Psychopharmacology (1989) 97:436–442

Psychopharmacology

Functional role of 5-HT_2 receptors in the regulation of sleep and wakefulness in the rat

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Abstract. Recently developed agents specifically acting on different 5-hydroxytryptamine (5-HT) receptor populations were used to analyze the functional role of 5-HT_2 receptor subtypes in the sleep-wakefulness cycle of the rat. The 5-HT_2 receptor antagonist ritanserin injected intraperitoneally (IP) (0.04–2.5 mg/kg) induced an increase in deep slow wave sleep (SWS2) duration at the expense of wakefulness (W), light slow wave sleep (SWS1) and paradoxical sleep (PS). The stimulation of 5-HT_2 receptors by 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) produced a dose-related increase in W and a dose-dependent decrease in both SWS2 and PS. Pretreatment with ritanserin (0.16–2.5 mg/kg) or with cinanserin (2.5–5 mg/kg), another 5-HT_2 receptor antagonist, dose-dependently reversed the W enhancement and the SWS2 deficit produced by DOM, but not the PS deficit. Sleep-wakefulness alterations (increase in W and SWS1 combined with a suppression of SWS2 and PS) observed after IP injection of two putative 5-HT_1 receptor agonists, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) (2.5 mg/kg) and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969) (0.63 mg/kg), were not modified by ritanserin pretreatment (0.16–2.5 mg/kg). These results further support the hypothesis that the serotonergic system plays an active role in the regulation of the sleep-wakefulness cycle in the rat and that 5-HT_2 receptors are involved in this action. In addition, it is suggested that 5-HT_1 receptor subtypes are unlikely to interact with 5-HT_2 receptors in the sleep-wakefulness modulation mediated through 5-HT_2 receptors.

Key words: Sleep – 5-Hydroxytryptamine (5-HT) – Antagonist – Agonist – Rat

Serotonergic mechanisms underlying the control of the sleep-wakefulness cycle have been extensively investigated. Historically, brain serotonin has been associated with the initiation and maintenance of sleep (Jouvet 1969). However, electrophysiological experiments on dorsal raphe nucleus unit activity (McGinty and Harper 1976; Trulson and Jacobs 1979) and studies on serotonin release during the sleep-wakefulness cycle (Cespuglio et al. 1979; Puizillout et al. 1979) have produced results which are inconsistent with this model. Recently, Jouvet (1983) proposed that serotonin released as a neurotransmitter during waking might also act as a neurohormone by inducing the synthesis and/or the liberation of hypnogenic factors.

In accordance with a direct role of serotonin in sleep triggering, the serotonin antagonists metergoline, methiothepine and methysergide have been reported to reduce both slow wave sleep (SWS) and paradoxical sleep (PS) in cats (Radulovacki 1982; Sallanon et al. 1982), and to have different sleep suppressing effects in rats (Fuxe and Kiianmaa 1978; Adrien et al. 1980; Laguzzi 1981). However, there seems to be a contradiction between data obtained in animals and clinical results. Methysergide reduces rapid eye movement (REM) sleep and stage 4 but increases stage 3 in humans (Mendelson et al. 1975). Sandoz FQ27-096 and FU29-245, which have been reported to display serotonin receptor blocking properties, increase SWS in human volunteers (Spiegel 1981; Oswald et al. 1982). On the other hand, systemic administration of the serotonin-mimetic quipazine also results in SWS and PS suppression in rats (Fornal and Radulovacki 1981). However, these compounds are relatively non-specific: methiothepine and metergoline have mixed serotonin/dopamine activities (Enjalbert et al. 1978; Hamon et al. 1981) and methysergide and metergoline have serotonin antagonistic/agonistic properties (Kehr 1977; Bourgoin et al. 1978). Therefore, it is difficult to draw definitive conclusions from experiments using these pharmacological tools.

