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Entrainment of Circadian Rhythms by S-20098, a Melatonin Agonist, Is Dose and Plasma Concentration Dependent

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MARTINET, L., B. GUARDIOLA-LEMAITRE AND E. MOCAER. *Entrainment of circadian rhythms by S-20098, a melatonin agonist, is dose and plasma concentration dependent.* PHARMACOL BIOCHEM BEHAV **54**(4) 713–718, 1996.— The present study determined first the dose–response (0.5 to 10 mg·kg^{-1}) to daily oral administration of S-20098, a melatonin agonist, in entraining circadian rhythms of rats free-running in constant darkness; second, the relation between entrainment and the plasma concentration of S-20098. Finally, responses to 8 mg·kg^{-1} of S-20098 were compared with those obtained with the same dose of melatonin and ipsapirone. Responses were classified as negative, transient, or true entrainment. The data indicated a clear dose-dependent response from 2.5 to 10 mg·kg^{-1} of S-20098 with an ED_{50} of 5.7 mg·kg^{-1} for true entrainment and a clear relation between entrainment and the plasma concentration of S-20098. S-20098 was as effective as melatonin to entrain free-running rhythms. Ipsapirone was ineffective in our experimental conditions.

Circadian rhythm Entrainment Dose-response S-20098 Melatonin Ipsapirone Rat

THE light–dark cycle is the major synchroniser or Zeitgeber entraining circadian rhythms. In mammals, melatonin, exclusively produced during the night by the pineal gland, has been proposed to act as an internal Zeitgeber by synchronizing multiple circadian rhythms (2). Since the first observations on the ability of melatonin in entraining free-running rhythms in rats (16), a parallel potential in human has been suspected opening up the possibility for using melatonin as therapeutic agent. In fact, circadian rhythm disorders in human, mostly sleep disorders, related to transmeridional air travel, shift work, blindness, or aging are well documented. The description of a phase response curve to melatonin, in opposite phase with that to light (11), suggests that phase delay and phase advance disorders may be treated by properly timed melatonin administration (1). Thus, the considerable interest in the physiological role of melatonin in regulating human circadian rhythms led to the synthesis of chronobiotic molecules by pharmacological Industry.

Among several synthetized melatonin naphtalenic bioisosters, the S-20098 analog, N[2-(>-methoxy-naphet-I-yl)ethyl]acetamid, a potent melatonin receptor agonist (22), has been shown to resynchronize rest–activity rhythms in free-running rats (4) and to shift the onset of activity in the negative phase angle paradigm (3) or after an 8-h phase advance (17).

To be used as a therapeutic agent for disorders of the human circadian system, the administration way of any molecule and its life duration in the circulation have to be taken into consideration. So, the main purpose of the present study was to determine the dose–response to daily oral administration of S-20098 and the plasma efficacious concentrations in entraining rats free-running in constant darkness. Furthermore, as the selective 5-HT_{1A} agonists 8-0H-DPAT and ipsapirone have been shown to phase shift wheel-running activity of rats and hamsters in constant darkness (7,9), the effect of oral administration of S-20098 and melatonin was compared to that of ipsapirone.

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METHOD

Animals

One-month-old male Long–Evans rats were purchased from a commercial supplier (CERJ, Le Genest sur Ile, France) and kept under a daily cycle of 12 L:12 D (LD 12:12). Temperature ranged from 20 to 23°C. Food pellets and water were continuously available.

Activity Recording

When 8-week-old rats were transferred into a light-tight, sound-attenuating room and maintained in individual cages equipped with a running wheel. Light intensity during the light phase was 50 $\mu w/cm^2$; red bulbs (6 $\mu w/cm^2$) during the dark phase allowed entrance in the room and animal care. Wheel-running activity was monitored using a chart recorder that reset each 24 h, so that consecutive days were plotted on a vertical axis.

