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Key Role of 5-HT$_{1B}$ Receptors in the Regulation of Paradoxical Sleep as Evidenced in 5-HT$_{1B}$ Knock-Out Mice

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The involvement of 5-HT$_{1B}$ receptors in the regulation of vigilance states was assessed by investigating the spontaneous sleep–waking cycles and the effects of 5-HT receptor ligands on sleep in knock-out (5-HT$_{1B}$−/−) mice that do not express this receptor type. Both 5-HT$_{1B}$−/− and wild-type 129/Sv mice exhibited a clear-cut diurnal sleep–wakefulness rhythm, but knock-out animals were characterized by higher amounts of paradoxical sleep and lower amounts of slow-wave sleep during the light phase and by a lack of paradoxical sleep rebound after deprivation. In wild-type mice, the 5-HT$_{1B}$ agonists CP 94253 (1–10 mg/kg, i.p.) and RU 24969 (0.25–2.0 mg/kg, i.p.) induced a dose-dependent reduction of paradoxical sleep during the 2–6 hr after injection, whereas the 5-HT$_{1B/1D}$ antagonist GR 127935 (0.1–1.0 mg/kg, i.p.) enhanced paradoxical sleep. In addition, pretreatment with GR 127935, but not with the 5-HT$_{1A}$ antagonist WAY 100635, prevented the effects of both 5-HT$_{1B}$ agonists. In contrast, none of the 5-HT$_{1B}$ receptor ligands, at the same doses as those used in wild-type mice, had any effect on sleep in 5-HT$_{1B}$−/− mutants. Finally, the 5-HT$_{1A}$ agonist 8-OH-DPAT (0.2–1.2 mg/kg, s.c.) induced in both strains a reduction in the amount of paradoxical sleep. Altogether, these data indicate that 5-HT$_{1B}$ receptors participate in the regulation of paradoxical sleep in the mouse.

Key words: serotonin; 5-HT$_{1B}$ receptor; paradoxical sleep; knock-out; mice

The idea that serotonin [5-hydroxytryptamine (5-HT)] is involved in the regulation of sleep–wakefulness cycles was proposed several decades ago (Koella et al., 1968; Jouvet, 1969) and has been further supported recently by using new means of investigations (Cespuglio et al., 1990; Portas and McCarley, 1994). The respective roles of various classes of central 5-HT receptors in this regulation have been investigated primarily by pharmacological means. Notably, it has been reported that 5-HT$_{1A}$ receptors are involved in the regulation of paradoxical sleep (PS) and wakefulness (W) (de Saint Hilaire-Kafi et al., 1987; Dzoljic et al., 1992; Tissier et al., 1993; Portas et al., 1996; Thakkar et al., 1998) and that 5-HT$_{2A}$ receptors participate in the control of slow-wave sleep (SWS) (Idzikowski et al., 1986; Dugovic et al., 1989).

Despite the development of numerous ligands in the past 15 years, it was not possible to investigate specifically the involvement of 5-HT$_{1B}$ receptors in the regulation of sleep–wakefulness cycles because of the paucity of selective agonists and antagonists able to cross the blood–brain barrier. Nevertheless, a few studies led to the suggestion that 5-HT$_{1B}$ receptor stimulation might exert a negative influence on PS (Dugovic et al., 1989; Dzoljic et al., 1992; Bjorvatn and Ursin, 1994).

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Gene targeting is another means that allows a selective approach to study the role of a specific receptor in sleep regulations. To date, several groups have reported behavioral modifications in transgenic mutants (Montkowski et al., 1995; Sollars et al., 1996; Zhang et al., 1996; Tobler et al., 1997), notably the 5-HT$_{1B}$ receptor gene knock-out (5-HT$_{1B}$−/−) mutant mice (Saudou et al., 1994; Crabbe et al., 1996; Dulawa et al., 1997; Rocha et al., 1997).

The 5-HT$_{1B}$ receptor is located on both presynaptic serotoninergic terminals (Boschert et al., 1994), where it modulates 5-HT release (Engel et al., 1986), and nonserotoninergic terminals, where it modulates the release of, notably, acetylcholine (ACh) (Maura and Raiteri, 1986) and GABA (Stanford and Lacey, 1996). Interestingly, the latter two are involved in sleep–waking regulations (Gillin et al., 1985) at mesopontine tegmental (McCarley and Massaquoi, 1992) and basal forebrain (Cape and Jones, 1998) levels and in the dorsal raphe (Nitz and Siegel, 1997a), the locus ceruleus (Nitz and Siegel, 1997b), and the hypothalamic preoptic (Mendelson, 1998) nuclei.

The aim of the present study was to investigate the role of 5-HT$_{1B}$ receptors in sleep and wakefulness in mice. For this purpose, the spontaneous sleep–waking cycles and the recovery after selective paradoxical sleep deprivation were examined in 5-HT$_{1B}$−/− mutants (Saudou et al., 1994) compared with wild-type 129/Sv mice. In addition, we analyzed in both strains the effects of treatments with 5-HT$_{1B}$ receptor ligands on the vigilance states. Studies were also performed with 5-HT$_{1A}$ receptor ligands, whose well characterized effects on sleep and wakefulness in rodents (Dzoljic et al., 1992; Tissier et al., 1993) were used as a reference.

MATERIALS AND METHODS

All the procedures involving animals and their care were conducted in conformity with the institutional guidelines, which are in compliance

with national and international laws and policies [Council Directive 87–848, October 19, 1987, from Ministère de l'agriculture et de la forêt, Service vétérinaire de la santé et de la protection animale, Permissions 0299 (to M.H.) and 0315 (to J.A.)].

Surgery

Wild-type (5-HT_{1B}+/+) and 5-HT_{1B}−/− mice, both with a pure 129/Sv genetic background (Ramboz et al., 1996), were used. At 2 months of age, when body weight was similar in both groups (range, 24–30 gm), animals were implanted under sodium pentobarbital anesthesia (70–75 mg/kg, i.p.) with the standard set of electrodes (made of enameled nichrome wire, 150 μm in diameter) for polygraphic sleep monitoring (Tissier et al., 1993). In brief, EEG electrodes were inserted through the skull onto the dura over the right cortex (2 mm lateral and 4 mm posterior to the bregma) and over the cerebellum (at midline, 2 mm posterior to lambda) (Tobler et al., 1997), electro-oculogram electrodes were positioned subcutaneously on each side of the orbit, and EMG electrodes were inserted into the neck muscles. All electrodes were anchored to the skull with superbond and acrylic cement (Limoge-Lendais et al., 1994) and soldered to a mini-connector also embedded in cement. After completion of surgery, animals were housed in individual cages (20 × 20 × 30 cm) and maintained under standard laboratory conditions: 12 hr light/dark cycle (light on at 7:00 A.M.), food and water available *ad libitum*, and 24 ± 1°C ambient temperature. The animals were allowed 7–10 d to recover, during which they were habituated to the recording conditions.