In the last decade, identification and classification of multiple 5-hydroxytryptamine (5-HT) receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_2) (Leysen et al. 1978; Peroutka and Snyder 1979; Pedigo et al. 1981) and the availability of drugs which selectively stimulate or block a given receptor site (see Bradley et al. 1986), have led to the hypothesis that each receptor subtype is differently involved in the regulation of physiological processes mediated by serotonin (see Conn and Sanders-Bush 1987). It has been recently shown that a specific serotonin antagonist at central 5-HT_2 receptors, ritanserin (Leysen et al. 1985), substantially increases SWS (stages 3 and 4) in human volunteers (Clarenbach et al. 1986; Idzikowski et al. 1986; Declerck et al. 1987) and in dysthymic patients (Paiva et al. 1988). This effect persisted during at least 2 weeks of chronic treatment (Idzikowski et al. 1987).

In an attempt to clarify whether 5-HT_2 receptors have a specific role in the modulation of sleep, sleep-wakefulness patterns were analyzed in rats after pharmacological blockade and/or stimulation of these receptors by ritanserin and

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the preferential 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Shannon et al. 1984). In addition, the interactions between ritanserin and two putative 5-HT_1 receptor agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969), suggested to bind on 5-HT_{1A} (Middlemiss and Fozard 1983) and 5-HT_{1B} (Sills et al. 1984) sites, respectively, were examined.

Materials and methods

Implantation procedure. A total of 37 male adult Wistar rats weighing 250 to 300 g were used in this study.

Under pentobarbital anesthesia [50 mg/kg injected intraperitoneally (IP)] electrodes for standard polygraphic monitoring were implanted. Electrodes were positioned on the frontal and parietal cortices, subcutaneously on each side of the orbit, and in the neck muscles. After surgery, the animals were housed in individual sound proof recording cages, maintained at a temperature of $22 \pm 2°$ C with free access to food and water and in a 12 h light-dark schedule (light on 9:30 a.m.).

Recording procedure. At the end of an 8–10-day recovery period from surgery and habituation to the environment, polygraphic recordings were started. The animals were connected by a cable to a rotating connector for polygraphic recordings of electroencephalogram, electro-oculogram and electromyogram.

The polygraphic activities were recorded on paper with a speed of 6 mm/s. Each 25-s epoch was visually classified as being wakefulness (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2) or paradoxical sleep (PS), using the criteria of Michel et al. (1961). The scores were entered into a computer (Hewlett-Packard 9816) which plotted the hypnograms and calculated the different sleep-wakefulness parameters (amount of time spent in these four states, number and duration of episodes for each state, SWS1, SWS2 and PS latencies).

Pharmacological procedure. The different pharmacological protocols (drug treatments, times of injection, doses used) are summarized in Table 1. Ritanserin was dissolved in 1 mM tartaric acid. DOM, cinanserin, 8-OH-DPAT and RU 24969 were dissolved in distilled water. All drugs were injected IP in a volume of 4 ml/kg body weight. Each rat never received the same dose of a drug more than once, except for the vehicle of ritanserin and saline which were injected three times. A minimum of 3 recovery days were allowed between two treatments.

Data analysis. Sleep-wakefulness parameters were analyzed for each of the two successive 4-h periods following the last injection and compared to baseline (vehicle injection under the same conditions). The amounts of the different states of vigilance were expressed in minutes and as per cent of baseline recordings which represent means of three baseline values per animal. In these conditions each rat was considered as its own control. Statistical tests were performed by means of the two-tailed Student *t* test. In addition, linear regression was calculated for drug dose-response effects.

Table 1. Summary of the different pharmacological protocols performed in this study. All compounds were administered intraperitoneally (IP). When the rats received two drugs in combination, a 30-min interval was allowed between the two treatments

Pharmacological treatment	Time IP injection	Dose range	Number of tests	Number of rats
5-HT_2 antagonist injected alone				
Ritanserin	9:30	0.04–2.5 mg/kg	65	11
5-HT_2 agonist injected alone				
DOM	9:30	0.16–2.5 mg/kg	24	4
5-HT_2 antagonists and 5-HT_2 agonist in combination				
Ritanserin +DOM	9:00 9:30	0.16–2.5 mg/kg 0.63 mg/kg	42	6
Cinanserin +DOM	9:00 9:30	2.5–5 mg/kg 0.63 mg/kg	24	4
5-HT_2 antagonist and 5-HT_1 agonists in combination				
Ritanserin +8-OH-DPAT	9:00 9:30	0.16–2.5 mg/kg 2.5 mg/kg	46	7
Ritanserin +RU 24969	9:00 9:30	0.16–2.5 mg/kg 0.63 mg/kg	35	5

Results

Dose-response effects of ritanserin

Ritanserin injected at the onset of the light period (9:30 a.m.) induced sleep-wakefulness modifications shown in Fig. 1.