Experimental Protocol

Activity was recorded under LD 12:12 for 2–3 weeks, then under constant darkness (DD) until stable free-running rhythms were established. In less than 5% of the rats, the free-running periods were shorter than 24 h; these rats were discarded. The remaining animals were handled every day at the same time for 7–10 days, then given daily oral administration within the oesophage through a probang (16 × 3″; Ealing, Les Ulis, France) of either S-20098 (6 doses: 0.5, 1, 2.5, 5, 8, and 10 $mg \cdot kg^{-1}$) or melatonin (Sigma, St. Louis, MO; 8 $mg \cdot kg^{-1}$) or ipsapirone (Bayer; 8 $mg \cdot kg^{-1}$) or vehicle only (hydroxiethylcellulose 1% in H_2O_2) for 16 to 21 days. As the results were similar when the same dose was tested in more than one series (vehicle; 1.0, 2.5, 5.0, and 10.0 $mg \cdot kg^{-1}$ S-20098), they were brought together.

Melatonin (3) and S-20098 (4,17) daily injections are effective in entraining rat free-running rhythms during a narrow time window, that is, at activity onset. So, in our protocol the first administration of the drugs was about 2 hrs after activity onset to obtain the coincidence between the time of administration and activity onset in a few days.

After treatment was discontinued, activity was recorded for a further 10 to 15 days except in a subset of rats in which 1 ml blood samples were collected under ether anesthesia from the jugular vein into heparinized tubes just before and 30, 120, and 240 min after the last administration. Blood samples were centrifuged (3500 tr/min, at 4°C for 10 min) and plasma stored below −18°C.

Activity Analysis

Activity charts were duplicated and double mounted to facilitate the visual inspection of rhythms over a 48-h time span. Free running was evidenced by eye-fitting a straight line through consecutive daily activity onsets. Activity onset, defined as the first bout of activity longer than 10 min, was used as a marker for determining alteration of the free-running rhythm by drugs.

Responses to the treatment were classified as negative or positive. Responses were negative when the eye-fitting straight line drawn from the daily activity onsets was not interrupted and its slope not modified. When positive, responses were divided as transient effect or true entrainment. An analysis of covariance was carried out with the General Linear model (SAS/STAT Guide for personnal computers, SAS Institute Inc., 1987) to test the difference between the slopes and the intercepts of the regression lines before and after the presumed period of effect. Entrainment of the activity onset component was effective when the intercepts but not the slopes were statistically different. In rats blood-sampled and sacrificed after the last administration, extrapolation was done from the slope of the free-running phase preceding the treatment; in fact, in the other rats the slopes before and after treatment were significantly different in only 10% of the population. The percentages of responses between doses and drugs were compared with the χ^2 test.

HPLC Assay of S-20098 in Plasma and Pharmacokinetic Analysis

The unchanged drug was measured in plasma samples according to the analytical method developed at Technologie Servier. One milliliter or 250 µl for the low and high concentration range, respectively, was spiked with the internal standard S-20242. Samples were alkalinized with 200 µl of a buffer, pH 13, (NaOH 0.2 M, KCl 0.2 M). A liquid–liquid extraction was performed with 5 ml diethylether-cyclohexane (60/40 v/v). Extracts were taken to dryness, reconstituted in the mobile phase (acetonitrile and potassium dihydrogen phosphate, between 40–60 and 25–75 v/v, added to 2% heptane sulfonic acid, v/v). The chromatographic system consisted of an intelligent pump L6200 A (Merck), a model WISP 710 automatic sample injector (Waters), a fluorescence detector model LC240 (Perkin-Elmer), and data acquisition Vax Mulichrom computer system (VG Laboratory systems, UK). The liquid chromatographic separation was performed on a reversed-phase column (Spherisorb C18, 250 × 4.6 mm i.d., particle size 5 µM, Interchim). The fluorescence detector was used with the excitation and emission wavelength set at 277 and 356 nm, respectively.

Recovery after extraction was 88 and 91% for S-20098 and the internal standard S-20242, respectively, and did not depend on the plasma concentration of the drug. The limit of quantitation was 0.05 $ng \cdot ml^{-1}$. The calibration curves were linear from 0.05 to 10 and 5 to 1000 $ng \cdot ml^{-1}$, respectively. Accuracy ranged from 1 to 6%. Intra- and interassay coefficient of variation ranged from 1 to 4 and 5 to 7%. Assays were performed without knowing activity recording results.