PS deprivation

Animals were placed for 12 hr, starting at the beginning of either the dark or the light period, on platforms (control conditions: 7.5 cm in diameter, 3 cm high; deprivation conditions: 3.5 cm in diameter, 4 cm high) surrounded by water (2 cm deep) (Pokk et al., 1996) at an ambient temperature of 25°C, with access to food and water *ad libitum*. At the end of this period, mice were returned to their home cage for 12 hr for recovery, the latter period thus occurring during the light or the dark period, respectively. Each mouse underwent the paired control and deprivation procedure (separated by at least 4 d), first during the dark period and second (at least 10 d later) during the light period.

Pharmacological procedures

Drugs were dissolved in 0.1 ml of saline, except CP 94253 [3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine], which was dissolved in warm distilled water. All injections were performed at 9:30–10:00 A.M. WAY 100635 [*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl)cyclohexane carboxamide], GR 127935 [2′-methyl-4′-(5-methyl-[1,2,4] oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazine-1-yl)-phenyl]amide], RU24969 [5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole], and CP 94253 were injected intraperitoneally, and 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)-tetralin] was injected subcutaneously. A 15 min interval separated the two injections when animals were treated with an antagonist and then an agonist. For baseline data, mice were injected intraperitoneally or subcutaneously with the vehicle only, as appropriate. In each case, a delay of at least 48 hr separated two successive pharmacological tests to allow complete washout of drugs (Frances and Monier,1991; Koe et al., 1992; Pauwels, 1997).

Polygraphic recording

For the study of spontaneous sleep–waking cycles, each animal was recorded during 48 hr, beginning at 7:00 P.M., i.e., at the onset of the dark period. For PS deprivation experiments, mice were recorded during 24 consecutive hours, beginning at 7:00 P.M. for the first paired series and at 7:00 A.M. for the second one. For pharmacological studies, sleep–wakefulness parameters were recorded during the 8 hr after injections, i.e., from 10:00 A.M. to 6:00 P.M.

Data analysis and statistics

Polygraphic recordings were scored manually every 30 sec epoch, using the criteria validated for mice (Valatx and Bugat, 1974). Data were fed into a computer according to a method described previously (Tissier et al., 1993).

Spontaneous sleep–waking cycles. For each animal, the amounts of vigilance states were calculated over 3 hr periods throughout 48 hr and were averaged for the light and the dark phases. The mean ± SEM of these amounts (expressed in minutes) for each strain of mice was then used for calculating the ANOVA for the factor genotype. In case of significance ($p < 0.05$), the F test was followed by Student's t test for mean comparisons.

PS deprivation. For each animal, the sleep amounts during the small platform condition and the following recovery period were compared with those during the large platform condition and the corresponding control recovery period, and expressed as percent of respective baseline. Two PS latencies were defined: one as the time interval between the beginning of the recovery phase and the first PS episode (PS latency) and the other as the time interval between the first SWS episode and the first PS one (intrasleep PS latency). Paired t tests were performed to assess statistical significance of the data.

Pharmacological experiments. The effects of each dose of a given compound on each state of vigilance were analyzed for every 2 hr period after injection and expressed in minutes as mean ± SEM. The PS latency was defined as the time interval between the end of injection and the onset of the first PS episode. For a given treatment, each animal was referred to its own baseline represented by the data obtained after injection of vehicle. Statistical analyses were performed using ANOVA for the factor treatment, and in case of significance ($p < 0.05$), the F test was followed by Student's t test (paired samples) for mean comparisons.

Chemicals

RU 24969 (0.25–5.0 mg/kg, i.p.) was obtained from Roussel-Uclaf (Romainville, France); WAY 100635 (0.05–1.0 mg/kg, i.p.) was from Wyeth Research (Princeton, NJ); 8-OH-DPAT (0.2–1.2 mg/kg, s.c.) was obtained from Research Biochemicals (Natick, MA); CP 94253 (1.0–10.0 mg/kg, i.p.) was from Pfizer Central Research (Groton, CT); and GR 127935 (0.1–1.0 mg/kg, i.p.) was from Glaxo-Wellcome (Ware, UK).

RESULTS

Previous studies have shown that 5-HT_{1B}−/− mice develop normally, have no histologically detectable defects of the CNS, and do not exhibit obvious behavioral impairments (Ramboz et al., 1996). In the present study, we confirmed that 5-HT_{1B}−/− mice had similar body weight as the wild-type mice and no apparent behavioral alterations.

Spontaneous sleep–wakefulness cycles

All mice exhibited a clear-cut diurnal sleep–waking rhythm, with larger amounts of sleep during the light period than during the dark one. Indeed, they spent ~70% of the time asleep during the light phase (70.6 ± 0.8 and 69.8 ± 1.6% in seven 5-HT_{1B}+/+ and eight 5-HT_{1B}−/− mice, respectively) compared with ~45% in the dark one (46.8 ± 1.8 and 44.0 ± 2.8%, respectively). However, the 5-HT_{1B}−/− mice differed significantly ($p < 0.05$) from the wild-type mice by a greater amount of PS (11.9 ± 0.7% of total time compared with 8.9 ± 0.3% in the 5-HT_{1B}+/+ group), at the expense of SWS (58.0 ± 1.3 and 61.8 ± 1.0%, respectively) during the 12 hr of the light phase (Table 1). No significant differences were found between the two groups during the dark phase.

The analysis per 3 hr period indicates that the major difference between the two groups was a peak of PS in the middle of the light phase in mutant mice but not in 5-HT_{1B}+/+ animals (Fig. 1).