Doses of 0.04 and 0.16 mg/kg produced no major effects on sleep-wakefulness patterns. At the dose of 0.63 mg/kg, ritanserin significantly increased SWS2 for 8 h. In the first 4 h following the treatment, the mean duration of SWS2 episodes was increased (7.2 ± 0.4 min, $P < 0.001$ versus 4.1 ± 0.2 min as the baseline value), whereas the number of episodes was reduced (23 ± 1, $P < 0.001$ versus 34 ± 1 as the baseline value). Concurrently, a significant decrease in W, SWS1 and PS was observed in the first 4-h period. PS reduction persisted into the second 4-h period. For each of these states the number of episodes was decreased while their mean duration tended to be increased. At the highest dose tested (2.5 mg/kg), the SWS2 increasing effect of ritanserin was less pronounced. The PS deficit was accentuated while no significant changes were observed in the amounts of W and SWS1. The SWS1 and SWS2 latencies were unchanged for all the doses of ritanserin tested. The latency for the first episode of PS was dose-dependently increased from the dose of 0.04 mg/kg upwards (136.1 ± 18.1 min, $P < 0.05$ to 188.8 ± 15.5 min, $P < 0.001$ versus 97.5 ± 9.2 min as the baseline value).

Dose-response effects of DOM

The administration of DOM (from 0.16 to 2.5 mg/kg) at the beginning of the light period was followed by a dose-related deficit in both SWS2 and PS during the first 4-h period (Fig. 2). This was due to a decrease in the number of episodes and of their mean duration. Concomitantly, W was dose-dependently increased from the dose of

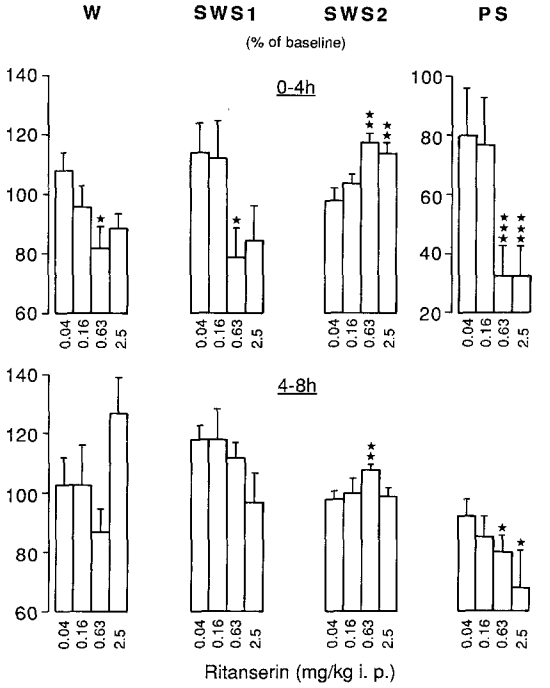

Fig. 1. Dose-response effects of ritanserin on sleep-wakefulness states in rats during the two 4-h periods following the treatment. Mean values ±SEM of 8–11 animals were expressed as per cent of baseline (vehicle injection under the same conditions). *W* wakefulness; *SWS1* light slow wave sleep; *SWS2* deep slow wave sleep; *PS* paradoxical sleep. $*P<0.05$, $**P<0.01$, $***P<0.001$ (two-tailed Student-*t*-test) as compared to baseline