ED_{50}

The ED_{50} was calculated from the 6 doses by probit analysis (10) with linear regression giving values of 4.1 and 5.7 $mg \cdot kg^{-1}$ PO.

Area Under the Curve

The AUCt of plasma concentration time from t0 to t240 min were calculated according to the log-linear trapezoidal rule. When plasma concentrations are rising or static:

$$AUCt_1 - t_2 = (t_2 - t_1) - \left(\frac{C_1 + C_2}{2}\right)$$

When plasma concentrations are decreasing:

$$AUCt_1 - t_2 = (t_2 - t_1) - \left(\frac{C_1 + C_2}{\ln C_1 - \ln C_2}\right)$$

with $AUCt_1 - t_2$ representing the fractional AUC calculated from t_1 to t_2. $AUCt$ was calculated as the sum of fractional area.

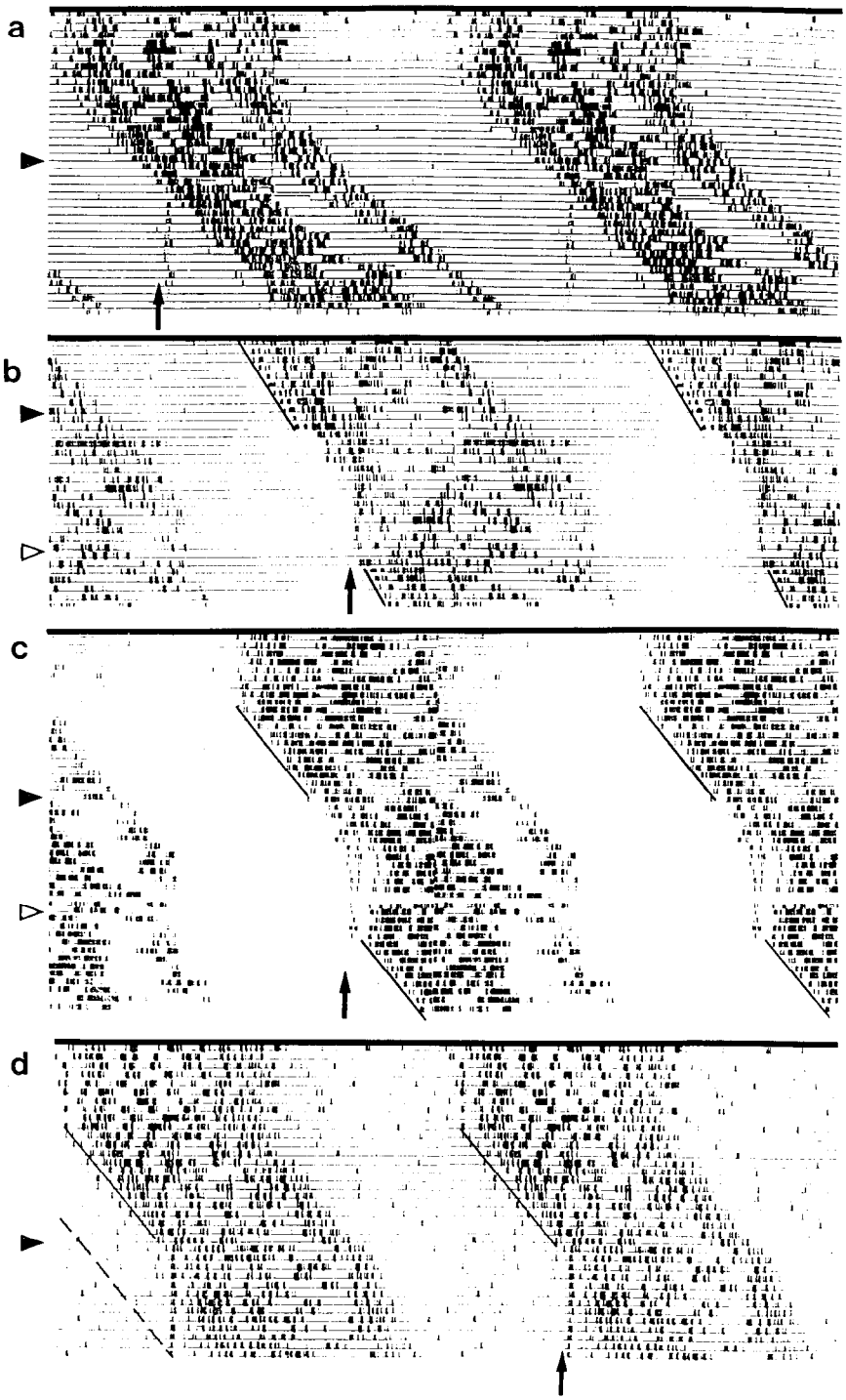

FIG. 1. Actograms of four representative rats free running in DD (horizontal black bars at the top of each panel). Successive days are double-plotted from top to bottom. Daily time of drug administration is indicated by the vertical arrow. Treatment started the day indicated by the black triangle and lasted until the day indicated by the open triangle. Plain diagonal bars indicate the free-running slopes and the dashed one that was extrapolated from the slope preceding the treatment and used for statistical analysis. Male a is representative of animals in which activity was not altered by the treatment. In male b, the drug had a transient effect, altering the activity rhythm without entrainment. True entrainment was observed on the actograms of male c and d.

TABLE 1

EFFECTS OF INCREASING DOSES OF S-20098 ON FREE-RUNNING RHYTHMS OF ACTIVITY; COMPARISON WITH MELATONIN AND IPSAPIRONE

Treatment mg/kg	n	Positive Response		True Entrainment	
		n	%	n	%
Vehicle	12	1	8%	0	0%
S-20098					
0.5	8	1	12%	0	0%
1.0	15	1	7%	0	0%
2.5–3.0	16	6	37%*	3	19%*
5.0	18	12	67%†	6	33%†
8.0	7	6	86%†	5	71%†
10.0	15	14	93%†	13	87%†
Melatonin					
8.0	7	7	100%†	5	71%†
Ipsapirone					
8.0	8	3	37%	1	14%

*$p < 0.05$, †$p < 0.01$ compared to vehicle-treated rats.

RESULTS

As the endogenous period of all the rats used in the present study was greater than 24 h, entrainment was due to small daily phase shift advances, activity onset being phase locked to treatment time. None of the rats displayed anticipatory activity before the time of the daily treatment. Most of them showed a bout of wheel running just after being returned to their cage (Fig. 1).

