*Pharmacology Biochemistry & Behavior*, Vol. 34, pp. 533–537. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

# The Light-Dark Cycle Modulates the Effects of Ritanserin on Sleep-Wakefulness Patterns in the Rat

CHRISTINE DUGOVIC,* JOSEE E. LEYSEN,† PAUL F. M. JANSSEN† AND ALBERT WAUQUIER*

**Department of Neuropsychopharmacology and †Department of Biochemical Pharmacology Janssen Research Foundation, B-2340 Beerse, Belgium*

Received 24 March 1989

DUGOVIC, C., J. E. LEYSEN, P. F. M. JANSSEN AND A. WAUQUIER. *The light-dark cycle modulates the effects of ritanserin on sleep-wakefulness patterns in the rat.* PHARMACOL BIOCHEM BEHAV **34**(3) 533–537, 1989.—The 5-$HT_2$ receptor antagonist ritanserin (0.63 mg/kg IP) produced differential effects on sleep-wakefulness patterns in rats when administered during the light or dark period: an increase in the duration of deep slow wave sleep at the expense of light slow wave sleep, paradoxical sleep and wakefulness when injected during the light period, and no major sleep alteration when given at dark onset. Since circadian variations in serotonin receptor density might modulate the sleep response, we examined the effects of ritanserin on sleep in rats exposed to continuous light for 10 days,and whether 5-$HT_2$ receptors were affected in separate groups of rats exposed to similar conditions. No significant changes in the $K_D$- and $B_{max}$-values of various receptors were found. However, ritanserin produced greater effects in continuous light conditions than when given during the light period in the 12-hr light-dark condition. This suggests a possible role of 5-$HT_2$ receptors in the organization of sleep when the environmental photoperiod is disturbed.

Circadian Sleep Wakefulness Ritanserin Rat

CIRCADIAN variations in the therapeutic response to a drug may result from diurnal rhythms in metabolism, concentration of brain neuromodulators and receptor site number and affinity (15, 21, 26). On the other hand, circadian rhythms in these biochemical factors may modulate behavioral parameters in many mammals, such as rest and activity related to the environmental photoperiod.

The recently developed selective 5-hydroxytryptamine-2 (5-$HT_2$) receptor antagonist ritanserin (18) has been shown to promote slow wave sleep (SWS) in humans (4, 5, 12, 24). Interestingly, a more pronounced effect was observed when the drug was given in the morning rather than in the evening (12). An increase in the duration of deep SWS (SWS2) was also found in rats when ritanserin was administered at the onset of the light period (6,7). These findings raised the question of whether this compound would produce differential effects during the resting and active periods in the rat that might be related to the light-dark cycle. Therefore, the effects of ritanserin on sleep-wakefulness patterns were tested during the dark and light periods in the same rats.

To further investigate the role of the light-dark cycle in the sleep-wakefulness changes induced by ritanserin, a separate experiment was carried out in animals first maintained on a 12-hr light-dark schedule, and then on a constant light schedule. Additionally, alterations in various receptor sites in the rat brain were studied following prolonged light exposure.

## METHOD

### *Sleep-Wakefulness*

Under pentobarbital anesthesia (50 mg/kg injected intraperitoneally) (IP) 18 adult male Wistar rats weighing 240 to 260 g were chronically implanted for standard sleep monitoring. Electrodes were positioned on the frontal and occipital cortices, subcutaneously on each side of the orbit and in the neck muscles for polygraphic recordings of electroencephalogram, electro-oculogram and electromyogram. After an 8–10-day recovery period from surgery and habituation to the environmental conditions (12-hr light-dark schedule, temperature maintained at 22 ± 2°C, food and water ad lib), pharmacological tests were started.