Paradoxical sleep deprivation

Only two mice (one in each strain) fell from the platform into the water during the deprivation protocol and were excluded from the analysis. During the deprivation periods (small platform), mice of both groups (n = 5–7) exhibited the same amounts of SWS but only 20–30% of PS (data not shown) compared with those observed under control conditions (large platform). Then, the amounts of PS in the wild-type group were significantly enhanced during the first 3 hr of the recovery period after PS deprivation for either the dark or the light phase (12 hr); in addition, the intrasleep PS latency (but not the PS latency) was reduced in wild-type mice (Fig. 2). In contrast, in the 5-HT_{1B} knock-out

Table 1. Amounts of wakefulness (W), slow-wave sleep (SWS), and paradoxical sleep (PS) in 5-HT$_{1B}$+/+ and 5-HT$_{1B}$−/− mice

State amounts (minutes)

Genotype	Period	W	SWS	PS
5-HT$_{1B}$+/+ (n = 7)	light	211.8 ± 5.5	444.8 ± 7.0	63.8 ± 2.3
	dark	382.9 ± 13.2	302.5 ± 13.4	34.8 ± 1.9
5-HT$_{1B}$−/− (n = 8)	light	216.8 ± 11.2	417.5 ± 9.3*	86.1 ± 5.2*
	dark	403.2 ± 20.2	280.8 ± 20.0	36.4 ± 3.2

Results are expressed as minutes (mean ± SEM of n animals) during the 12 hr light (7:00 A.M. to 7:00 P.M.) and dark (7:00 P.M. to 7:00 A.M.) periods.

*$p < 0.05$, significantly different from 5-HT$_{1B}$$^{+/+}$ group; Student's t test.

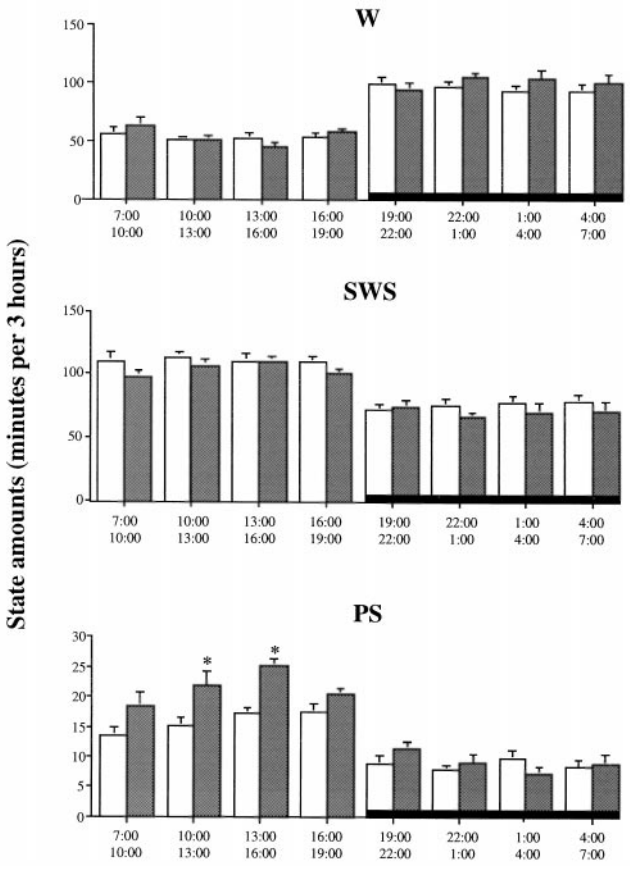

Figure 1. Diurnal variations of wakefulness (*W*), slow-wave sleep (*SWS*), and paradoxical sleep (*PS*) during 12 hr light/dark cycle (light on from 7:00 A.M. to 7:00 P.M.) in 5-HT$_{1B}$+/+ (*open bars*) and 5-HT$_{1B}$−/− (*filled bars*) mice. Data are expressed as min/3 hr (mean ± SEM of 7 and 8 animals, respectively). *$p < 0.05$, significantly different from the 5-HT$_{1B}$+/+ group; Student's t test.

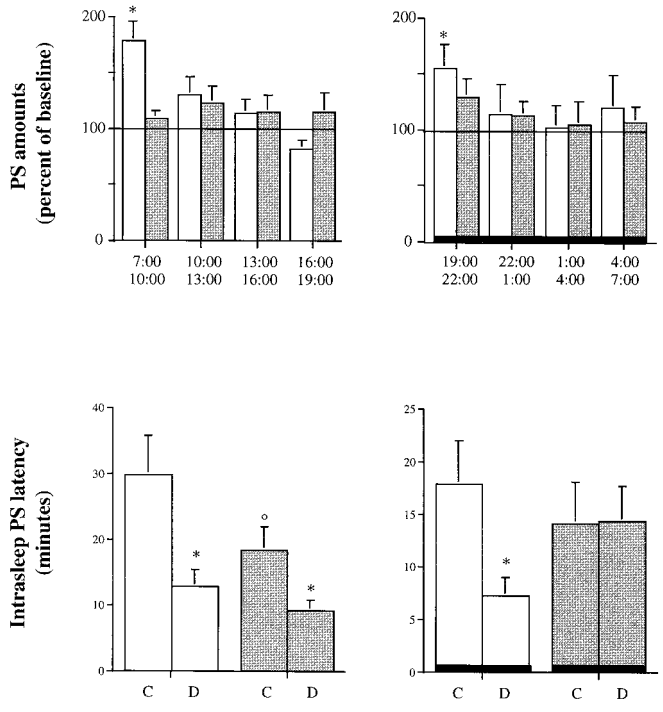

Figure 2. Paradoxical sleep characteristics observed after a 12 hr PS deprivation performed during either the preceding dark period (*left*) or the preceding light period (*right*) in 5-HT$_{1B}$+/+ (*open bars*) and 5-HT$_{1B}$−/− (*filled bars*) mice. *Top*, PS amounts (mean ± SEM of 5 and 7 animals, respectively) are expressed as percent of the paired values obtained under control conditions (large platform). *Bottom*, Intrasleep PS latency observed at recovery is expressed as minutes (mean ± SEM) after control (*C*, large platform) or deprivation (*D*, small platform) conditions. *$p < 0.05$, significantly different from control conditions; paired Student's t test. °$p < 0.05$, significantly different from the 5-HT$_{1B}$+/+ group; Student's t test.

group, no significant increase in PS was observed for the recovery period (except for a trend after deprivation performed during the light phase), and the intrasleep PS latency was significantly reduced only after the deprivation performed during the dark phase (Fig. 2).

Under control conditions in which mice were on the large platform, differences between the two groups were observed only during the light period. Thus, PS amounts were larger (+25.1%; $p < 0.05$; data not shown), and intrasleep PS latencies were smaller (−30.0%; $p < 0.05$) (Fig. 2) in 5-HT$_{1B}$−/− mutants than in wild-type 5-HT$_{1B}$+/+ mice.