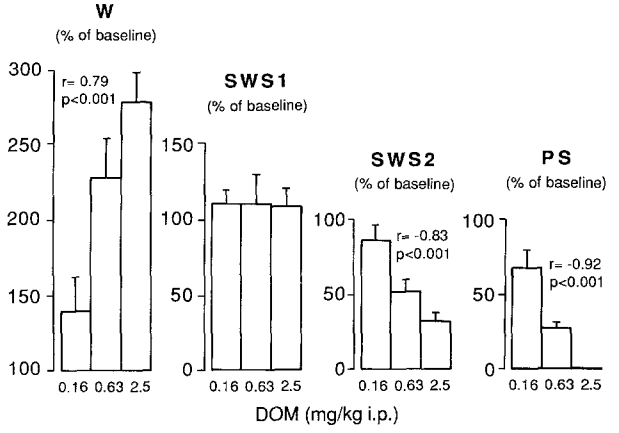

Fig. 2. Dose-response effects of DOM on sleep-wakefulness states in rats during the first 4-h period following the treatment. Mean values ±SEM of four animals were expressed as per cent of baseline (vehicle injection under the same conditions). *W* wakefulness; *SWS1* light slow wave sleep; *SWS2* deep slow wave sleep; *PS* paradoxical sleep. Linear regression (*r* = regression coefficient) was calculated for drug dose-response effects on W, SWS2 and PS

0.16 mg/kg upwards (Fig. 2). The number of W episodes was not modified but their mean duration was enhanced. SWS1 amounts were unaffected by all the doses of DOM tested. In the second 4-h period the amounts of the different states of vigilance returned to baseline values, except PS which remained reduced with doses of 0.63 (−42%, $P<0.01$) and 2.5 mg/kg (−44%, $P<0.05$). SWS1, SWS2 and

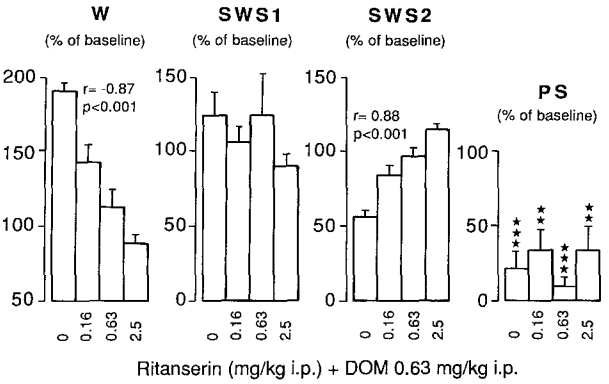

Fig. 3. Dose-response effects of ritanserin on sleep-wakefulness states in DOM-treated rats (0.63 mg/kg IP) during the first 4-h period following the treatment. Mean values ±SEM of six animals were expressed as per cent of baseline (vehicle injection under the same conditions). *W* wakefulness; *SWS1* light slow wave sleep; *SWS2* deep slow wave sleep; *PS*, paradoxical sleep. Linear regression (*r* = regression coefficient) was calculated for drug dose-response effects on W and SWS2. Two-tailed Student-*t*-test was performed for effects on SWS1 and PS $**P<0.01$, $***P<0.001$ as compared to baseline

PS latencies were prolonged in a dose-dependent manner (SWS1 latency: 20.7±8.8 min, $P<0.05$ and 109.6±16.5 min, $P<0.001$ versus 7.2±1.6 min; SWS2 latency: 50.3±6.0 min, $P<0.01$ and 156.4±4.8 min, $P<0.001$ versus 21.8±2.8 min; PS latency: 120.3±15.3 min, NS and 253.3±4.0 min, $P<0.01$ versus 82.5±17.6 min for 0.16, 2.5 mg/kg and baseline, respectively).

Effects of ritanserin combined with DOM

In a first group of rats treated with the 5-HT_2 receptor agonist DOM, dose-response effects of the 5-HT_2 receptor antagonist ritanserin were examined. The results are presented in Fig. 3. The administration of DOM (0.63 mg/kg) in rats pretreated with the vehicle of ritanserin produced similar effects to those obtained in the experiment where the dose-response curve of DOM was investigated. In the first 4-h period, SWS2 and PS amounts were significantly reduced, W was markedly increased whereas SWS1 was not altered. The pretreatment with ritanserin (0.16–2.5 mg/kg) prevented the DOM-induced SWS2 deficit and W enhancement in a dose-dependent manner. This occurred from the dose of 0.16 mg/kg ritanserin, a dose which by itself had no effect on sleep-wakefulness patterns. Complete antagonism was obtained at the dose of 0.63 mg/kg ritanserin. In contrast, ritanserin did not modify the PS deficit produced by DOM. During the second 4-h period, PS amounts remained lower than control values and in the same range for all the doses of ritanserin tested (PS was reduced by about 30%). SWS1 and SWS2 latencies, which were enhanced after DOM, progressively decreased towards control values with increasing doses of ritanserin (Table 2). The latency of the first PS episode was delayed following DOM injection, and was not modified by the pretreatment with ritanserin (Table 2).

