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Melatonin modulates the sensitivity of 5-hydroxytryptamine-2 receptor-mediated sleep–wakefulness regulation in the rat

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The interaction between melatonin and two 5-hydroxytryptamine (5-HT_2) compounds was studied on sleep patterns in rats. Administration of the 5-HT_2 receptor antagonist ritanserin (0.63 mg/kg, i.p.) resulted in a significant increase of deep slow wave sleep (SWS2) and a decrease of paradoxical sleep (PS). The 5-HT_2 receptor agonist DOM (1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane) (0.63 mg/kg, i.p.) produced a significant reduction of both SWS2 and PS. Melatonin (1 mg/kg, i.p.) alone did not alter sleep but counteracted the sleep effects induced by ritanserin as well as DOM. It is proposed that melatonin modulates the sensitivity of 5-HT_2 receptor-mediated sleep response probably by an indirect route.

Recently, it has been suggested that the 5-hydroxytryptamine-2 (5-HT_2) receptor subtypes could be involved in the regulation of the sleep–wakefulness cycle in the rat [4, 6]. The 5-HT_2 receptor antagonist ritanserin [10] increased the duration of deep slow wave sleep (SWS2) and dose-dependently reversed the SWS2 deficit produced by the 5-HT_2 receptor agonist DOM (1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane) [18]. However, the effectiveness of ritanserin depended on the time of drug administration during the light–dark cycle in humans [9] and in rats [5]. Interestingly, serotonin and the endogenous hormone melatonin are biochemically related (serotonin is the precursor of melatonin) and the melatonin rhythm is synchronized to the light–dark cycle [1, 2]. Therefore, the differential sleep effects of ritanserin during the light and dark periods in the rat could be related to the circadian variations in melatonin production. In order to test this hypothesis, sleep–wakefulness patterns were analyzed after pharmacological blockade or stimulation of 5-HT_2 receptors by ritanserin and DOM, respectively in melatonin-pretreated rats.

Pharmacological treatments were performed in adult male Wistar rats (240–260

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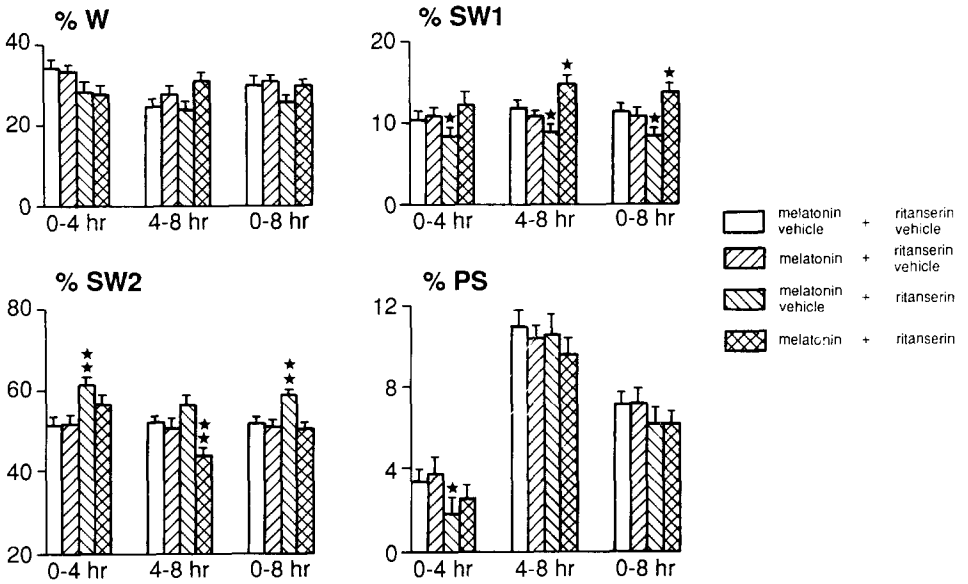

Fig. 1. Effects of ritanserin (0.63 mg/kg i.p.) on sleep and wakefulness states in melatonin (1 mg/kg, i.p.)-pretreated rats for each of the two 4-h periods following the treatment. Values (mean ± S.E.M. of 6 animals) of wakefulnes (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) are expressed as percentage of the recording time. $^{*}P<0.05$, $^{**}P<0.01$ (two-tailed Student t-test) as compared to baseline (vehicle injection under the same conditions).

g) which were chronically implanted for standard polygraphic monitoring of sleep as previously described [6], and maintained on a 12-h light–dark schedule (light on at 09.30 h). The rats received 1 mg/kg of melatonin dissolved in hydroxypropyl-β-cyclodextrine 10% and injected intraperitoneally (i.p.) 15 min before light onset, and 15 min later 0.63 mg/kg i.p. of either ritanserin ($n=6$) or DOM ($n=7$). Ritanserin was dissolved in 1 mM tartaric acid and DOM was dissolved in saline. Polygraphic recordings were scored visually and classified as being either wakefulness (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) or paradoxical sleep (PS), using the criteria of Michel et al. [14]. Sleep–wakefulness patterns were analyzed for each of the two successive 4-h periods following the last injection and were compared to baseline (vehicle injection under the same conditions). Statistical significance of the data was assessed by means of the two-tailed Student t-test.