In the first experiment, 8 animals received 0.63 mg/kg of ritanserin injected IP, either at the onset of the dark period (5:00 p.m.) or 4 hr post light onset (9:00 a.m.). In the second experiment, 10 animals were first maintained on a 12-hr light-dark schedule (LD), and then on a constant light schedule (LL) during 14 days. Ritanserin (0.63 mg/kg IP) was injected at the onset of the light period (9:30 a.m.) when the rats were kept in LD, and at the same time after 10 days in LL. Ritanserin was dissolved in 1 mM tartaric acid and injected in a volume of 4 ml/kg body weight. The dose of 0.63 mg/kg ritanserin was chosen because in a previous study (7) rats treated with ritanserin (0.04–2.5 mg/kg IP) at the onset of the light period exhibited the most pronounced sleep-wakefulness

response at this dose.

Polygraphic recordings were performed for 8 or 24 hr following the treatment. They were scored visually and classified in either wakefulness (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) or paradoxical sleep (PS), according to the criteria of Michel *et al.* (20). Sleep and wakefulness amounts were expressed as percentage of the recording time and were compared to baseline values: vehicle injections under the same conditions as the first experiment; vehicle injection after 0, 9 and 14 days in LL for the second experiment. Statistical significance of the data was assessed by means of the paired two-tailed Student *t*-test.

*Receptor-Binding Assays*

For the biochemical experiments, male Wistar rats weighing 200 to 220 g were housed in groups of 8 per cage under a temperature of 22 ± 2°C with free access to food and water. The rats were maintained either in LD (light on at 7:00 a.m.) or in LL for 10 days. The animals were sacrificed by decapitation at 9:00 a.m. and various brain areas were rapidly dissected, frozen in liquid nitrogen and stored at −80°C.

Radioligand binding assays for the following receptors were performed in twice washed total membrane fractions: 5-$HT_2$: [$^3$H]ketanserin in the frontal cortex and in the total cortex; 5-$HT_{1A}$: [$^3$H]N,N-dipropyl-8-hydroxy-2-aminotetralin in the hippocampus; dopamine-$D_2$: [$^3$H]spiperone in the presence of R 43 448 in the striatum; adrenergic-$\beta_1$: [$^3$H]dihydroalprenolol in the presence of ICI 118-551 in the total cortex. Experimental conditions and materials were as described in Leysen *et al.* (17,19). Saturation binding curves, using at least 8 radioligand concentrations, were measured in tissue pools of 3 rats. $K_D$- and $B_{max}$-values were derived by Scatchard analysis as in Leysen *et al.* (19). The experiment was repeated independently five times.

## RESULTS

In the first group of rats, ritanserin (0.63 mg/kg) injected at the onset of the dark period (Fig. 1, left) produced no change in the amounts of W, SWS1 and SWS2 throughout the 24-hr recording period. PS amounts were not modified during the first two 4-hr periods, but were significantly increased during the last 4 hr of the dark period (+92%, $p<0.05$) and in the subsequent 12-hr light period (+15%, $p<0.01$). When administered during the light period (Fig. 1, right) ritanserin induced a significant increase of SWS2 during 12 hr. The SWS2-enhancing effect mainly occurred in the first 4 hr following the injection (+16%, $p<0.001$) at the expense of SWS1 (−34%, $p<0.05$) and PS (−34%, $p<0.01$).

In the second group of animals first maintained in LD, ritanserin (0.63 mg/kg) had similar effects to those obtained in the first group of rats. During the 8-hr recording period following the treatment at the onset of the light period, rats exhibited an increase of SWS2 (+14%, $p<0.001$) combined with a suppression of W (−15%, $p<0.05$), SWS1 (−20%, $p<0.05$) and PS (−27%, $p<0.01$ in the first 4 hr).

Continuous light exposure induced marked alterations in the sleep-wakefulness distribution. After 9 or 14 days in LL, W amounts almost doubled, whereas SWS2 and PS amounts halved as compared to baseline values obtained in the same 8-hr recording period when the rats were in LD (Fig. 2). All values obtained on day 14 were not significantly different from those obtained on day 9.