Effects of cinanserin combined with DOM

In order to know whether 5-HT_2 receptor antagonists with differing chemical structure, affinity and profile, affect the

Table 2. Dose-response effects of ritanserin on light slow wave sleep (SWS1), deep slow wave sleep (SWS2) and paradoxical sleep (PS) latencies in DOM-treated rats (0.63 mg/kg IP). Mean values ±SEM of six animals were expressed in minutes

	Ritanserin (mg/kg IP) in DOM-treated rats (0.63 mg/kg IP)				
	Baseline	0	0.16	0.63	2.5
SWS1 latency	7.2 ±1.1	58.8 ±14.7*	29.0 ±7.5*	16.9 ±3.7*	13.4 ±1.9*
SWS2 latency	26.8 ±2.9	109.7 ±6.3***	62.9 ±11.5*	34.1 ±4.1	33.4 ±6.7
PS latency	98.1 ±3.6	222.9 ±29.6**	194.7 ±41.3*	233.5 ±21.3**	218.9 ±50.2*

*$P<0.05$, **$P<0.01$, ***$P<0.001$ (two-tailed Student-*t*-test) as compared to baseline (vehicle injection under the same conditions)

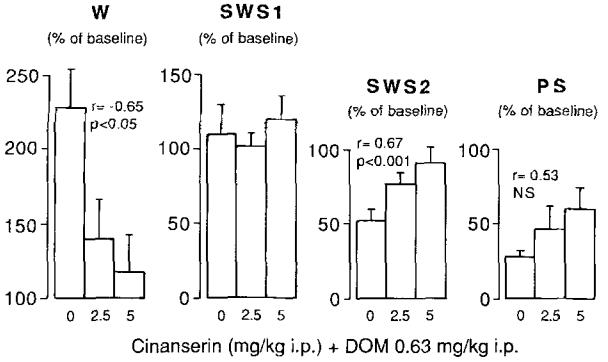

Fig. 4. Dose-response effects of cinanserin on sleep-wakefulness states in DOM-treated rats (0.63 mg/kg IP) during the first 4-h period following the treatment. Mean values ±SEM of four animals were expressed as per cent of baseline (vehicle injection under the same conditions). *W* wakefulness; *SWS1* light slow wave sleep; *SWS2* deep slow wave sleep; *PS* paradoxical sleep. Linear regression (*r*=regression coefficient) was calculated for drug dose-response effects on W, SWS2 and PS

sleep-wakefulness states in a similar way, a second group of rats underwent the same pharmacological procedure with cinanserin, another 5-HT_2 receptor antagonist (Leysen et al. 1981). Cinanserin alone was previously tested in these animals. Administration of 2.5 mg/kg cinanserin had no significant effect on sleep-wakefulness patterns, but at a higher dose (5 mg/kg) PS dropped by 40% ($P<0.001$) during 8 h (not shown). In combination with DOM (0.63 mg/kg) cinanserin dose-dependently prevented the decrease of SWS2 and the increase of W due to the 5-HT_2 receptor agonist (Fig. 4). The lowest effective dose of cinanserin for antagonizing the SWS2 deficit and the W enhancement was 2.5 mg/kg and complete antagonism was obtained at the dose of 5 mg/kg. PS deficit produced by DOM was less pronounced after pretreatment with cinanserin, though PS amounts remained substantially reduced (Fig. 4).