Effects of 5-HT$_{1B}$ receptor ligands

Agonists

The 5-HT$_{1A/1B}$ agonist RU 24969 (Hoyer et al., 1994) (Figs. 3, 4) and the selective 5-HT$_{1B}$ agonist CP 94253 (Koe et al., 1992) (Table 2, Fig. 4) induced a dose-related inhibition of PS during essentially the 2–6 hr after injection in wild-type mice (ANOVA; during the 0–4 hr period after treatment; RU 24969, $F_{(4,31)} = 78.68$; $p < 0.05$; and CP 94253, $F_{(5,30)} = 39.5$; $p < 0.05$) (Fig. 4). PS latency was significantly increased up to 257.8 ± 16.0 min

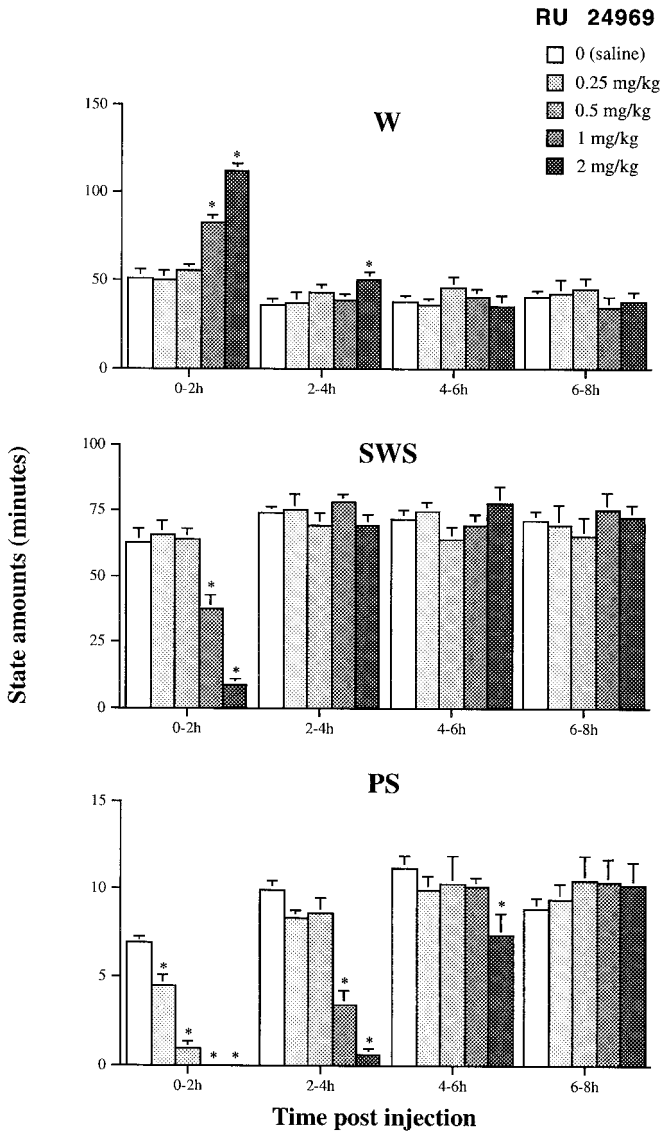

Figure 3. Effects of the 5-HT$_{1A/1B}$ agonist RU 24969 at various doses on sleep and wakefulness in 5-HT$_{1B}$+/+ mice during the four successive 2 hr periods after injection. Results are expressed as min/2 hr (mean ± SEM of 8 animals; 6–8 tests for each dose). *$p < 0.05$, significantly different from baseline (*open bars*); paired Student's *t* test.

(mean ± SEM; $n = 8$) with the highest dose of RU 24969 (2 mg/kg, i.p.), and 233.0 ± 14.8 min (mean ± SEM; $n = 6$) with that of CP 94253 (10 mg/kg, i.p.) compared with 45.3 ± 3.1 and 54.2 ± 5.3 min ($p < 0.05$; data not shown) after administration of the vehicle, respectively. In contrast, wakefulness and SWS were not modified, except at 1 and 2 mg/kg of RU 24969 (Fig. 3) and at 10 mg/kg of CP 94253 (Table 2) for which SWS was reduced during 2 and 4 hr, respectively, at the benefit of wakefulness. Finally, for both RU 24969 and CP 94253, no modification of sleep–waking cycles was observed during the 6–8 (Fig. 3) and 4–8 (data not shown) hr periods after treatment, respectively.

In the 5-HT$_{1B}$−/− group, neither RU 24969 nor CP 94253, in the same dose ranges as those used in the 5-HT$_{1B}$+/+ group, induced any significant alteration of sleep–wakefulness cycles (Fig. 4, Table 2). However, at 3 and 5 mg/kg (data not shown), RU 24969 induced an inhibition of PS during 4 hr after injection

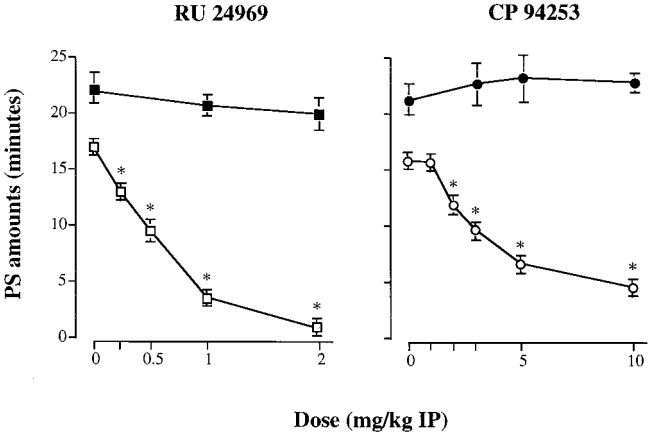

Figure 4. Effects of RU 24969 (*left*) and CP 94253 (*right*) at various doses on PS in 5-HT$_{1B}$+/+ (*open symbols*) and 5-HT$_{1B}$−/− (*filled symbols*) mice during the 4 hr after injection in which an effect was observed. Results are expressed as minutes (mean ± SEM of 8 mice in each group for RU 24969 and 6 mice for CP 94253; 5–8 tests for each dose). *$p < 0.05$, significantly different from baseline (*0* on *abscissa*); paired Student's *t* test. Complete set of data is available on request.