The effects of ritanserin injected after 10 days of continuous light exposure were more pronounced than in the LD condition. During the 8-hr recording period SWS2 amounts were increased by 33% ($p<0.001$), whereas W and SWS1 amounts were reduced

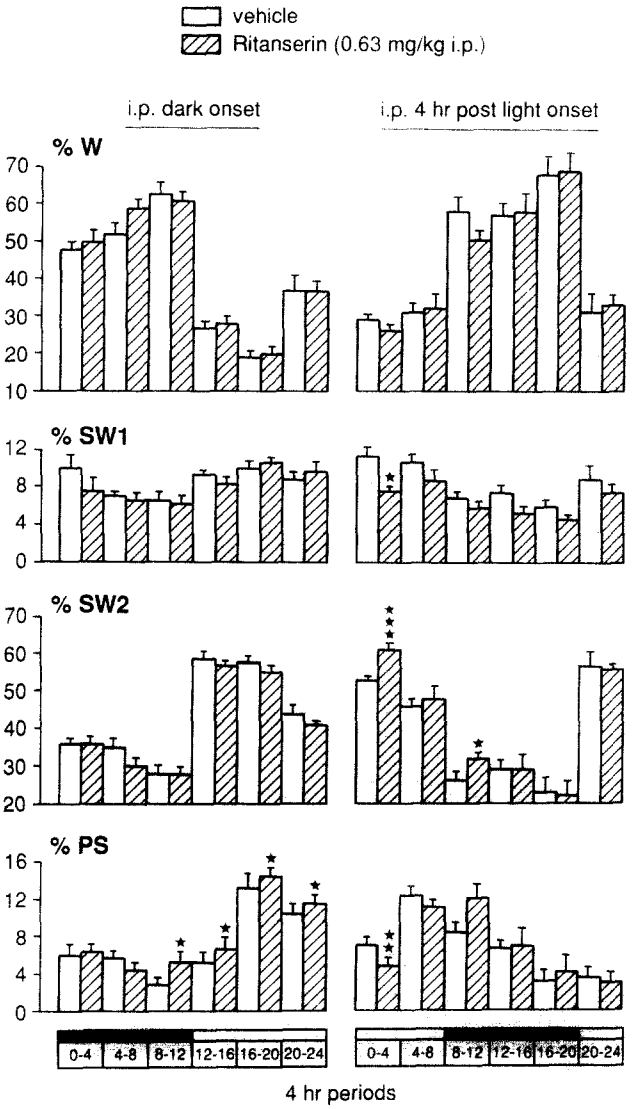


FIG. 1. Effects of ritanserin (0.63 mg/kg IP) injected either at dark onset or 4 hr post light onset on sleep-wakefulness states in rats during the six 4-hr periods following the treatment. Values (mean ± SE of 8 animals) of wakefulness (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) are expressed as percentage of the recording time. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (paired two-tailed Student *t*-test) as compared to baseline (vehicle injection under the same conditions).

by 17% ($p<0.001$) and 34% ($p<0.001$) respectively as compared to the day before ritanserin treatment (Fig. 2). Conversely, PS, which was suppressed in the LD condition, was enhanced by 53% ($p<0.01$) in the LL condition (Fig. 2).

Sleep latencies were modified as shown in Table 1. Continuous light exposure produced no major effects on sleep latencies. Under ritanserin treatment, the SWS2 latency was significantly reduced only in the LL condition and the PS latency was significantly prolonged only in the LD condition.

$K_D$- and $B_{max}$-values of various receptor sites in brain membranes of rats maintained for 10 days in LD and LL conditions are presented in Table 2. The maximal number of 5-$HT_2$, 5-$HT_{1A}$, dopamine-$D_2$ and adrenergic-$\beta_1$ receptor sites as well as the