Effects of ritanserin combined with 8-OH-DPAT and RU 24969

Dose-response effects of the 5-HT_2 receptor antagonist ritanserin were analyzed in rats treated with two different

Table 3. Dose-response effects of ritanserin on sleep and wakefulness duration in 8-OH-DPAT-treated rats (2.5 mg/kg IP) during the first 4-h period following the treatment. Mean values ±SEM of 5–7 animals were expressed in minutes

	Ritanserin (mg/kg IP) in 8-OH-DPAT-treated rats (2.5 mg/kg IP)				
	Baseline	0	0.16	0.63	2.5
W	71.5 ±5.6	128.4 ±7.3***	113.1 ±11.0**	112.3 ±15.3*	114.7 ±11.6*
SWS1	30.7 ±2.2	46.1 ±2.4***	52.9 ±5.7**	51.1 ±6.2*	47.1 ±1.6**
SWS2	128.0 ±4.8	64.9 ±7.1***	73.1 ±8.5***	75.8 ±17.2*	77.8 ±11.7*
PS	9.8 ±2.1	0.6 ±0.6**	1.0 ±1.0**	0.8 ±0.8*	0.4 ±0.4*

W, wakefulness; *SWS1* light slow wave sleep; *SWS2* deep slow wave sleep; *PS* paradoxical sleep
*$P<0.05$, **$P<0.01$, ***$P<0.001$ (two-tailed Student-*t*-test) as compared to baseline (vehicle injection under the same conditions)

Table 4. Dose-response effects of ritanserin on sleep and wakefulness duration in RU 24969-treated rats (0.63 mg/kg IP) during the first 4-h period following the treatment. Mean values ±SEM of five animals were expressed in minutes

	Ritanserin (mg/kg IP) in RU24969-treated rats (0.63 mg/kg IP)				
	Baseline	0	0.16	0.63	2.5
W	63.9 ±3.5	124.6 ±18.5*	121.3 ±17.5*	103.8 ±14.1*	123.1 ±21.1*
SWS1	26.2 ±3.1	35.7 ±7.2	42.6 ±9.0	52.9 ±2.5**	47.1 ±5.7
SWS2	136.8 ±6.0	79.7 ±11.5**	76.1 ±10.6***	83.3 ±14.7*	69.8 ±16.4**
PS	13.1 ±1.3	0	0	0	0

W, wakefulness; *SWS1* light slow wave sleep; *SWS2* deep slow wave sleep; *PS* paradoxical sleep
*$P<0.05$, **$P<0.01$, ***$P<0.001$ (two-tailed Student-*t*-test) as compared to baseline (vehicle injection under the same conditions)

5-HT_1 receptor agonists. As illustrated in Tables 3 and 4, the administration of 8-OH-DPAT (2.5 mg/kg) and RU 24969 (0.63 mg/kg) induced a significant decrease of SWS2 and a concomitant increase of W and SWS1 during the first 4-h period following treatment. PS was markedly decreased after 8-OH-DPAT (Table 3) and completely suppressed after RU 24969 (Table 4). The sleep-wakefulness architecture showed a reduction in state transfers, consistent with a decrease in the number of all episodes and an increase in their mean duration. In the second 4-h period the amounts of the different states of vigilance returned to control values, except PS which was still abolished in RU 24969-treated rats. Pretreatment with ritanserin (0.16–2.5 mg/kg) did not modify the sleep-wakefulness alterations produced by 8-OH-DPAT (Table 3) and RU 24969 (Table 4). The amounts of time spent in the dif-

ferent sleep-wakefulness states remained in the same range for all the tested doses of ritanserin.

Discussion

Using compounds which interact at different serotonin binding sites, the present study investigated the functional role of 5-HT_2 receptors in the modulation of the sleep-wakefulness cycle of the rat.