(PS amounts of 8.9 ± 1.1 min; $n = 8$; and 0.3 ± 0.2 min; $n = 5$, respectively; compared with 22.0 ± 1.6 min after saline; $p < 0.05$). At 5 mg/kg, a consecutive PS rebound was observed during the 6–8 hr period after injection (PS amounts of 17.2 ± 1.6 min compared with 11.8 ± 1.8 min in saline-treated mice; mean ± SEM; $n = 5$; $p < 0.05$). Concomitantly, an increase in PS latency was observed in 5-HT$_{1B}$−/− mice injected with 3 and 5 mg/kg RU 24969 (138.7 ± 11.1 min; $n = 8$; and 277.6 ± 23.6 min; $n = 5$, respectively; compared with 40.1 ± 3.2 min after saline; $p < 0.05$). The other states of vigilance were not affected, except wakefulness, which was significantly increased (+26 ± 8%; $p < 0.05$) during the first 2 hr after injection of 5 mg/kg RU 24969 (data not shown).

Antagonist

In 5-HT$_{1B}$+/+ mice, the 5-HT$_{1B/1D}$ antagonist GR 127935 (Pauwels, 1997), at the doses of 0.1, 0.5 (data not shown), and 1.0 (Fig. 5) mg/kg induced no modification of sleep–wakefulness during the first 2 hr period after treatment. Thereafter, a dose-dependent enhancement of PS amounts was observed (ANOVA; $F_{(3,26)} = 9.93$; $p < 0.05$), in particular at 0.5 (data not shown) and 1.0 (Fig. 5) mg/kg. The other states of vigilance were not affected (data not shown).

In 5-HT$_{1B}$−/− mice, GR 127935 at 0.5 (data not shown) and 1.0 (Fig. 5) mg/kg had no effect on PS. However, an increase of SWS, at the expense of W, was observed for the first 2 hr after the administration of 0.5 mg/kg of this drug (data not shown).

In 5-HT$_{1B}$+/+ mice, the effects of both RU 24969 (0.5 mg/kg) and CP 94253 (5 mg/kg) on sleep–wakefulness cycles were prevented by pretreatment with GR 127935 at the dose of 1 mg/kg (Fig. 6, Table 3). In contrast, in 5-HT$_{1B}$−/− mice, 1 mg/kg of GR 127935 (Table 3) did not prevent the effects of RU 24969 at the dose of 3 mg/kg (i.e., the dose inducing the same PS inhibition in 5-HT$_{1B}$−/− mice as 0.5 mg/kg in wild-type mice) (Fig. 4).

Effects of 5-HT$_{1A}$ receptor ligands

Agonist

The 5-HT$_{1A}$ agonist 8-OH-DPAT (Hoyer et al., 1994) induced in all mice a dose-dependent inhibition of PS during the first 2 hr

Table 2. Effects of the 5-HT$_{1B}$ agonist CP 94253 at various doses on sleep and wakefulness in 5-HT$_{1B}$+/+ and 5-HT$_{1B}$−/− mice during the 4 hr after injection

		State amounts (minutes)			
		5-HT$_{1B}$+/+		5-HT$_{1B}$−/−	
	CP 94253 (mg/kg)	0–2 hr	2–4 hr	0–2 hr	2–4 hr
W	0	43.8 ± 4.8	30.0 ± 2.9	48.3 ± 2.7	40.5 ± 1.4
	1	39.7 ± 2.8	36.0 ± 4.3		
	2	45.1 ± 1.9	31.5 ± 2.4		
	3	41.4 ± 3.2	41.8 ± 3.6	50.2 ± 4.6	35.0 ± 2.9
	5	48.6 ± 5.0	36.7 ± 3.5	44.9 ± 3.1	39.8 ± 2.6
	10	105.4 ± 4.5*	56.2 ± 5.7*	46.0 ± 1.9	35.1 ± 3.1
SWS	0	69.8 ± 4.9	80.4 ± 3.2	62.8 ± 2.4	67.7 ± 2.1
	1	73.8 ± 2.8	74.5 ± 4.3		
	2	71.6 ± 2.0	80.2 ± 2.9		
	3	77.0 ± 2.9	71.0 ± 4.0	61.8 ± 4.0	71.0 ± 1.7
	5	71.1 ± 4.9	78.3 ± 3.2	65.7 ± 3.3	66.9 ± 2.3
	10	14.6 ± 4.5*	61.2 ± 6.0*	65.4 ± 1.6	71.2 ± 2.8
PS	0	6.4 ± 0.2	10.3 ± 0.6	8.8 ± 0.6	11.7 ± 0.6
	1	6.5 ± 0.5	9.5 ± 1.0		
	2	3.1 ± 0.5*	8.3 ± 0.7		
	3	1.7 ± 0.5*	7.1 ± 0.8*	8.1 ± 1.1	14.0 ± 1.6
	5	0.2 ± 0.2*	4.9 ± 0.8*	9.4 ± 0.7	13.2 ± 2.2
	10	0*	2.6 ± 0.7*	8.6 ± 0.6	13.8 ± 0.9

Results are expressed as min/2 hr period (mean ± SEM of 6 animals in each group; 5–6 tests for each dose) for the time after injection in which the effects were observed.

*$p < 0.05$, significantly different from baseline (0); paired Student's *t* test.

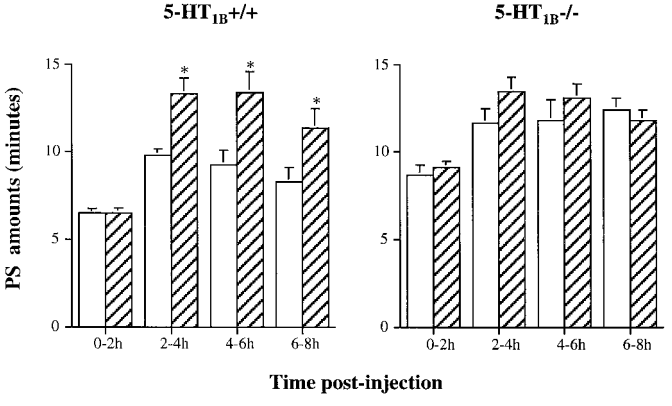

Figure 5. Effects of the 5-HT$_{1B/1D}$ antagonist GR 127935 (*hatched bars*) on PS in 5-HT$_{1B}$+/+ (*left*) and 5-HT$_{1B}$−/− (*right*) mice during the four successive 2 hr periods after injection. Results are expressed as min/2 hr (mean ± SEM of 8 and 6 animals, respectively). *$p < 0.05$, significantly different from baseline (*open bars*); paired Student's *t* test.

