.9%. Venous blood samples (0.4–0.9 ml) were collected through the catheter after various time intervals (0.5, 1, 2, 4, 10, 20, 40, 90, 180 and 360 min) in EDTA-K^{3+} tubes and centrifuged (at 13 000 × g) for 5 min within 2 min following sampling. The plasma was divided into 4 aliquots for radioactivity counting, thin-layer chromatography (TLC), protein determination and plasma protein-binding studies (according to sampling interval). Total volume sampled was 5.5 ml/rat.

2.2.2. Experiment 2

5 rats were exposed to constant light for 18–30 weeks, starting on arrival at the laboratory. Temperature and activity were monitored in all rats of each group for 3–12 weeks prior to melatonin administration. 3 days after jugular catheter implantation, the rats were housed in metabolism cages and received a 6-h infusion of control

solution (ethanol 0.25%-NaCl 0.9%) or melatonin (dissolved in 0.25% ethanol) from 22:00 to 04:00 h, followed by NaCl 0.9% infusion for 18 h, from 04:00 to 22:00 h. Each rat received the control solution or melatonin on 2 consecutive days in random sequence (melatonin first: 1 rat; control solution first: 4 rats). A programmable four-reservoir pump (IntelliJect, Aguettant, Lyon, France) insured the automatic delivery of melatonin, control solution or NaCl 0.9%. The daily melatonin dose was 1 mg/kg for 2 rats and 0.01 mg/kg for 3 rats and the total daily infusion volume was 10 ml. The plasma melatonin pattern was estimated from the time curve of its main urinary metabolite, 6-sulfatoxymelatonin. Urine samples were collected every 2 h for 4 days using a FRAC1000 collector (Pharmacia, France).

2.3. Melatonin

Melatonin was purchased from Sigma (St. Quentin Fallavier, France).

2.3.1. Experiment 1

Radioactive [^{3}H]melatonin (80 μl, 1 mCi/ml; 83 Ci/mmol; Amersham, UK) was added to a 10% ethanol solution of cold melatonin (2 mg/ml). A dose of 1 mg/kg was injected. The nature of the radioactivity in the biological samples was checked by chromatography (TLC; silicagel 60F-precoated plate; Merck-Clevenot, Nogent-sur-Marne, France) using chloroform, methanol and acetic acid (85/15/1). Melatonin, 6-hydroxy-melatonin, *N*-acetylserotonin and 5-methoxytryptamine were used as migration standards with respective R_f values of 0.64, 0.54, 0.47 and 0.08. The bands were scraped off and their radioactivity was quantitated by scintillation counting (Beckman LS1800).

2.3.2. Experiment 2

Melatonin was dissolved in a 0.25–0.5% ethanol solution. The urinary 6-sulfatoxymelatonin concentration was measured in duplicate using radioimmunoassay (Harthé et al., 1991).

2.4. Binding to rat plasma proteins

Protein binding of [^{3}H]melatonin was measured in pooled Sprague-Dawley rat plasma, obtained between 09:00 and 12:00 h from 8 rats kept in LD 12:12 and 8 rats exposed to continuous light. This pool contained ~ 74 g/l total proteins, including 573 μM serum albumin, and was stored at −25°C. [^{3}H]Melatonin binding was measured by equilibrium dialysis using a Dianorm apparatus. At equilibrium, the concentrations in each compartment of the dialysis cell were measured by liquid scintillation counting (Packard Tricarb 1600 TR). Total, free and bound molar concentrations of the drugs were determined and the binding parameters (N, concentration of binding sites) and K_a (affinity constant) were calculated as previously described (Zini et al., 1990).

2.5. Data analysis

Pharmacokinetic parameters were calculated using Micropharm software and were derived from a mean plasma-concentration curve over time following melatonin injection (Urien, 1995). The total area under the plasma-concentration vs. time curve (AUC or AUMC, area under mean curve) was calculated using the trapezoidal rule. Systemic clearance (Cl_s) was calculated as dose/AUC. Mean residence time (MRT) was calculated using $t_{1/2}$/ln2. The apparent volume of distribution at steady state (V_{ss}) was calculated from the relation: systemic clearance * mean residence time.