In the two groups of rats, melatonin (1 mg/kg) had no effect on the sleep–wakefulness states throughout the 8-h recording period (Figs. 1 and 2). Administration of ritanserin (0.63 mg/kg) resulted in a significant increase of SWS2 (+20%, $P<0.01$) at the expense of SWS1 (–23%, $P<0.05$), PS (–44%, $P<0.05$) and W (–18%, N.S.) in the first 4 h following the treatment (Fig. 1). DOM injection (0.63 mg/kg) produced a reduction of both SWS2 (–41%, $P<0.01$) and PS (–74%, $P<0.01$) combined with an enhancement of W (+72%, $P<0.01$) during the first 4 h (Fig. 2). PS deficit persisted into the second 4-h period (–44%, $P<0.05$). As illustrated in Fig. 1, pretreat-

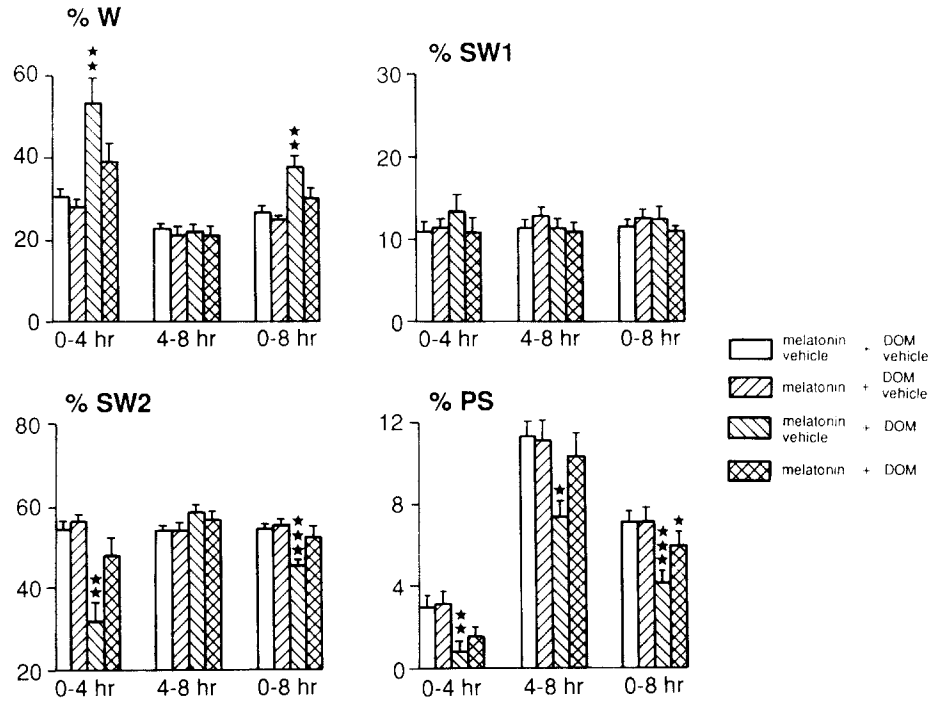

Fig. 2. Effects of DOM (0.63 mg/kg, i.p.) on sleep and wakefulness states in melatonin (1 mg/kg, i.p.)-pretreated rats for each of the two 4-h periods following the treatment. Values (mean ± S.E.M. of 7 animals) of wakefulness (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) are expressed as percentage of the recording time. $*P<0.05$, $**P<0.01$, $***P<0.001$ (two-tailed Student's t-test) as compared to baseline (vehicle injection under the same conditions).

ment with melatonin prevented the ritanserin-induced SWS2 increase and PS reduction. Similarly, melatonin counteracted the SWS2 and PS deficit produced by DOM (Fig. 2). Sleep latencies were modified as shown in Table I. After ritanserin treatment the PS latency was significantly prolonged, and in melatonin-pretreated rats it tended to reach baseline values. DOM prolonged the SWS1, SWS2 and PS latencies, and in melatonin-pretreated rats they were shortened.

In agreement with previous data obtained in the rat [4, 6], ritanserin increased the duration of SWS2 at the expense of SWS1, PS and W, whereas the stimulation of the 5-HT_2 receptors by DOM produced a reduction of both SWS2 and PS combined with an increase of W.