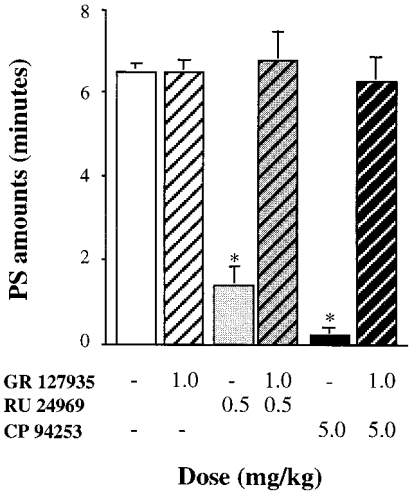

Figure 6. Effects of the 5-HT$_{1B/1D}$ antagonist GR 127935 (*hatched bars*) on PS inhibition induced by RU 24969 (*gray bars*) or CP 94253 (*black bars*) in 5-HT$_{1B}$+/+ mice during the 2 hr period after injection in which an effect was observed. Results are expressed as minutes (mean ± SEM of 8 animals; 8 and 6 tests for each treatment, respectively). *$p < 0.05$, significantly different from baseline (*open bar*); paired Student's *t* test. Complete set of data is available on request.

after injection (ANOVA; wild-type, $F_{(4,27)} = 33.93$; $p < 0.05$; and knock-out, $F_{(4,30)} = 49.46$; $p < 0.05$) (Table 4). This effect was significantly more pronounced in 5-HT$_{1B}$−/− mice than in wild-type animals ($p < 0.05$; unpaired *t* test). In addition, in the 5-HT$_{1B}$−/− group, a consecutive PS rebound was observed during the 4–6 hr after administration of 0.8 mg/kg 8-OH-DPAT, whereas such a rebound was observed at the dose of 1.2 mg/kg in the 5-HT$_{1B}$+/+ group (Table 4). In both groups, 8-OH-DPAT also induced during the first 2 hr after injection an increase of W (ANOVA; 5-HT$_{1B}$+/+ mice, $F_{(4,27)} = 14.08$; $p < 0.05$; and 5-HT$_{1B}$−/− mutants, $F_{(4,30)} = 14.89$; $p < 0.05$), concomitant with a decrease of SWS (ANOVA; $F_{(4,27)} = 10.27$; $p < 0.05$; and $F_{(4,30)} = 10.98$; $p < 0.05$, respectively) (data not shown).

Antagonist

The 5-HT$_{1A}$ antagonist WAY 100635 (Fletcher et al., 1996), at the doses of 0.05–1.0 mg/kg, induced no significant modifications of sleep–waking cycles in any group of mice (data not shown).

Table 3. Effects of the 5-HT$_{1B/1D}$ antagonist GR 127935, in association with the 5-HT$_{1A/1B}$ agonist RU 24969, on sleep and wakefulness in 5-HT$_{1B}$+/+ and 5-HT$_{1B}$−/− mice during the first 2 hr after injection

	State amounts (minutes)							
	5-HT$_{1B}$+/+				5-HT$_{1B}$−/−			
	GR 127935 (mg/kg)	RU 24969 (mg/kg)	*n*	0–2 hr	GR 127935 (mg/kg)	RU 24969 (mg/kg)	*n*	0–2 hr
W	0	0	8	42.3 ± 3.9	0	0	6	51.9 ± 3.2
	—	0.5	7	56.6 ± 1.6	—	3	6	52.8 ± 4.2
	1	0.5	8	39.2 ± 2.6	1	3	6	48.8 ± 7.8
SWS	0	0	8	71.2 ± 3.9	0	0	6	60.0 ± 3.6
	—	0.5	7	62.0 ± 2.0	—	3	6	66.3 ± 4.0
	1	0.5	8	74.0 ± 2.8	1	3	6	70.4 ± 7.4
PS	0	0	8	6.5 ± 0.2	0	0	6	8.9 ± 0.7
	—	0.5	7	1.4 ± 0.6*	—	3	6	0.8 ± 0.4*
	1	0.5	8	6.8 ± 0.7	1	3	6	0.8 ± 0.5*

Results are expressed as minutes (mean ± SEM of 8 and 6 animals, respectively; *n* tests for each dose) during the 2 hr after injection in which the effects were observed.
*$p < 0.05$, significantly different from baseline (0); paired Student's *t* test.

Table 4. Effects of the 5-HT$_{1A}$ agonist 8-OH-DPAT at various doses on paradoxical sleep in 5-HT$_{1B}^{+/+}$ and 5-HT$_{1B}^{-/-}$ mice during the 8 hr after injection

	PS amounts (minutes)									
8-OH-DPAT (mg/kg)	5-HT$_{1B}$+/+					5-HT$_{1B}$−/−				
	n	0–2 hr	2–4 hr	4–6 hr	6–8 hr	*n*	0–2 hr	2–4 hr	4–6 hr	6–8 hr
0	8	6.6 ± 0.2	10.2 ± 0.5	9.8 ± 1.0	9.1 ± 0.7	8	8.6 ± 0.5	13.2 ± 1.5	12.2 ± 0.9	12.2 ± 0.8
0.2	7	5.6 ± 0.4	10.6 ± 1.2	10.9 ± 1.2	11.0 ± 0.8	6	5.2 ± 0.5*	12.4 ± 1.2	12.3 ± 1.2	11.6 ± 0.5
0.4	7	3.6 ± 0.6*	10.4 ± 1.0	11.1 ± 1.0	9.9 ± 1.2	8	2.8 ± 0.5*	10.5 ± 0.9	11.1 ± 1.1	10.3 ± 0.4
0.8	8	2.7 ± 0.5*	11.6 ± 1.4	11.7 ± 1.4	9.1 ± 0.7	7	0.9 ± 0.4*	12.6 ± 1.0	16.5 ± 0.9*	12.4 ± 1.8
1.2	8	0.3 ± 0.3*	12.2 ± 0.6	15.7 ± 2.4*	12.2 ± 0.5*	6	0*	9.0 ± 1.4	12.2 ± 1.0	10.4 ± 1.0

Results are expressed as min/2 hr period (mean ± SEM of 8 and 6 animals, respectively; *n* tests for each dose). Complete set of data is available on request.
*$p < 0.05$, significantly different from baseline (0); paired Student's *t* test.

However, when WAY 100635 (0.05 and 1 mg/kg) was used as a pretreatment to 8-OH-DPAT (0.4 mg/kg), it prevented the effects of the latter 5-HT$_{1A}$ agonist on sleep–wakefulness cycles in both groups of mice (Fig. 7*A*).