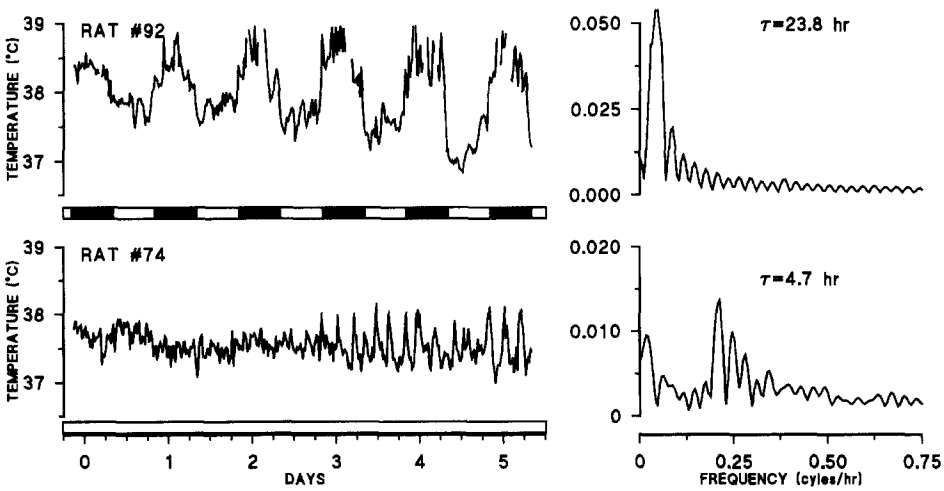

Fig. 1. Body temperature records of rats maintained either under a 12-h light 12-h dark schedule (upper left panel) or having had 14 weeks of continuous light exposure (LL, lower left panel). The figure displays 4 days of continuous monitoring with the corresponding power spectra in panels on the right.

Table 1
Values for rhythm parameters of body temperature and locomotor activity

		LD 12:12	LL
Temperature	τ (h)	24±0.03	5.7±0.5
	Mean (°C)	37.93±0.07	37.67±0.12
	Amplitude (°C)	0.66±0.06	0.13±0.01
Activity	τ (h)	23.8±0.18	5.7±0.5
	Mean (U)	24.6±5	21±7
	Amplitude (%)	64±11	43±2

Data are the mean±S.E.M. for 3 rats.

Urinary 6-sulfatoxymelatonin concentration (ng/ml) and excretion (ng/h) were plotted against time. The data were analysed with an analysis of variance and least-square cosine regression.

Temperature and activity data were analysed using Dataquest III software. 7-day time series from each rat were analyzed with 3 methods (Nelson et al., 1979; De Prins and Hecquet, 1991; Deprés-Brummer et al., 1996). 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. The period which corresponded to the highest percentage rhythm (highest amplitude) was considered as the dominant one, if $P < 0.001$. The values for mean circadian parameters (±S.E.M.) were computed from individual parameters.

3. Results

3.1. Experiment 1

3.1.1. Temperature and activity rhythms

The mean values for body temperature or locomotor activity were similar whether the rats were maintained in LD or in continuous light. Circadian rhythms were found for both variables in rats kept in LD 12:12, but not in those maintained under continuous light as was expected (Fig. 1, Table 1). No correlation was observed between light intensity and extent of circadian system suppression.

Table 2
Pharmacokinetic parameters, mean values analyzed with logarithmic trapezoids

	Total plasma		Melatonin-TLC	
	LD	LL	LD	LL
AUC (μg·min/ml)	3.1	3.3	1.2	2.7
Cl_s (ml/min/kg)	328	304	817	373
V_{ss} (l)	49.9	44.6	119	67
MRT (min)	152	147	145	178
$t_{1/2ss}$ (min)	106	102	100	124

Rats were maintained either under a 12-h light 12-h dark schedule (LD) or under continuous light (LL). AUC, area under the curve for plasma concentration vs. time; Cl_s, systemic clearance; V_{ss}, volume of distribution at steady state; MRT, mean residence time; $t_{1/2ss}$, elimination half-life at steady state; TLC, thin-layer chromatography.

3.1.2. Plasma pharmacokinetics

Melatonin clearance from blood showed an apparent classical biphasic pattern, corresponding to a rapid initial distribution phase, followed by an elimination phase in both LD and continuous light-maintained rats (Fig. 2). The values for pharmacokinetic parameters of mean total plasma radioactivity and mean radiolabeled melatonin obtained from TLC are shown in Table 2. No difference was apparent for total plasma radioactivity with different lighting conditions. However, the mean AUC was higher with continuous light than with LD 12:12 and clearance (Cl_s) and distribution volume (V_{ss}) of radiolabeled melatonin were ~halved with continuous light as compared to LD.