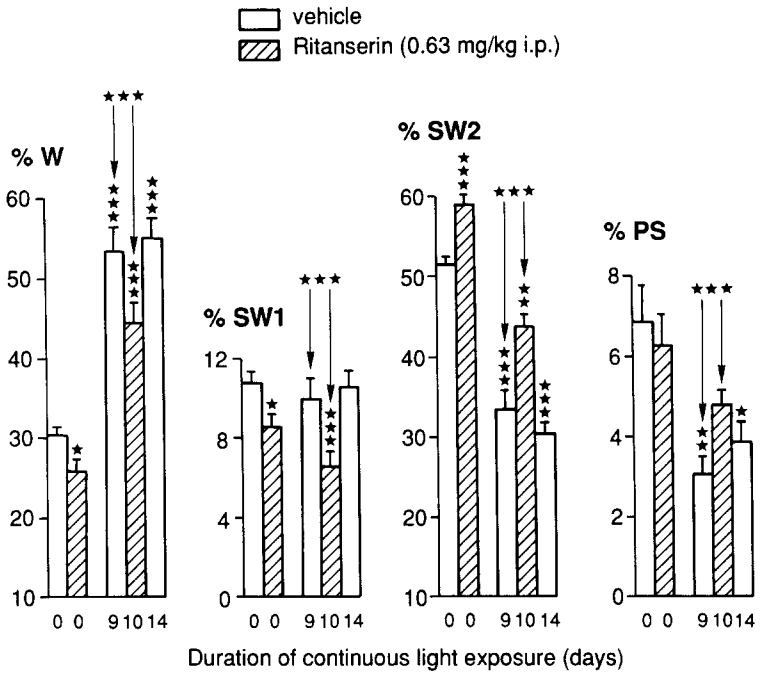


FIG. 2. Effects of ritanserin (0.63 mg/kg IP) during 8 hr following the treatment on sleep-wakefulness states in rats first maintained in LD, and then in LL. Values (mean ± SE of 10 animals) of wakefulness (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) are expressed as percentage of the recording time. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (paired two-tailed Student $t$-test) as compared to the values obtained after vehicle injection in LD, except otherwise indicated.

$K_D$-values were not altered after a 10-day period in the LL condition as compared to the LD condition.

## DISCUSSION

Ritanserin acted differentially on sleep-wakefulness patterns when administered during the light or dark period in the rat. Rats treated during the light period exhibited an increase in the duration of SWS2 at the expense of W, SWS1 and PS. The present results confirm those previously obtained (6,7). Conversely, ritanserin produced no major alteration of sleep-wakefulness patterns when injected at the onset of the dark period, except an enhancement of PS amounts in the subsequent light period. Thus, it appears that the SWS2-increasing property of ritanserin is only evident during the physiological rest period of the rat.

In addition, the onset and duration of ritanserin's effects on sleep-wakefulness states depended on the time of drug administration. After injection in the light period the sleep response started within 4 hr following treatment and lasted for 12 hr. However, after injection at the onset of the dark period, an increase in PS duration occurred only after an 8-hr delay period following treatment and lasted for at least 16 hr. In a wide range of common pharmacological tests in rats carried out during the light period, ritanserin had its peak effect close to 2 hr after subcutaneous or oral administration and was relatively long acting (1).The above observations reflect the fact that ritanserin occupies 5-$HT_2$ receptors for more than 24 hr in rat frontal cortex even though plasma levels of drug were below detection level after 24 hr (18).

It has been suggested that during the light period in rats, when sleep amounts are the highest, it would be more difficult to increase sleep above baseline levels (14). In line with this idea, delta-sleep-inducing peptide, prostaglandin $D_2$ and uridine

TABLE 1

SLEEP LATENCIES AFTER RITANSERIN TREATMENT IN RATS FIRST MAINTAINED IN LD, AND THEN IN LL

| Treatment: | Vehicle | Ritanserin | Vehicle | Ritanserin | Vehicle |
|---|---|---|---|---|---|
| Duration of Continuous Light Exposure (days): | 0 | 0 | 9 | 10 | 14 |
| SWS1 latency (min) | 12.1 ± 1.8 | 10.5 ± 2.8 | 17.1 ± 3.3 | 12.7 ± 1.8 | 19.4 ± 3.2* |
| SWS2 latency (min) | 31.6 ± 4.9 | 30.1 ± 2.5 | 35.0 ± 9.6 | 18.7 ± 1.9* | 34.7 ± 3.4 |
| PS latency (min) | 123.5 ± 25.8 | 175.9 ± 23.1* | 125.6 ± 23.4 | 82.8 ± 7.4 | 110.6 ± 25.7 |

Values of light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) latencies are means ± SE of 10 animals.

