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Successful Use of S20098 and Melatonin in an Animal Model of Delayed Sleep-Phase Syndrome (DSPS)

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ARMSTRONG, S. M., O. M. McNULTY, B. GUARDIOLA-LEMAITRE AND J. R. REDMAN. *Successful use of S20098 and melatonin in an animal model of delayed sleep-phase syndrome (DSPS).* PHARMACOL BIOCHEM BEHAV **46**(1) 45–49, 1993. — In human delayed sleep-phase syndrome (DSPS), sleep onset and wake times occur far later than normal. In the population, DSPS may be an important contributor to complaints of sleep onset insomnia. We previously reported an animal model of DSPS in laboratory rats in which the onset of nocturnal activity is delayed by several hours [negative phase angle difference (PAD)]. The effect of melatonin 1 mg/kg SC and S20098 (Servier) 1 and 3 mg/kg on the negative PAD was investigated over 22 days of injections. In comparison to control injections of dimethylsulfoxide (DMSO), both melatonin and S20098 over approximately 9 days phase advanced the onset of activity toward the onset of darkness. At cessation of injections, activity onset delayed over approximately 11 days back toward, but as a group did not reach the original PAD. This effect of melatonin on the phase angle of entrained rats is consistent with its effects on delayed sleep in humans. It is likely, therefore, that S20098 may be of use to ameliorate DSPS in humans

Sleep disorders Melatonin Pineal gland S20098 Blind humans Circadian rhythmicity Rats

THE recent expansion of investigations into the effects of exogenous melatonin on the sleep-wake cycle has been prompted by the potential for its use in ameliorating human sleep disorders of the circadian type. These include jetlag, shiftwork, delayed sleep-phase syndrome (DSPS), advanced sleep-phase syndrome (ASPS), non-24-h sleep-wake cycles, and irregular sleep-wake patterns (30). In dyssomnias of the circadian type, the sleep mechanisms per se are functionally intact but there is a temporal misalignment between the timing of the sleep-wake cycle and that of the norm for society.

Of particular interest to the present investigators was the potential use of melatonin and its analogs and agonists for phase advancing sleep onset in DSPS as well as stabilising the sleep pattern of certain blind subjects (7). In DSPS, sleep onset occurs much later than is normal (e.g., 0300 h or later) (30) and a similar lag occurs in some blind subjects although others have irregular sleep onsets and even free run (18). While the use of timed bright light has been advocated in DSPS (11,13), this procedure cannot be used in the blind and may be inconvenient to use in the sighted. Therefore, the search for a pharmaceutical preparation capable of stabilising a misaligned human circadian pacemaker is warranted (7).

Since the first report that exogenous melatonin administration could advance the circadian rhythm of rat locomotor activity (9) and in humans increase evening fatigue in a manner consistent with an advance in the sleep-wake cycle (4,5), there have been a number of investigations that confirmed and extended on these findings in animals (23) and both blind and sighted humans (19,24,25,32). The phase advance of the sleep-wake cycle induced by oral melatonin ingestion has been confirmed in simple case studies of blind subjects with DSPS (27,31) and in eight sighted DSPS subjects in a 4-week double-blind crossover trial (12).

In all the studies cited above and others (3,14,22), the exact timing of melatonin administration for optimising the advance of sleep onset is crucial but can be deduced from the published phase-response curve to melatonin for humans (17). In this context, the onset of sleep is not itself a reliable marker for estimating circadian phase, so ideally the pattern of the endogenous melatonin rhythms needs to be established (16).

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In the present experiment, timed administration of S20098 was compared with melatonin for phase advancing the running-wheel locomotor rhythm of laboratory rats. S20098 is a naphthalenic bioisostere of melatonin selected for behavioural studies from a group of 15 such analogs for its high affinity and high specificity, binding to melatonin receptor sites in the sheep pars tuberalis with an affinity of $K_d = 8.1 \times 10^{-11}$ (15,33). Such studies of melatonin analogs help clarify the nature of the structure-binding relationship and mode of interaction at the melatonin receptor in addition to providing a new tool for examining the physiological functions of melatonin. The animal model for DSPS was developed in this laboratory in the early 1980s. Rats are maintained in constant darkness (DD) for several months and allowed to free run. On