3.1.3. Protein binding

At concentrations ranging from 1 to 100 μM, [^{3}H]melatonin was weakly bound to rat plasma proteins:

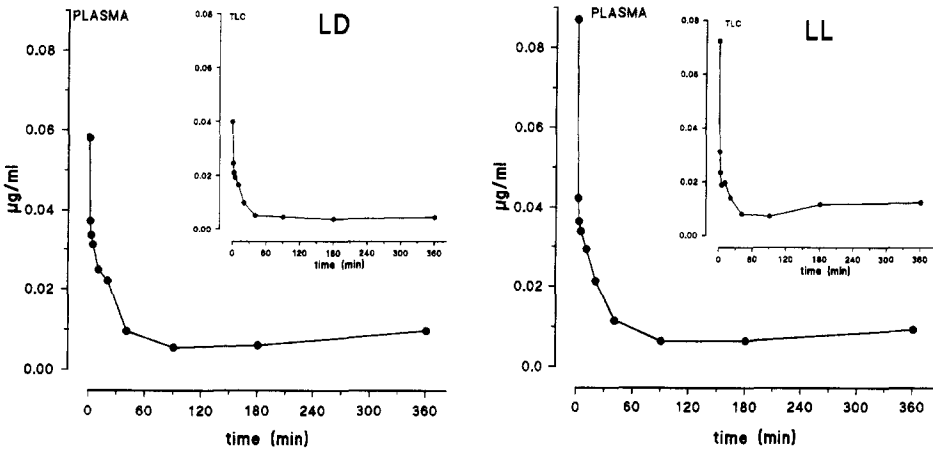

Fig. 2. Plasma concentrations of total radioactivity and radiolabeled (TLC) melatonin (mean ± S.E.M.) after an i.v. bolus injection of 1 mg/kg [^{3}H]melatonin between 09:00 and 12:00 h.

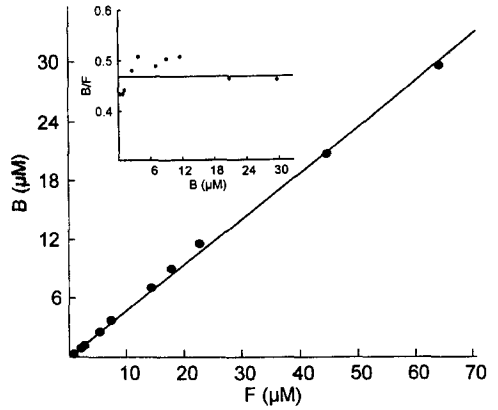

Fig. 3. Direct and Scatchard plots for [^{3}H]melatonin binding to rat serum. Free (F) and bound (B) molar concentrations of melatonin.

32.5 ± 0.4%. The binding percentage to rat serum albumin also remained similar over the same concentration range. The Scatchard plot suggested that [^{3}H]melatonin binding was non-saturable (NKa = 0.49 ± 0.02) and that rat serum albumin was the only protein responsible for this binding (Fig. 3). There was no difference in [^{3}H]melatonin binding to plasma proteins between LD- and continuous light-maintained rats (32.6 ± 0.5 and 32.0 ± 0.4%, respectively). [^{3}H]melatonin binding was also analyzed in plasma samples obtained 0.5, 180 and 360 min after its i.v. injection (Table 3). The binding percentage varied neither with time nor according to lighting schedule.

3.2. Experiment 2

3.2.1. Temperature and activity rhythms

Both body temperature and locomotor activity showed suppressed circadian rhythms and dominant ultradian rhythms with continuous light. 1 week prior to melatonin infusion the mean ± S.E.M. periods were 5.9 ± 0.7 h for body temperature and 4.2 ± 0.4 h for locomotor activity.

Table 3
Proportion of [^{3}H]melatonin bound to plasma proteins in samples obtained at various intervals following melatonin injection in rats maintained under LD 12:12 or continuous light (LL; mean ± S.E.M.)

Environment schedule	Rats (n)	Interval after [^{3}H]melatonin injection (min)		
		0.5	180	360
LD	7	36.9 ± 3	33 ± 8.4	34.1 ± 1.7
LL	6	33.4 ± 2.1	32.7 ± 3.8	36.7 ± 3.1

3.2.2. Urinary 6-sulfatoxymelatonin

Urine from 5 rats was collected prior to melatonin administration. The mean ± S.E.M. 6-sulfatoxymelatonin concentration and excretion rate were 27.2 ± 3 ng/ml and 6.3 ± 1 ng/h, respectively. There was no significant group variation with time. Individual least-square cosine regression analysis showed a significant circadian rhythm for 6-sulfatoxymelatonin excretion rate or concentration in 2 of 5 rats.