Conflicting results have been reported after melatonin treatment in rats. Mendelson et al. [13] have shown that melatonin (0.8 mg/kg, i.p.) decreased total sleep when injected 15 min before light onset. Conversely, an increase in both SWS and PS duration have been found following 10 mg/kg i.p. of melatonin injected 4 h post light onset [8] or during continuous melatonin infusion (10 μg/day) via subcutaneous implants [15]. In the two separate groups of rats used in the present experiment, melatonin (1 mg/kg, i.p.) did not alter sleep. The physiological effects of melatonin may

TABLE I

SLEEP LATENCIES AFTER RITANSERIN (0.63 mg/kg, i.p.) OR DOM (0.63 mg/kg, i.p.) ADMINISTRATION IN MELATONIN (1 mg/kg, i.p.)-PRETREATED RATS

Values (mean ± S.E.M. of 6 and 7 animals for each of the two experiments, respectively) of light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) latencies are expressed in minutes.

	SWS1 latency (min)	SWS2 latency (min)	PS latency (min)
Melatonin vehicle + ritanserin vehicle	12.8 ± 2.1	30.5 ± 5.2	116.2 ± 16.9
Melatonin + ritanserin vehicle	16.8 ± 2.8	30.5 ± 5.5	117.1 ± 19.7
Melatonin vehicle–ritanserin	9.8 ± 1.7	19.8 ± 3.9	159.7 ± 18.5*
Melatonin + ritanserin	13.8 ± 2.4	30.0 ± 3.5	145.3 ± 15.6
Melatonin vehicle + DOM vehicle	7.0 ± 0.5	21.2 ± 3.4	130.8 ± 12.2
Melatonin + DOM vehicle	9.6 ± 3.2	23.3 ± 4.1	103.9 ± 17.5
Melatonin vehicle + DOM	49.7 ± 14.4*	76.5 ± 15.4*	228.6 ± 27.8*
Melatonin + DOM	36.7 ± 11.2*	57.7 ± 14.9	172.3 ± 18.1*

*$P < 0.05$ (two-tailed Student t-test) as compared to baseline (vehicle injection under the same conditions).

depend on the dose used and/or the time of drug administration as observed in the electrophysiological response of rat suprachiasmatic neurones [12].

Melatonin pretreatment produced an antagonism of both ritanserin- and DOM-induced sleep–wakefulness changes suggesting a bidirectional modulation of the 5-HT_2 receptor-mediated sleep response by melatonin. This is supported by the study in which ritanserin failed to increase SWS2 and to reduce PS when administered at the onset of the dark period in the rat [5], e.g. when the peak of melatonin secretion occurs. Diurnal variations in the 5-HT_2 receptor-mediated head-twitch response in the mouse [16] and in the sensitivity of suprachiasmatic and lateral geniculate neurones to 5-HT in the rat [11] might also be related to the present results. On the other hand, an action of ritanserin on melatonin secretion is unlikely since melatonin levels in the rat pineal gland were unchanged within a 10-h period following ritanserin treatment (0.63 mg/kg, i.p.) at the onset of the light period (N. Sarda and A. Gharib, unpublished results).

In order to investigate whether ritanserin or DOM and melatonin could compete at the same receptor site, the affinity of melatonin for 5-HT_2 receptor sites was examined. In in vitro binding assays using [^{3}H]ketanserin, melatonin did not interact with 5-HT_2 receptor sites in rat frontal cortex at concentrations up to 10^{-4} M. In addition, two recently described melatonin receptor antagonists luzindole [3] and N-(3,5-dinitrophenyl)-5-methoxytryptamine [19] had no affinity for 5-HT_1 and 5-HT_2 receptor sites. In turn, compounds with high affinity for the 5-HT_2 site exhibit a very low affinity for melatonin binding sites in hamster brain [7]. Thus, there is probably no direct competition between melatonin and ritanserin or DOM at the same receptor site. An alternative explanation is that melatonin might induce modifications in the release of pineal and/or hypothalamic hormones such as arginine-vasotocin [17] and that

they would alter the sleep response to 5-HT_2 drugs (W.B. Mendelson, personal communication).

In both diurnal and nocturnal species, melatonin secretion occurs at nighttime and is rather associated with sleep in diurnal species and with wakefulness in nocturnal species [13]. Thus, an inverse correlation exists between the sleep–wakefulness cycle and the rhythm of melatonin secretion in humans and in rats. In humans, ritanserin increased SWS duration (stages 3 and 4) by 115% when given in the morning and by 57% when given in the evening [9]. Circadian variations in melatonin production may also account for the differential effects of ritanserin in humans. Therefore, it would be interesting to examine whether the combination of ritanserin with a melatonin receptor antagonist produces an increase of SWS2 in rats during the dark period. Differential effects of ritanserin might also be suppressed by using pinealectomized rats.

In conclusion, the present study indicates that melatonin modulates the sensitivity of 5-HT_2 receptor-mediated sleep–wakefulness regulation in the rat. Melatonin acts upon the sleep response produced by a 5-HT_2 receptor antagonist as well as a 5-HT_2 receptor agonist probably by an indirect route.

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