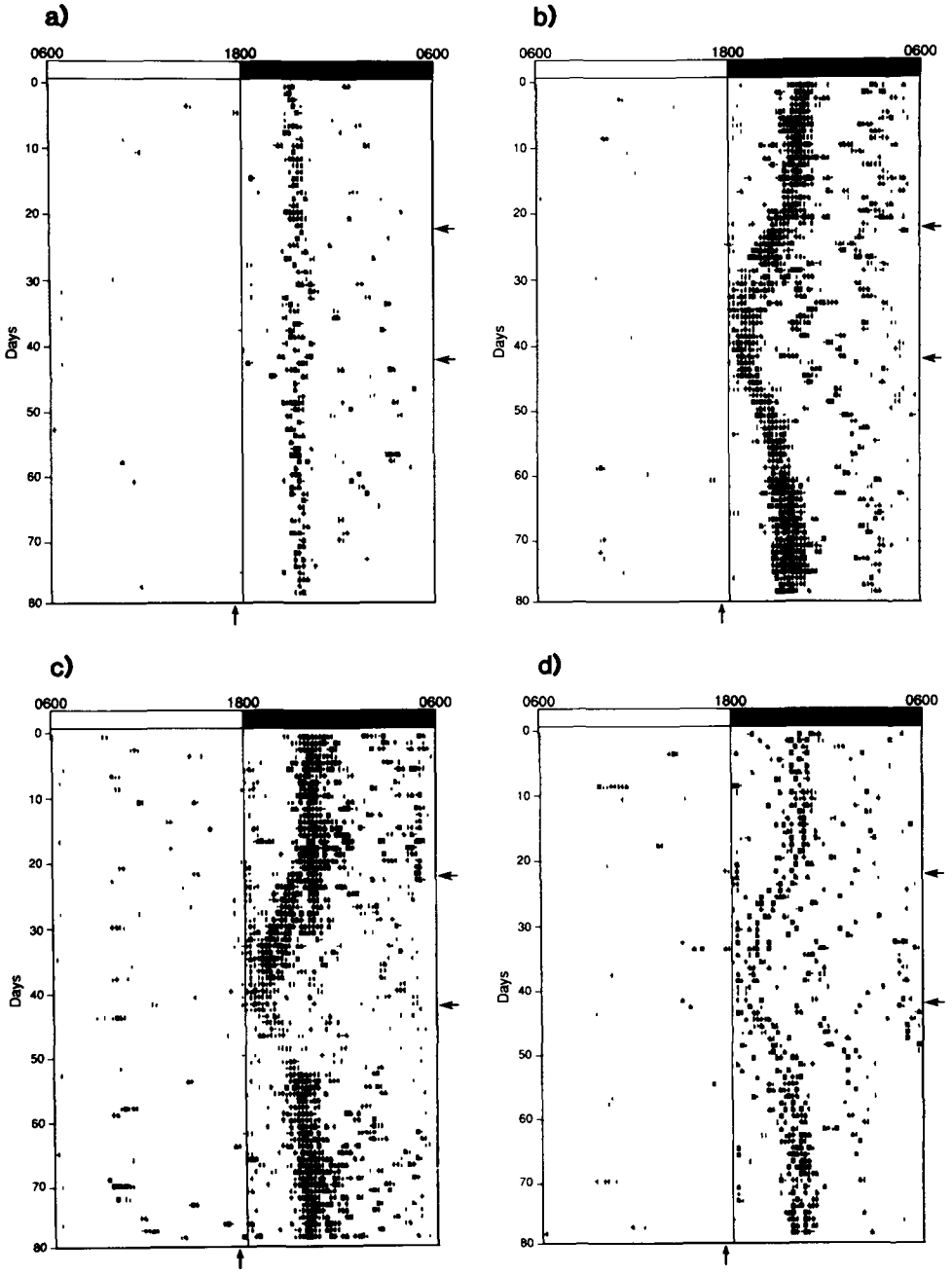

FIG. 1. Actograms of four rats treated with (a) 50% dimethylsulfoxide (DMSO), (b) 1 mg/kg melatonin in 50% DMSO, (c) 1 mg/kg S20098 in 50% DMSO, and (d) 3 mg/kg S20098 in 50% DMSO. Note the marked reduction in negative phase angle difference (PAD) resulting from melatonin and S20098 injections compared to the lack of effect of the control DMSO-treated rat. Rats were injected in the last 30 min of the light phase (indicated by vertical arrow below actogram) for 22 days (indicated by horizontal arrows on right-hand side of actogram).