There was a dose-dependent difference for both 24-h mean concentration and excretion of 6-sulfatoxymelatonin (± S.E.M.): 11732 ± 4288 ng/ml and 1813 ± 678 ng/h in rats receiving 1 mg/kg/day and 295 ± 33 ng/ml and 33 ± 6 ng/h in rats receiving 0.01 mg/kg/day, respectively. A significant circadian rhythm for both variables, irrespective of dose level, was statistically validated, with analysis of variance and least-square cosine regression. The circadian acrophase of urinary 6-sulfatoxymelatonin was located near the end of melatonin delivery, at 5^{25} h ± 60 min (1 mg/kg) or at 5^{10} h ± 20 min (0.01 mg/kg). However, urinary 6-sulfatoxymelatonin concentration and excretion remained 2–13 times higher than the normal physiological values 18 h after the end of melatonin

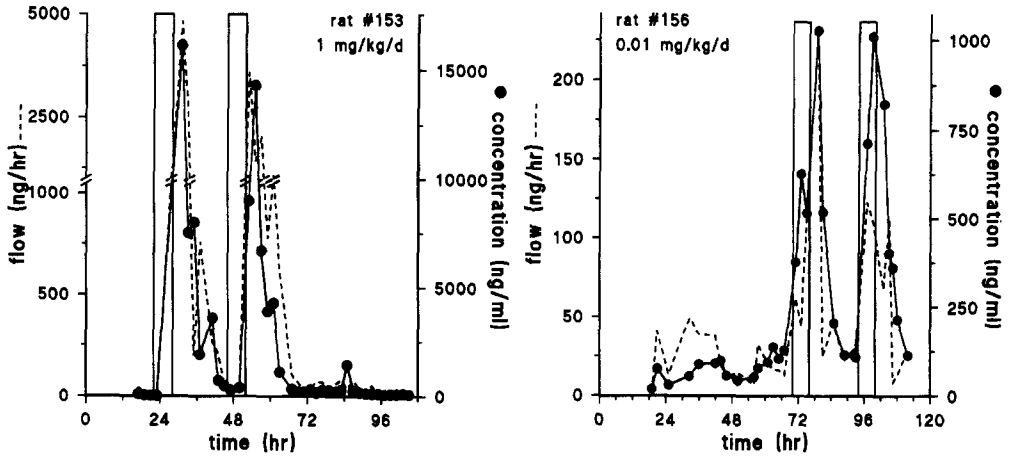

Fig. 4. Urinary 6-sulfatoxymelatonin (flow (- - -) and concentration (●)) before, during and after melatonin i.v. infusion between 22:00 and 04:00 h in 2 rats at 1 mg/kg/day (left panel) or 0.01 mg/kg/day (right panel).

infusion with a dose of 1 mg/kg/day. Conversely, 6-sulfatoxymelatonin concentration and excretion returned to their normal physiological low levels during the treatment-free interval in rats receiving 0.01 mg/kg/day of melatonin (Fig. 4).

4. Discussion

Prolonged constant light exposure induces suppression of the circadian rhythms of body temperature and locomotor activity while a plasma melatonin rhythm can still be detected (Deprés-Brummer et al., 1995a). Under these conditions, alteration of melatonin pharmacodynamics and or kinetics can be expected.

The fact that s.c. melatonin could not bring about clear synchronization of the activity rhythm in continuous light could be explained on the basis of the route of administration. The short elimination half-life of melatonin makes this drug a good candidate for i.v. administration. However, modulation of the infusion rate could be of importance, since continuous s.c. administration failed to synchronize disrupted activity rhythms (Cheung and McCormack, 1982).

In a first experiment, we compared the pharmacokinetics of an i.v. bolus injection of a pharmacologic dose of melatonin (1 mg/kg) in rats maintained under LD 12 : 12 (in the first half of the L phase) or constant light. The pharmacokinetics of melatonin in rats followed the usual two-compartment model with an initial distribution phase, followed by an elimination phase. Elimination half-lives were similar with LD and continuous light, ~ 100 min as calculated by the trapezoidal rule. The elimination half-life of melatonin has most frequently been reported, with the exception of Brown et al. (1985), to be ~ 30 min (Table 4). All studies, however, involved modelization (curve-peeling, two-compartment models, rotating iterative procedure or slope calculations) of the data in order to calculate half-lives. This could explain the higher value for the steady-state elimination half-life found in the present study ($t_{1/2ss}$ ~ 100 min). No difference in pharmacokinetic parameters of total plasma radioactivity was observed between rats maintained under LD and those under continuous light (Table 2). However, TLC allowed further quantitation of the radiolabeled melatonin concentration itself. Steady-state distribution volume and clearance of melatonin were ~ halved with continuous light as compared to LD. This, however, had few consequences for the melatonin elimination half-life, which remained near 100 min. Likewise, elimination half-life was similar in rats receiving melatonin at mid-light or at mid-dark, but distribution volume and metabolic clearance were significantly lower at mid-light than at mid-dark (Chan et al., 1984). Prolonged exposure to continuous light could alter the circadian coordination of melatonin metabolism and result in decreased clearance.