Respective contributions of 5-HT$_{1B}$ and 5-HT$_{1A}$ receptors to the effects of RU 24969 on sleep–wakefulness cycles

Because RU 24969 is a mixed 5-HT$_{1A/1B}$ agonist (Hoyer et al., 1994), we examined whether PS inhibition induced by large doses (3 and 5 mg/kg) of this ligand in 5-HT$_{1B}$−/− mice could be caused by its action at 5-HT$_{1A}$ receptors. Thus, the 5-HT$_{1A}$ antagonist WAY 100635 was used as pretreatment to RU 24969 at doses equivalent for their effect on PS in the respective groups, i.e., 0.5 mg/kg in 5-HT$_{1B}$+/+ mice (Fig. 3) and 3 mg/kg in 5-HT$_{1B}$−/− mutants (Fig. 7*B*). WAY 100635 at doses of 0.15–1.0 mg/kg prevented totally the effect of RU 24969 in 5-HT$_{1B}$−/− mice but not in wild-type animals (Fig. 7*B*). At the highest dose tested, 1 mg/kg, WAY 100635, in combination with either 8-OH-DPAT or RU 24969, produced a significant enhancement of PS in 5-HT$_{1B}$−/− mice but not in wild-type animals (Fig. 7*A*,*B*).

DISCUSSION

Knock-out mice lacking the 5-HT$_{1B}$ receptor (5-HT$_{1B}$−/−) and the corresponding wild-type controls (5-HT$_{1B}$+/+) exhibit similar diurnal sleep–waking cycles, with predominance of wakefulness during the dark period and sleep during the light one. These data are comparable with those reported previously in various strains of mice (Mitler et al., 1977; Kitahama and Valatx, 1980; Oliverio, 1980; Richardson et al., 1985; Tobler et al., 1997).

Interestingly, it was found here that 5-HT$_{1B}$−/− mice exhibited during the light period significantly larger amounts of PS and smaller amounts of SWS than 5-HT$_{1B}$+/+ animals and no PS rebound after selective PS deprivation. Whether such alterations of spontaneous sleep characteristics and homeostatic processes (Barbato and Wehr, 1998) are a direct consequence of the 5-HT$_{1B}$ receptor gene disruption or are attributable to other factors is open to discussion.

Various adaptive mechanisms resulting from the absence of the 5-HT$_{1B}$ receptors might have occurred during development in 5-HT$_{1B}$−/− mice. Indeed, in the latter mutants, the lack of expression of the 5-HT$_{1B}$ heteroreceptor might facilitate cholinergic (Maura and Raiteri, 1986) and GABAergic (Stanford and Lacey, 1996) neurotransmission and thus induce an enhancement of PS amounts (Gillin et al., 1985; McCarley and Massaquoi, 1992; Nitz and Siegel, 1997a,b; Cape and Jones, 1998). In addition, because 5-HT$_{1B}$ receptors are also autoreceptors on serotoninergic terminals (Engel et al., 1986; Boschert et al., 1994), their absence might also have an influence, in turn, on 5-HT$_{1A}$

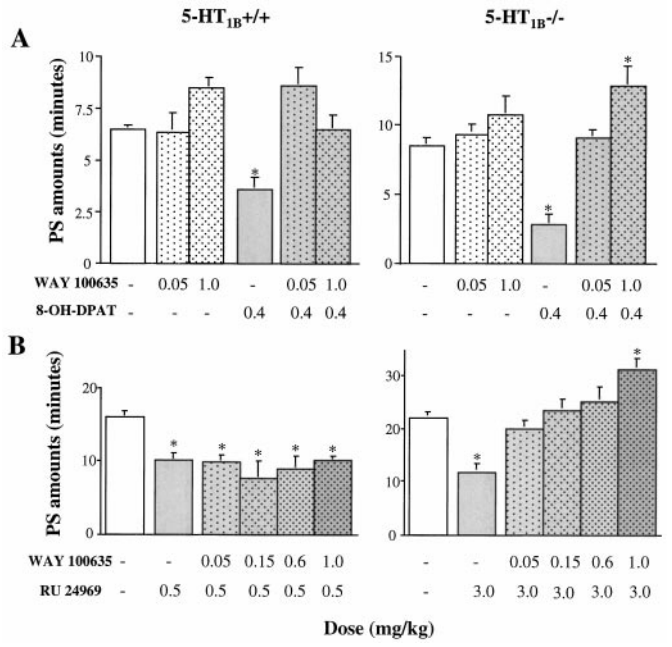

Figure 7. *A*, Effects of the 5-HT$_{1A}$ antagonist WAY 100635 (*dotted bars*) on the 8-OH-DPAT-induced inhibition of PS in 5-HT$_{1B}$+/+ (*left*) and 5-HT$_{1B}$−/− (*right*) mice during the 2 hr period after injection in which an effect was observed. Results are expressed as percent of baseline (mean ± SEM of 7 animals in each group; 6–7 tests for each dose). *B*, Effects of the 5-HT$_{1A}$ antagonist WAY 100635 (*dotted bars*) on the RU 24969-induced inhibition of PS in 5-HT$_{1B}$+/+ (*left*) and 5-HT$_{1B}$−/− (*right*) mice during the 4 hr after injection. Results are expressed as percentage of baseline (mean ± SEM of 7 and 9 animals, respectively; 4–5 tests for each dose). *$p < 0.05$, significantly different from baseline (*open bars*); paired Student's *t* test. Complete set of data is available on request.

receptors, which participate in the regulation of PS (de Saint Hilaire-Kafi et al., 1987; Dzoljic et al., 1992; Tissier et al., 1993; Portas et al., 1996; Thakkar et al., 1998).

With respect to the lack of PS rebound observed in the 5-HT$_{1B}$−/− group after deprivation, it should be noted that, in contrast to another study (Gonzalez et al., 1996) in which the platforms were of smaller size than the ones used here, mice were not totally deprived of PS under our conditions. In fact, 5-HT$_{1B}$−/− animals might exhibit a significant PS rebound after more drastic PS deprivation, but we purposely did not choose such an experimental design to minimize possible stress factors involved in this paradigm (Pokk et al., 1996). Still, after major PS deprivation for 12 hr, 5-HT$_{1B}$+/+, but not 5-HT$_{1B}$−/−, mice exhibited a significant PS rebound. The absence of 5-HT$_{1B}$ receptors, notably at the level of the locus ceruleus in which these receptors are expressed normally (Weissmann-Nanopoulos et al., 1985; Bobker and Williams, 1989; Clement et al., 1992), might account in part for this phenomenon. Indeed, lesion by *N*-(2-chloroethyl)-*N*-ethyl-2-gromobenzylamine of noradrenergic neurons in the locus ceruleus has been reported to suppress PS rebound in rats subjected to PS deprivation (Gonzalez et al., 1996). In contrast, rebound after pharmacologically induced PS inhibition persisted after such a lesion (Gonzalez et al., 1996), like that observed in 5-HT$_{1B}$−/− mice after RU 24969 or 8-OH-DPAT treatment. This suggests that the lack of rebound after PS deprivation in 5-HT$_{1B}$−/− mice is probably not because of some ceiling effect but rather of impairment of homeostatic regulation of PS. Finally, although the target areas for these phenomena have not yet been characterized, possible alterations in 5-HT, ACh, and GABA neurotransmission in 5-HT$_{1B}$−/− mice might account for the differences in PS regulations between the two genotypes.