*$p<0.05$ (paired two-tailed Student $t$-test) as compared to the values obtained after vehicle injection in LD.

TABLE 2

RECEPTOR-BINDING ASSAYS IN RATS MAINTAINED IN LL

| Receptor | Tissue | 12-hr Light-Dark Schedule $K_D$ (nM) | 12-hr Light-Dark Schedule $B_{max}$ (fmoles/mg tissue) | Constant Light Schedule for 10 Days $K_D$ (nM) | Constant Light Schedule for 10 Days $B_{max}$ (fmoles/mg tissue) |
|---|---|---|---|---|---|
| 5-$HT_2$ | Frontal cortex | 0.52 ± 0.03 | 32.4 ± 1.5 | 0.52 ± 0.05 | 30.8 ± 1.5 |
| | Total cortex | 0.44 ± 0.04 | 13.1 ± 0.7 | 0.58 ± 0.04 | 14.3 ± 0.3 |
| 5-$HT_{1A}$ | Hippocampus | 1.24 ± 0.04 | 26.2 ± 1.6 | 1.16 ± 0.04 | 23.8 ± 0.4 |
| Dopamine-$D_2$ | Striatum | 0.029 ± 0.002 | 27.2 ± 1.3 | 0.03 ± 0.01 | 28.6 ± 0.7 |
| Adrenergic-$\beta_1$ | Total cortex | 1.23 ± 0.30 | 4.6 ± 1.0 | 1.46 ± 0.18 | 4.8 ± 0.3 |

Values are means ± SE of 5 independent determinations.

increased sleep in rats when given nocturnally when the animals sleep less (13), but had little effect when given diurnally (14). The present study with ritanserin demonstrated the opposite, since the compound was only active in the light period. Other drugs such as nomifensine and amitryptyline also increased SWS only when given during the light period (16,23). Drugs which are expected to alter sleep-wakefulness patterns should, therefore, be tested both at the beginning of the light and the dark periods. In addition, in order to be relevant to human studies, it is more appropriate to measure drug effects during the "rest" period of the rat.

Circadian variations in drug effects might be related to daily rhythms in several factors including fluctuations in neurotransmitter metabolism and receptor binding sites (15, 21, 26). Differential sleep-wakefulness changes induced by the 5-$HT_2$ receptor antagonist ritanserin during the light-dark cycle in the rat could result from daily variations in serotonin levels, synthesis and release (9, 10, 25), as well as in 5-$HT_2$ binding sites (3) in the rat brain. In particular, elevated numbers of 5-$HT_2$ binding sites have been observed during light as compared to dark in the adult rat (3). This could be related to the results of Moser and Redfern (22) who have demonstrated that the 5-$HT_2$ receptor-mediated head-twitch response in the mouse reached a maximum during the light period and a minimum during the dark period. Therefore, it would be interesting to examine whether the 5-$HT_2$ receptor agonist DOM [1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane], which dose-dependently suppressed SWS2 and PS when administered at light onset (7), produces differential effects during the light and dark periods in rats.

In order to investigate whether the light would have a facilitating action on the sleep-wakefulness response induced by ritanserin, the effects of ritanserin were tested in rats exposed to continuous light for 10 days. During prolonged exposure to LL (9 to 14 days) the circadian rhythm of sleep-wakefulness was disrupted. These data confirm results observed by Borbély and Neuhaus (2) and by Eastman and Rechtschaffen (8) in rats. In our study using these experimental conditions both the SWS2-promoting action and the W-suppressing effects of ritanserin were enhanced. Furthermore, ritanserin seemed to be able to directly induce SWS2 since the SWS2 latency was significantly reduced. Unlike the results obtained in the LD condition, PS amounts were increased and PS latency even tended to be shortened. In fact, ritanserin tended to normalize the disturbed sleep-wakefulness organization in LL rats. Recent clinical data have demonstrated the effectiveness of ritanserin in counteracting jet-lag after transatlantic flights (11). Therefore, further studies of phase-shifts in the photoperiod may reveal a role of 5-$HT_2$ receptors in sleep-wakefulness synchronization with the photoperiod.