Ritanserin appears to be a selective and potent 5-HT_2 receptor antagonist which easily crosses the blood-brain barrier, since a low dose (0.08 mg/kg) injected subcutaneously produces a 50% 5-HT_2 receptor occupation in the frontal cortex in ex vivo and in vivo binding experiments in the rat (Leysen et al. 1985). Thus, sleep-wakefulness modifications can be attributed to a central action of ritanserin. In agreement with preliminary data obtained in the rat (Dugovic and Wauquier 1987), systemic administration of moderate doses of ritanserin increased SWS2 at the expense of W, SWS1 and PS (Fig. 1). Although the number of episodes was significantly reduced in all the states of vigilance, the marked prolongation of SWS2 episodes led to an increase in the amounts of SWS2, whereas the decrease in the number of W, SWS1 and PS episodes, associated with only a slight increase in their mean duration, resulted in a reduction of the time spent in these states. This suggests that ritanserin alters sleep-wakefulness maintenance and has probably no direct sleep-inducing action. This hypothesis is reinforced by the fact that the SWS latency was not changed. The most pronounced effects were observed at the dose of 0.63 mg/kg, which occupies more than 80% of frontal cortical 5-HT_2 sites for 6 h (Leysen et al. 1985). At a higher dose (2.5 mg/kg), it cannot be excluded that, despite a more than 90% inhibition of 5-HT_2 receptor labeling, a histaminergic component could be involved since a 60% inhibition of histamine-H_1 receptor is observed at this dose in ex vivo binding experiments in guinea-pig brain. Nevertheless, ritanserin exhibits a 50-fold higher affinity for 5-HT_2 receptors than for the histamine-H_1 receptor (Leysen et al. 1985).

It could be argued that ritanserin may also modulate sleep-wakefulness patterns indirectly via effects on blood pressure or on body temperature. However, the compound does not reduce blood pressure following acute or chronic administration in spontaneously hypertensive rats (Gradin et al. 1985) and in patients with essential hypertension (Hedner et al. 1987). Also, ritanserin fails to antagonize serotonin-induced hypothermia (Colpaert et al. 1985) and does not alter locomotor activity at doses up to 40 mg/kg (Awouters et al. 1988) in rats.

An interesting point is that the SWS2-increasing effect of ritanserin in the rat is consistent with the reported clinical findings that the drug promotes stages 3 and 4 in human volunteers (Clarenbach et al. 1986; Idzikowski et al. 1986; Declerck et al. 1987). On the other hand, the decrease in PS amounts in the rat under ritanserin treatment apparently contrasts with the absence of effects on REM sleep observed in humans. Doses and species differences in the affinity for 5-HT_2 receptors could account for the different results.

The stimulation of the same receptors by using the selective 5-HT_2 receptor agonist DOM (Shannon et al. 1984) produced opposite effects on W and SWS2 to those obtained with ritanserin. Indeed, DOM dose-dependently increased W and decreased SWS2 (Fig. 2). This is in accordance with the reported finding that DOM elicitates hyperactivity which is associated with an activation of the EEG in rats, cats and rabbits (Florio et al. 1969). A dose-related PS deficit was also observed (Fig. 2), which seems to be a common response to the stimulation or the blockade of 5-HT_2 receptors. With regard to other binding sites, DOM has a 30-fold selectivity for 5-HT_2 sites as compared with 5-HT_1 sites (Shannon et al. 1984), but it is only 10-fold more selective for 5-HT_2 receptors than adrenergic-α_2 receptors (Leysen 1988). It is possible that an adrenergic-α_2 effect of DOM would have an activating influence and mediate the effect on PS. Indeed, the administration of the adrenergic-α_2 agonist clonidine is followed by a PS inhibition in rats, cats and humans (see Gaillard 1983).

The fact that ritanserin clearly manifested a dose-related antagonism of the DOM-induced W enhancement and SWS2 deficit (Fig. 3) is consistent with a role for 5-HT_2 receptors in the modulation of W and SWS2. Also, the selective 5-HT_2 receptor antagonist cinanserin (Leysen et al. 1981) dose-dependently prevented these effects of DOM (Fig. 4). However, cinanserin alone failed to alter the amount of W and SWS2. Apart from the fact that cinanserin and ritanserin come from different chemical series, it is noteworthy that cinanserin has a 7-fold lower affinity for the 5-HT_2 receptor. The restricted dose range presently studied may have been below the threshold for intrinsic effects of cinanserin on W and SWS2. At the highest dose tested (5 mg/kg) a small but non-significant increase in SWS2 was seen. Higher doses may therefore have similar effects to ritanserin. On the other hand, an interaction with histamine-H_1-related mechanisms could be considered, even though DOM as well as cinanserin show very low affinity for the histamine-H_1 receptor (Leysen 1988). It would be interesting to examine whether ritanserin counteracts the effects of a histamine-H_1 agonist on sleep and wakefulness.