A second reason for the differences in spontaneous sleep and PS rebound between the two strains might be a difference in genetic background (Gerlai, 1996; Valatx and Bugat, 1974; Kitahama and Valatx, 1980; Tobler et al., 1997) rather than the specific 5-HT$_{1B}$ receptor gene disruption. However, because backcrossing was performed with the strain that gave embryonic stem cells for homologous recombination, i.e., 129/Sv (Saudou et al., 1994), both 5-HT$_{1B}$−/− and 5-HT$_{1B}$+/+ mice have the same pure genetic background (Ramboz et al., 1996).

In fact, our pharmacological data provide strong support to the idea that the increased amounts of spontaneous PS in knock-out mice can be accounted for by the absence of 5-HT$_{1B}$ receptors. In particular, blockade of the latter by GR 127935 (Pauwels, 1997) induced an increase of PS amounts in 5-HT$_{1B}$+/+, but not 5-HT$_{1B}$−/−, mice (Fig. 5), so that the wild-type mice exhibited the same levels of PS as those occurring spontaneously in the 5-HT$_{1B}$−/− strain.

In addition, stimulation of 5-HT$_{1B}$ receptors by the selective agonist CP 94253 (Koe et al., 1992) and the mixed 5-HT$_{1A/1B}$ agonist RU 24969 (Hoyer et al., 1994) induced a dose-dependent decrease of PS in 5-HT$_{1B}$+/+ mice (Table 2, Fig. 4). That the inhibitory action of CP 94253 and RU 24969 on PS actually resulted from 5-HT$_{1B}$ receptor activation was confirmed by the fact that GR 127935 (Fig. 6), but not the 5-HT$_{1A}$ antagonist WAY 100635 (Fig. 7*B*), prevented this action and that, in 5-HT$_{1B}$−/− mice, the same compounds in the same dose range altered neither sleep nor wakefulness. Previous studies in rats also supported the idea that 5-HT$_{1B}$ receptors are involved in a negative modulation of PS (Bjorvatn and Ursin, 1994; Monti et al., 1995).

At the largest doses of the 5-HT$_{1B}$ agonists used, both a decrease of SWS and an enhancement of W were observed in 5-HT$_{1B}$+/+ mice, concomitantly with the PS reduction. These effects are similar to those reported in the rat (Dugovic et al., 1989; Dzoljic et al., 1992) and are probably not secondary to some hyperlocomotor activity triggered by this compound, notably because the doses of RU 24969 used here (0.25–2.0 mg/kg) were 10-fold lower than those required for the latter effect to occur in rodents (Green et al., 1984). However, if the action of 5-HT$_{1B}$ agonists on sleep and wakefulness can be accounted for by selective activation of 5-HT$_{1B}$ receptors, the effects of RU 24969 at higher doses (3 and 5 mg/kg) in 5-HT$_{1B}$ knock-out mice deserve some comments. These effects (reduction of PS and increase of W) could not be secondary to hyperlocomotion or ascribed to an action of RU 24969 at some residual 5-HT$_{1B}$ receptors, because they were not prevented by the 5-HT$_{1B/1D}$ antagonist GR 127935 (Table 3). Rather, the effects on PS of large doses of RU 24969 in 5-HT$_{1B}$−/− mice would be attributable to the 5-HT$_{1A}$ component of this ligand. Indeed, RU 24969 binds to 5-HT$_{1A}$ receptors with an affinity only fivefold lower than to 5-HT$_{1B}$ receptors (Peroutka, 1986; Hoyer et al., 1994), and the use of large doses of this ligand in 5-HT$_{1B}$−/− mice might activate 5-HT$_{1A}$ receptors sufficiently to induce a PS decrease, as expected of a 5-HT$_{1A}$ agonist (de Saint Hilaire-Kafi et al., 1987; Dzoljic et al., 1992; Tissier et al., 1993). In agreement with this interpretation, the effect of RU 24969 on PS in 5-HT$_{1B}$−/− mice could be completely prevented by the selective 5-HT$_{1A}$ antagonist WAY 100635 (Fletcher et al., 1996).

In the absence of 5-HT$_{1B}$ receptors, 5-HT$_{1A}$ receptors might have exhibited some adaptive changes in comparison with those in wild-type animals. However, in both groups of mice, the 5-HT$_{1A}$ agonist 8-OH-DPAT induced a dose-dependent reduction of PS during the 2–4 hr after injection (Table 4), associated with an increase of W at the expense of SWS at the largest doses used (data not shown). These effects, which are similar to those observed in the rat (de Saint Hilaire-Kafi et al., 1987), were probably not caused by 8-OH-DPAT-induced hypothermia (Goodwin et al., 1985). Indeed, body temperature monitoring after subcutaneous injection of 0.2–0.8 mg/kg 8-OH-DPAT showed that hypothermia was maximum (−1 and −3°C) 15–30 min after injection and disappeared within the following 15–30 min in both 5-HT$_{1B}$−/− and wild-type mice (B. Boutrel and J. Adrien, unpublished observations). In contrast, the effects of 8-OH-DPAT on sleep persisted during 2–3 hr, well beyond the duration of drug-induced hypothermia. Interestingly, an increased reactivity of sleep to 8-OH-DPAT (Table 4) and WAY 100635 (Fig. 7*A*,*B*) was observed in 5-HT$_{1B}$−/− compared with wild-type mice. This would suggest that 5-HT$_{1A}$ receptors developed some functional supersensitivity in 5-HT$_{1B}$−/− mice, but further studies are needed to directly address this question.

In conclusion, the lack of effects of 5-HT$_{1B}$ receptor agonists on the vigilance states in 5-HT$_{1B}$−/− mice demonstrated that PS inhibition by these ligands in wild-type mice actually resulted from the specific stimulation of 5-HT$_{1B}$ receptors. Both the larger amounts of PS during the light phase in 5-HT$_{1B}$−/− mice and the PS increase in response to 5-HT$_{1B}$ receptor blockade in wild-type mice support the idea that 5-HT$_{1B}$ receptors mediate a 5-HT-dependent tonic inhibitory control of PS under physiological conditions.

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