Continuous light exposure for 10 days did not affect the $B_{max}$- and $K_D$-values of various receptor sites in rat brain, including the 5-$HT_2$ sites. Thus, modifications in the sleep-wakefulness response induced by ritanserin in the LL condition versus the LD condition are apparently not due to an alteration in 5-$HT_2$ receptors. Moreover, the fact that ritanserin had greater effects in LL than during the light period in LD rats suggests that circadian variations in 5-$HT_2$ receptor density are not responsible for the differential effects of ritanserin during the light-dark cycle. However, we measured the total number of receptor sites in brain membrane preparations, i.e., the sum of externalized and internalized receptors. Since only externalized receptors are physiologically active, it is still possible that subtile variations in externalized receptors occurred in the LL condition.

At this time, the molecular mechanisms underlying the differential effects of ritanserin on sleep-wakefulness patterns of the rat during the light and dark periods, and continuous light have not been elucidated. Nevertheless, sleep-wakefulness changes induced by ritanserin are clearly related to the environmental photoperiod. Ritanserin may facilitate the physiological mechanisms of sleep by enhancing the sleeping behavior normally associated with the light phase in nocturnal species.

ACKNOWLEDGEMENT

We are grateful to Dr. David Ashton for his very helpful comments and review of the manuscript.

## REFERENCES

1. Awouters, F.; Niemegeers, C. J. E.; Megens, A. A. H. P.; Meert, T. F.; Janssen, P. A. J. Pharmacological profile of ritanserin: a very specific central serotonin $S_2$-antagonist. Drug Dev. Res. 15:61–73; 1988.
2. Borbély, A. A.; Neuhaus, H. U. Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. J. Comp. Physiol. 128:37–46; 1978.
3. Bruinink, A.; Lichtensteiger, W.; Schlumpf, M. Ontogeny of diurnal rhythms of central dopamine, serotonin and spirodecanone binding sites and of motor activity in the rat. Life Sci. 33:31–38; 1983.
4. Clarenbach, P.; Birmanns, B.; Krätzschmar, S.; Jaursch-Hancke, C. Sleep pattern and nocturnal plasma profiles of HGH, prolactin and cortisol in man after the serotonin-antagonist ritanserin and the GABA-agonist gabapentin. Sleep Res. 15:29; 1986.
5. Declerck, A. C.; Wauquier, A.; Van der Ham-Veltman, P. H. M.; Gelders, Y. Increase in slow-wave sleep in humans with the serotonin-