Dose-Dependent Effect

Daily handling and oral administration of the vehicle did not induce any change in the free-running rhythm, except in 1 male out of 12, which displayed a transient response for 5 successive days. Daily administration of 0.5 and 1.0 mg·kg^{-1} S-20098 were also ineffective in altering free-running rhythms. The threshold of sensitivity was between 1 and 2.5 mg·kg^{-1} because the percentage of positive responses to 2.5–3.0 mg·kg^{-1} was significantly higher than that observed with lower doses or vehicle only. A dose-dependent effect was observed from 2.5 to 10 mg·kg^{-1} (Table 1). The ED_{50} for a positive response and for a true entrainment were 4.1 and 5.7 mg·kg^{-1}, respectively.

The incidence of rats entrained by 8 mg·kg^{-1} of either S-20098 or melatonin was identical. In contrast, the free-running rhythms were not entrained by the same dose of ipsapirone (Table 1).

Concentration-Dependent Effect

Entrainment was clearly related to the plasma concentration of S-20098 (Table 2) and the area under the curve (Fig. 2). Taking in account individual data, the threshold of maximal concentrations and areas under the curve inducing true entrainment were 41 ng·ml^{-1} and 40 ng·ml·h^{-1}. The animal that was not entrained by a dose of 5 mg·kg^{-1} showed a low C_{max} and AUC (Fig. 2, upper panel); inversely, two rats entrained by 2.5 mg·kg^{-1} (Fig. 2, middle panel) had a low concentration and an area under the curve similar to those of rats entrained by 5 mg·kg^{-1} (Fig. 2, lower panel).

DISCUSSION

The potential use of the melatonin receptor agonist, S-20098, to counteract circadian rhythm disturbances in humans is supported by the present study. In fact, the entrainment of circadian rhythms in free-running rats by a daily oral administration of S-20098 was identical to that obtained previously by peripheral injections of melatonin or S-20098 (4). Whether given IM (4,17) or orally (this study), the same order of potency was observed with both melatonin and S-20098.