The plasma protein-binding study revealed that melatonin was weakly bound to plasma proteins, albumin being the only melatonin-binding protein. These results confirmed those reported earlier by Cardinali et al. (1972). This binding was non-saturable, indicating that no variation of the binding percentage (~ 33%) was likely to occur at various melatonin plasma concentrations. Furthermore, plasma protein binding of melatonin was similar in continuous light- and in LD 12 : 12-maintained animals.

In summary, this first experiment demonstrated that prolonged constant light exposure modified the distribution (reduced V_{ss}) and elimination (reduced Cl_s) of a bolus injection of 1 mg/kg melatonin without modifying its elimination half-life.

These pharmacokinetic data served to develop a 24-h intermittent delivery schedule consisting of a 6-h constant infusion of melatonin from 22:00 to 04:00 h, followed by an 18-h drug-free interval from 04:00 to 22:00 h. It was expected that the plasma level of melatonin would reach its peak near the end of the melatonin infusion, then would gradually decrease to reach < 1% of its peak concentration after 7 half-lives, ~ 12 h later. Melatonin concentration would remain at basal physiological levels for the 6 additional h of the treatment-free interval. Such a schedule was thus expected to mimic the circadian rhythm of plasma melatonin concentration, which usually follows an asymmetric pattern (sharp rise, slow decline). In order to avoid

Table 4
Biological half-lives previously reported

Route	Animal	CT/ Halo	Dosage	Animals (n)	t_α (min)	t_β (min)	$t\gamma$ (min)	Authors
S.c.	Hamster LD 14 : 10	3	RIA	7	17	1500		Brown et al. (1985)
I.a.	Sprague-Dawley rat	6	RIA	8		17		Gibbs and Vriend (1981)
			^{3}H			23		
I.v.	Ewe	5	RIA	4	3	12	24	English et al. (1987)
I.v.	Mice	?	^{3}H	8	2	35		Kopin et al. (1961)
I.v.	Sprague-Dawley rat	4	RIA	11	3	19		Chan et al. (1984)
			^{3}H		4	26		
		17	RIA		4	26		
I.v.	Sprague-Dawley rat	8	^{14}C	13	0.2	6	48	Vitte et al. (1988)

catheter obstruction, NaCl 0.9% was infused during the 18-h melatonin-free interval from 04:00 to 22:00 h.

The effectiveness of this melatonin infusion schedule to yield the desired rhythm pattern of plasma melatonin concentration was assessed in unrestrained rats. Urinary 6-sulfatoxymelatonin, the major metabolite of melatonin, was monitored every 2 h for 4 days, therefore, allowing an acceptable definition of the circadian profile. Two daily dose levels were tested (1 and 0.01 mg/kg). While both resulted in a circadian pattern in 6-sulfatoxymelatonin excretion, only the lower dose allowed a return to physiologic low levels ~ 12 h after the end of each 6-h melatonin delivery span (Fig. 4). Indeed, in a previous experiment, 24-h sinusoidal administration of melatonin (1 mg/kg/day) did not yield such physiologic low levels at trough delivery. Instead, melatonin accumulation was suggested to occur, probably due to the lack of an infusion-free interval (P. Deprés-Brummer, unpublished observations). The absence of a synchronizing effect of this schedule on suppressed temperature and activity rhythms could be related to an inadequate administration schedule.

Artificial induction of a urinary melatonin rhythm was reported earlier from studies with programmed s.c. microinfusion in pinealectomized Sprague-Dawley rats (Lynch et al., 1980). However, administration by means of an external device is more precise and i.v. administration achieves more reliable drug exposure than does the s.c. route.

Further studies with this schedule of melatonin infusion are ongoing in continuous light-maintained rats that show functional suppression of their circadian system. The results may guide attempts to restore by pharmacological mean rhythms in patients with advanced cancer, in whom suppressed circadian rhythms have been observed (Bailleul et al., 1986; Bénavides, 1991; Touitou et al., 1995).

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