5-$HT_2$ antagonist ritanserin (The first exploratory polygraphic sleep study). Curr. Ther. Res. 41:427–432; 1987.
6. Dugovic, C.; Wauquier, A. 5-$HT_2$ receptors could be primarily involved in the regulation of slow-wave sleep in the rat. Eur. J. Pharmacol. 137:145–146; 1987.
7. Dugovic, C.; Wauquier, A.; Leysen, J. E.; Marrannes, R.; Janssen, P. A. J. Functional role of 5-$HT_2$ receptors in the regulation of sleep and wakefulness in the rat. Psychopharmacology (Berlin) 97:436–442; 1989.
8. Eastman, C.; Rechtschaffen, A. Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. Physiol. Behav. 31:417–427; 1983.
9. Faradji, H.; Cespuglio, R.; Jouvet, M. Voltammetric measurements of 5-hydroxyindole compounds in the suprachiasmatic nuclei. Circadian fluctuations. Brain Res. 279:111–119; 1983.
10. Hery, F.; Rouer, E.; Glowinski, J. Daily variations of serotonin metabolism in the rat brain. Brain Res. 43:445–465; 1972.
11. Hoppenbrouwers, M. L.; Vanden Bussche, G.; Gelders, Y. Is ritanserin, a serotonin receptor blocker, able to counteract jet lag? A placebo-controlled pilot investigation. Psychopharmacology (Berlin) 96(Suppl.):245; 1988.
12. Idzikowski, C.; Mills, F. J.; Glennard, R. 5-Hydroxytryptamine-2-antagonist increases human slow wave sleep. Brain Res 378: 164–168; 1986.
13. Inoué, S.; Honda, K.; Komoda, Y.; Uchizono, K.; Ueno, R.; Hayaishi, O. Differential sleep-promoting effects of five sleep substances nocturnally infused in unrestrained rat. Proc. Natl. Acad. Sci. USA 81:6240–6245; 1984.
14. Inoué, S.; Honda, K.; Komoda, Y.; Uchizono, K.; Ueno, R.; Hayaishi, O. Little sleep-promoting effect of three sleep substances diurnally infused in unrestrained rats. Neurosci. Lett. 49:207–211; 1984.
15. Kafka, M. S.; Wirz-Justice, A.; Naber, D. Circadian and seasonal rhythms in α- and β-adrenergic receptors in the rat brain. Brain Res. 207:409–419; 1981.
16. Lelkes, Z.; Obàl, F., Jr.; Benedek, G.; Rubicsek, G.; Alföldi, P.; Obàl, F. Effects of acute and chronic treatment with an atypical antidepressant drug, nomifensine, on the sleep-wake activity in rats. Naunyn Schmiedebergs Arch. Pharmacol. 335:149–153; 1987.
17. Leysen, J. E.; Gommeren, W.; Eens, A.; de Chaffoy de Courcelles, D.; Stoof, J. C.; Janssen, P. A. J. The biochemical profile of risperidone, a new antipsychotic. J. Pharmacol. Exp. Ther. 247: 661–670; 1988.
18. Leysen, J. E.; Gommeren, W.; Van Gompel, P.; Wynants, J.; Janssen, P.; Laduron, P. M. Receptor binding properties in vitro and in vivo of ritanserin: a very potent and long-acting serotonin-5-$HT_2$ antagonist. Mol. Pharmacol. 27:600–611; 1985.
19. Leysen, J. E.; Van Gompel, P.; Gommeren, W.; Woestenborghs, R.; Janssen, P. A. J. Down regulation of serotonin-$S_2$ receptor sites in rat brain by chronic treatment with the serotonin-$S_2$ antagonists: ritanserin and setoperone. Psychopharmacology (Berlin) 88:434–444; 1986.
20. Michel, F.; Klein, M.; Jouvet, D.; Valatx, J. L. Etude polygraphique du sommeil chez le rat. C R Soc. Biol. (Paris) 155:2389–2392; 1961.
21. Moore Ede, M. C. Circadian rhythms of drug effectiveness and toxicity. Clin. Pharmacol. Ther. 14:925–935; 1973.
22. Moser, P. C.; Redfern, P. H. Circadian variation in behavioural responses to central 5-HT receptor stimulation in the mouse. Psychopharmacology (Berlin) 86:223–227; 1985.
23. Obàl, F., Jr.; Benedek, G.; Lelkes, Z.; Obàl, F. Effects of acute and chronic treatment with amitryptyline on the sleep-wake activity of rats. Neuropharmacology 24:223–229; 1985.
24. Paiva, T.; Arriaga, F.; Wauquier, A.; Lara, E.; Largo, R.; Leitao, J. N. Effects of ritanserin on sleep disturbances of dysthymic patients. Psychopharmacology (Berlin) 96:395–399; 1988.
25. Semba, J.; Toru, M.; Mataga, N. Twenty-four hour rhythms of norepinephrine and serotonin in nucleus suprachiasmaticus, raphe nuclei, and locus coeruleus in the rat. Sleep 7:211–218; 1984.
26. Walker, C. A.; Soliman, M. R. I.; Soliman, K. F. A. Chronopharmacology and chronotoxicology of CNS drugs: interrelationships with neuromodulators. J. Am. Coll. Toxicol. 2:359–370; 1983.