The dose-dependent effect observed with peripheral melatonin injections (4,5) was also found with oral doses of S-20098 between 2.5 and 8 mg·kg^{-1}, followed by a plateau from 8 to 10 mg·kg^{-1}. It is worth noting that interseries variation in the incidence of entrainment was less than 10%, at least with doses of 2.5, 5.0, and 10.0 mg·kg^{-1}.

The most striking result was the positive relation between plasma concentrations of S-20098, 30 min after administration and its efficacy in entraining free-running rhythms; a similar relation was found between entrainment and the area under the curve. Such a relation has never been reported for melatonin, although the concentrations of plasma melatonin following SC injections of 1 μg and 1 mg·kg^{-1} melatonin have been measured and half lifes calculated (5).

The close parallelism between melatonin and S-20098 ability to resynchronize circadian rhythms in rats likely comes from their direct action on the circadian clock located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (6) through high affinity melatonin receptors present in human (20) as well as in rat SCN (21). S-20098, which binds to these receptors (4), decreases the firing rate of SCN neurons in vitro (13) and in vivo (Mason, personal communication) as does melatonin (14). In vitro, S-20098 induces a phase advance of

TABLE 2

CONCENTRATION OF S-20098 (NG/ML) IN THE PLASMA OF RATS 30 MIN AFTER ORAL ADMINISTRATION OF S-20098 INCREASING DOSES (mean ± s.d.)

Dose	No Effect			Transient Effect			Entrainment		
	30 min	2 h	4 h	30 min	2 h	4 h	30 min	2 h	4 h
2.5	26 ± 9	1.0 ± 0.2	0.7 ± 0.6	14	5.2	1.6	?	41*	6.6*
				n=1	n=1	n=1	60*	1.1*	0.6*
5.0	17	6.5	0.5	92	4.7	0.3	77 ± 8.2	2.6 ± 1.3	0.5 ± 0.2
	n=1	n=1	n=1	n=1	n=1	n=1			
10.0	—	—	—	71	7.3	2.2	258 ± 105	4.1 ± 2.6	1.0 ± 0.6
				n=1	n=1	n=1			

*Individual data.