return to a conventional 12 L : 12 D cycle, the onset of activity lags behind the onset of darkness by 3–4 h. This negative phase angle difference (PAD) can be eliminated by daily injections of melatonin given just prior to dark onset for approximately 1 week (8,9); the onset of α (the active phase) phase advances until it reaches dark onset and can be held at this phase by continuing daily injections. The shifting of α in a nocturnally active species such as rats is the equivalent to the shifting of the inactive phase in diurnally active species such as humans. Therefore, this rat model is an excellent one for testing pharmaceutical compounds likely to be effective in counteracting human DSPS.

METHOD

Subjects

Twenty-four male outbred strain Long-Evans hooded rats (350–430 g) obtained from Monash University Central animal house were individually housed in wire cages arranged in four rows of six cages per row under a 12 L : 12 D cycle. Food and water were available ad lib. Wheel-running activity was sampled every 15 min by a computer situated in an adjacent room. To facilitate injection during the dark period, one 15-W Osram red light, 0.1–0.4 lux depending upon cage position, remained on constantly. The light was positioned so as not to shine directly at rats. Cage positions of S20098-injected, melatonin-injected, and control-injected rats were randomly assigned, thereby avoiding any systematic variation in illumination between animals.

Injection Solutions

S20098 is not soluble in either water or 1% ethanol (the usual vehicle for melatonin). Therefore, dimethylsulphoxide (DMSO) 50% in water was used as the vehicle for both melatonin and S20098 and was used alone as the control solution.

Experimental Procedure

Rats were held for 3 months in DD with a dim red light (< 1 lux) and then in a 12 L : 12 D cycle and those animals that exhibited a large, negative PAD were selected to take part in the experiment. After at least 23 days of an LD baseline to ensure that the large PADs were stable, rats were injected daily, 0.5 h before dark onset, with one of the following solu-

TABLE 1

CHANGES IN NEGATIVE PAD FOLLOWING ADMINISTRATION AND CESSATION OF INJECTIONS OF MELATONIN, S20098, AND DMSO

Treatment	Animal	Preinjection PAD (min)	Injection PAD (min)	Latency to Steady State (days)	Postinjection PAD (min)	Latency to Steady State (days)
DMSO	1	186	186	0	163	9
	9	233	256	2	210	10
	17	204	204	0	204	0
	22	163	157	1	169	0
	24	198	198	0	198	0
	29	166	166	0	166	0
Mean ± SD		191.7 ± 5.1	194.5 ± 5.9	0.5 ± 0.9	185.0 ± 4.6	3.2 ± 2.2
1 mg melatonin	5	215	41	8	198	9
	7	186	47	8	175	10
	11	227	0	9	186	10
	15	216	41	8	222	12
	23	221	0	11	178	8
	26	181	6	8	145	13
Mean ± SD		207.7 ± 4.4	22.5 ± 4.8	8.7 ± 1.1	184.0 ± 5 1	10.3 ± 1.4
1 mg S20098	10	244	25	14	250	19
	13	251	0	8	239	13
	16	198	0	11	181	16
	19	198	56	7	157	9
	20	274	99	10	216	5
	25	198	0	12	192	12
Mean ± SD		227.2 ± 5.8	30.0 ± 6.4	10.3 ± 1.6	205.8 ± 6.0	12.3 ± 2.2
3 mg S20098	2	157	26	9	169	12
	4	212	12	7	186	9
	6	233	70	10	192	9
	12	181	0	8	157	11
	21	222	58	9	181	10
	29	204	35	9	210	10
Mean ± SD		201.5 ± 5.3	33.5 ± 5.2	8.7 ± 1 0	182.5 ± 4.3	10.2 ± 1.1

tions: a) S20098 1 mg/kg in 50% DMSO (n = 6); b) S20098 3 mg/kg in 50% DMSO (n = 6); c) melatonin 1 mg/kg in 50% DMSO (n = 6); or d) 50% DMSO control solution (n = 6). Daily injections given at this time of day were continued for a total of 22 days. After injections were discontinued, activity was monitored for a further 25 days.