The involvement of 5-HT_2 receptors in PS is less clear. DOM induced a PS deficit which was neither potentiated nor antagonized by ritanserin pretreatment (Fig. 3) and was only partially antagonized by cinanserin pretreatment (Fig. 4). This makes it possible that 5-HT_2 receptors are not directly implicated in regulating PS mechanism. However, in neonatal and juvenile rats treated with ritanserin, all the sleep-wakefulness states return to control values after administering the 5-HT_2 receptor agonist (1-(2,5-dimethyoxyphenyl-4-bromo)-2-aminopropane (DOB) (Titeler et al. 1985) instead of DOM (Davenne et al. 1988). The fact that DOB exhibits a higher affinity for 5-HT_2 receptors than DOM (Shannon et al. 1984) could account for the discrepancy observed between DOM and DOB in PS effects. On the other hand, most of drugs which alter serotonin-mediated transmission reduce PS. Thus, even though 5-HT_2 receptor antagonists delay the onset of PS at lower doses than those increasing SWS2, it is difficult to accept that PS alterations are a primary consequence of 5-HT_2 receptor blockade. Changes in PS amount after serotonergic drugs may also result from indirect interactions with the noradrenergic system, which plays an important role in PS regulation (Jouvet 1969).

In the next phase of this study, tests with putative 5-HT_1 receptor agonists in combination with ritanserin were conducted in order to investigate a possible interaction between 5-HT_1 and 5-HT_2 receptor sites in modulating sleep-wakefulness patterns. 8-OH-DPAT and RU 24969 are serotonin

agonists that display a preferential affinity for 5-HT_{1A} (Middlemiss and Fozard 1983) and 5-HT_{1B} (Sills et al. 1984) sites, respectively. Both compounds induced a substantial increase in W and SWS1 at the expense of SWS2 and PS (Tables 3 and 4). These data confirm results observed by Dzoljic et al. (1987) and by Depoortere and Riou-Merle (1988) in rats. As sleep-wakefulness alterations produced by 8-OH-DPAT and RU 24969 were not changed by ritanserin pretreatment (Tables 3 and 4), it may be hypothesized that 5-HT_1 receptors are not involved in the expression of 5-HT_2 receptor-mediated modulation of sleep-wakefulness patterns. However, we should be cautious in interpreting these results. First, administration of 8-OH-DPAT or RU 24969 in rats produces behavioural changes including hyperlocomotion that have been proposed to reflect activation of the 5-HT_1 receptor (Green et al. 1984; Tricklebank et al. 1984). This behavioural response could have contributed to the increase in W observed with these drugs and is probably resistant to blockade by ritanserin. Second, on the basis of recent biochemical data it has been suggested that the 5-HT_{1A} receptor is located on both pre- and postsynaptic sites in rat brain (Gozlan et al. 1983) and that either the 5-HT_{1A} or the 5-HT_{1B} receptor is involved in the control of serotonin release (see Conn and Sanders-Bush 1987). Also, 8-OH-DPAT can act as an antagonist of serotonin in rat hippocampus (Colino and Halliwell 1986). In addition, since an antagonist to either 8-OH-DPAT or RU 24969 is not available (Bradley et al. 1986), it is premature to precisely define the functional role of 5-HT_1 sites in sleep-wakefulness regulation.

In conclusion, the present work further supports the proposition that the serotonergic system plays an important role in the maintenance of the sleep-wakefulness cycle and demonstrates that 5-HT_2 receptors are functionally involved. A relationship between the functional state of 5-HT_1 receptor subtypes and the states of vigilance may also exist. However, 5-HT_1 receptors do not interact with 5-HT_2 receptors in the sleep-wakefulness modulation mediated through 5-HT_2 receptors.

Acknowledgement. Partial support for this study was provided by I.W.O.N.L. We are grateful to Dr. David Ashton for his very helpful comments and review of the manuscript.

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Received August 16, 1988 / Final version November 7, 1988