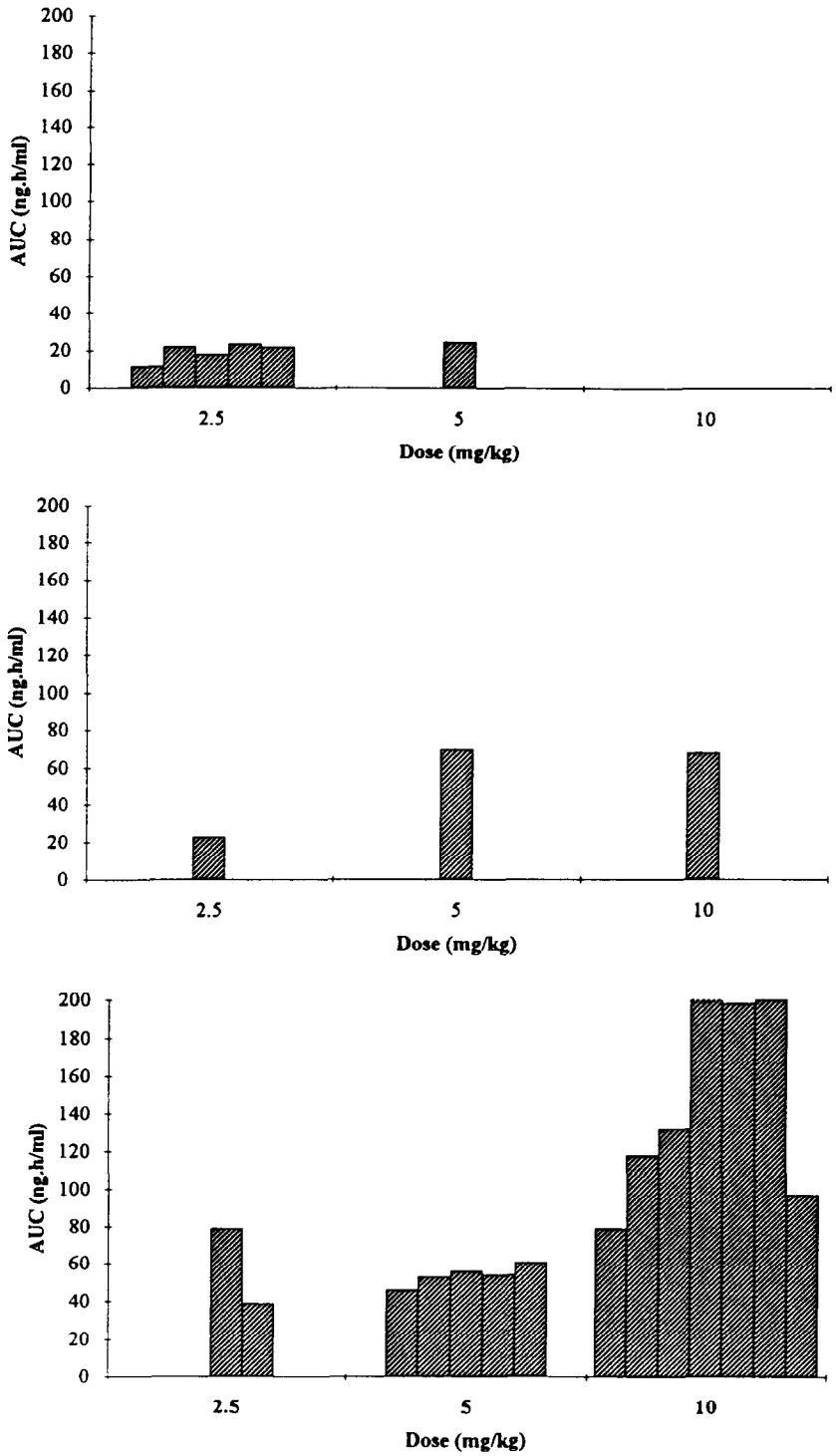

FIG. 2. Variation of the area under the curve (AUC) related to the dose of S-20098 in animals showing no entrainment (upper panel), transient (middle panel) and true (lower panel) entrainment.

the firing rate between CTO8 and CT12 similar to that observed in vivo in mice and hamsters (19). Furthermore, SCN-lesioned rats did not entrained anymore to daily injections of 1 and 10 mg·kg^{-1} S-20098 as do intact controls (18) showing that S-20098 acts on melatonin receptors within SCN.

It seems that besides the melatonin periodic signal feeding back upon the SCN, neurotransmitters, namely serotonin, can modulate the circadian clock activity rhythm (12,15). In our study, rats given ipsapirone orally between CT12 and CT14, at the onset of their subjective night, did not entrain. 5-HT$_{1A}$

agonists might induce phase shifts only when given during the subjective day (7,9). Furthermore, 8 $mg \cdot kg^{-1}$ given per os may be not sufficient when compared to the 5 $mg \cdot kg^{-1}$ delivered intraperitoneally (7).

The practical implication of S-20098 to resynchronize circadian rhythms (4,8,17) by resetting the circadian clock (13) seems as widespread as melatonin. The demonstration of its efficacy when administered orally supports its therapeutic usefulness as a chronobiotic to alleviate circadian rhythm disorders in humans.