RESULTS

Daily injections of melatonin and S20098, administered 0.5 h before dark onset, phase advanced the entrained activity rhythms, reducing the negative PAD to zero (Figs. 1b-1d). Control injections did not alter the phase angle difference (Fig. 1a). There was no dose-related effect in the two S20098 groups.

The mean negative PAD during the preinjection baseline ranged from 191 min in the DMSO control groups to 227 min in the 1-mg/kg melatonin group (Table 1). Daily injections of DMSO had no discernible effect on PAD except for increasing the difference slightly in one rat (#9) and decreasing it slightly in another (#22). In contrast, daily injections of melatonin, administered 0.5 h before dark onset, phase advanced the onset of activity, reducing PAD to 22.5 min. In two cases (#11, #23), PAD was reduced to zero and in a third case to 6 min (#26). S20098 appeared equally effective as melatonin, substantially reducing PAD in the majority of rats (Table 1). The time taken to reach the new steady state was approximately equivalent in all three drug groups (mean 8.7, 10.3, and 8.7 days for melatonin, 1 mg, and 3 mg S20098, respectively).

At the cessation of drug injections, activity onsets slowly delayed back toward the original positions but as a group did not reach the previous PAD (column 5, Table 1). Fifteen of the 18 rats stabilised at a PAD shorter than in the baseline preinjection stage. Number of postinjection days taken to reach the new PAD ranged from 10.3-12.3, slightly longer than the number of days of injection taken to change the original PAD. However, a tendency toward a shorter postinjection PAD compared to preinjection PAD was also observed in the DMSO control group, suggesting that a drift in the phase of entrainment was occurring irrespective of injection solution.

DISCUSSION

Both S20098 and melatonin phase advanced the activity rhythm of rats with large negative baseline PADs. In several cases, these differences were large, up to 4.5 h. This strongly suggests that if S20098 is as powerful in humans as it is in rats it should prove to be a substance of great clinical utility for treatment of human sleep disorders of the circadian type in both sighted and blind people. Because both 1- and 3-mg/kg doses were equally effective in this experiment, further investigations to determine the ED_{50} of S20098 are warranted. In addition, the optimal times in relation to the onset of activity and phase of the LD cycle need to be explored.

In humans (17), but apparently not rats (10), melatonin induces both phase delays and phase advances depending upon the time of day of melatonin administration. If S20098 mimics melatonin's action due to its agonist properties, then the potential use in ASPS by phase-delaying rhythms when administered at subjective dawn cannot be determined from our rat model. Nevertheless, the fact that S20098 may counteract DSPS is significant given the population that suffers from this disorder either in its full form or in what has been termed sub-DSPS (7). Sub-DSPS refers to the "owls" in society who habitually retire to bed later than average and who may in fact suffer from a milder form of DSPS.

Evening, oral administration of melatonin to sighted and blind humans has been shown to advance evening fatigue and sleep onset (4,5,12,19,24,25,27,31,32) under a variety of conditions ranging from single case studies to controlled double-blind trials. In some of these studies, there are confounding effects between chronobiotic (circadian rhythm shifting) and hypnotic (sleep inducing) properties of melatonin, particularly at the doses chosen, and often a further complication of timing of administration in relation to the endogenous melatonin profile. However, it is now clear from the work of the Portland, OR group that melatonin at doses far below those exerting hypnotic effects and administered at a time of day when endogenous melatonin levels are low can induce both phase advances and delays in blind as well as sighted people (17).

The use of melatonin in sleep disorders of the circadian type have tended to focus on jetlag (1,2,20,22,28,29). However, in terms of chronic long-term use of melatonin or agonists such as S20098 disorders such as DSPS and ASPS (particularly in the aged and in certain types of depression) represent a more important concern to the community at large and thus should be focused on by chronobiologists. The present work on rats suggests that S20098 should be as effective as melatonin.

Both melatonin and S20098 failed to permanently change the phase angle difference. Therefore, it is likely that the indole and agonist do not alter the entrainment pathway of light to the clock (suprachiasmatic nucleus) but act as competing zeitgeber. Once injections cease, the LD cycle reasserts its original entrainment phase on the clock. However, in some blind humans with DSPS it is possible that longer lasting effects of melatonin or its analogs on entrainment may occur due to the lack of competition by the LD zeitgeber and reinforcement by social zeitgebers.

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