REFERENCES

1. Arendt, J. Clinical perspectives for melatonin and its agonists. Biol. Psychiatry 35:1–2; 1994.
2. Armstrong, S. M. Melatonin: A link between the environment and behavior. Integr. Psychiatry 5:19–21; 1987.
3. Armstrong, S. M.; McNulty, O. M.; Guardiola-Lemaitre, B.; Redman, J. R. Successful use of S20098 and melatonin in an animal model of delayed sleep-phase syndrome (DSPS). Pharmacol. Biochem. Behav. 46:45–49; 1993.
4. Bonnefond, C.; Martinet, L.; Lesieur, D.; Adam, G.; Guardiola-Lemaitre, B. Characterization of S-20098, a new melatonin agonist. In: Touitou, Y.; Arendt, J.; Pevet, P., eds. Melatonin and the pineal gland. From basic science to clinical application. New York: Elsevier Science; 1993:123–126.
5. Cassone, V. M.; Chesworth, M. J.; Armstrong, S. M. Dose-dependent entrainment of rat circadian rhythms by daily injection of melatonin. J. Biol. Rhythms 1:219–229; 1986.
6. Cassone, V. M.; Chesworth, M. J.; Armstrong, S. M. Entrainment of rat circadian rhythms by daily injection of melatonin depends upon the hypothalamic suprachiasmatic nuclei. Physiol. Behav. 36:1111–1121; 1986.
7. Cutrera, R. A.; Ouarour, A.; Pévet, P. Effects of the 5-HT_{1a} receptor agonist 8-OH-DPAT and other nonphotic stimuli on the circadian rhythm of wheel-running activity in hamsters under different constant conditions. Neurosci. Lett. 172:27–30; 1994.
8. Defrance, R.; Mocaër, E. The role of melatoninergic agonists in the clinic. Proc. XIIIth Int. Symp. Med. Chem.; 1994.
9. Edgar, D. M.; Miller, J. D.; Prosser, R. A.; Dean, R. R.; Dement, W. C. Serotonin and the mammalian circadian system: II. Phase-shifting rat behavioral rhythms with serotonergic agonists. J. Biol. Rhythms 8:17–31; 1993.
10. Finney, D. J. The probit procedure. In: SAS/STAT user guide. vol. 2, 4th edition. Cary, NC: SAS Institute; 1990:1325–1335.
11. Lewy, A. J.; Ahmed, S.; Latham Jackson, J. M.; Sack, R. L. Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiol. Int. 9:380–392; 1992.
12. Mason, R. Circadian variation in sensitivity of suprachiasmatic and lateral geniculate neurons to 5-hydroxytryptamine in the rat. J. Physiol. 377:1–13; 1986.
13. Mason, R.; Delagrange, P.; Guardiola, B. Melatonin and S-20098 mediated resetting of rat suprachiasmatic nuclei clock in vitro. Br. J. Pharmacol. Proc. Suppl. 108:32P; 1993.
14. McArthur, A. J.; Gillette, M. U.; Prosser, R. A. Melatonin directly resets the rat suprachiasmatic circadian clock in vitro. Brain Res. 565:158–161; 1991.
15. Prosser, R. A.; Miller, J. D.; Heller, C. A serotonin agonist phase-shifts the circadian clock in the suprachiasmatic nuclei in vitro. Brain Res. 534:336–339; 1990.
16. Redman, J. R.; Armstrong, S. M.; Ng, K. T. Free-running activity rhythms in the rat: Entrainment by melatonin. Science 219:1081–1091; 1983.
17. Redman, J. R.; Guardiola-Lemaitre, B. The melatonin agonist, S-20098: Effects on the rat circadian system. In: Touitou, Y.; Arendt, J.; Pevet, P.; eds. Melatonin and the pineal gland. From basic science to clinical application. New York: Elsevier Science; 1993:127–130.
18. Redman, J. R.; Andrew, F.; Smith, A. Possible sites of action of the melatonin agonist S-20098. Neuropsychopharmacology 10:173S; 1994.
19. Van Reeth, O. S-20098, a melatonin agonist, phase-shifts the circadian clock of mice and Syrian hamsters according to a phase-response-curve. Soc. Neurosci. Abstr. 20:1440; 1994.
20. Weaver, D. R.; Stehle, J. H.; Stopa, E. G.; Reppert, S. M. Melatonin receptors in human hypothalamus and pituitary: Implications for circadian and reproductive responses to melatonin. J. Clin. Endocrinol. Metab. 76:295–301; 1993.
21. Weaver, D. R.; Rivkees, S. A.; Reppert, S. M. Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. J. Neurosci. 9:2581–2590; 1989.
22. Yous, S.; Andrieux, J.; Howell, H. E.; Morgan, P. J.; Renard, P.; Pfeiffer, B.; Lesieur, D.; Guardiola-Lemaitre, B. Novel napthalemic ligands with high affinity for the melatonin receptor. J. Med. Chem. 35:1484–1486; 1992.