

5-HT_{1A/1B} Receptor-Mediated Effects of the Selective Serotonin Reuptake Inhibitor, Citalopram, on Sleep: Studies in 5-HT_{1A} and 5-HT_{1B} Knockout Mice

Christelle Monaca¹, Benjamin Boutrel¹, René Hen², Michel Hamon¹ and Joëlle Adrien*¹

¹INSERM U288, NeuroPsychoPharmacologie Moléculaire Cellulaire et Fonctionnelle, CHU Pitié-Salpêtrière-91, Boulevard de l'Hôpital, 75013 Paris, France; ²Center for Neurobiology and Behavior, Columbia University, New York, NY, USA

Selective serotonin reuptake inhibitors (SSRIs) are extensively used for the treatment of depression. Aside from their antidepressant properties, they provoke a deficit in paradoxical sleep (PS) that is most probably mediated by the transporter blockade-induced increase in serotonin concentration in the extracellular space. Such an effect can be accounted for by the action of serotonin at various types of serotonergic receptors involved in PS regulation, among which the 5-HT_{1A} and 5-HT_{1B} types are the best candidates. According to this hypothesis, we examined the effects of citalopram, the most selective SSRI available to date, on sleep in the mouse after inactivation of 5-HT_{1A} or 5-HT_{1B} receptors, either by homologous recombination of their encoding genes, or pharmacological blockade with selective antagonists. For this purpose, sleep parameters of knockout mice that do not express these receptors and their wild-type counterparts were monitored during 8 h after injection of citalopram alone or in association with 5-HT_{1A} or 5-HT_{1B} receptor antagonists. Citalopram induced mainly a dose-dependent inhibition of PS during 2–6 h after injection, which was observed in wild-type and 5-HT_{1B}–/– mice, but not in 5-HT_{1A}–/– mutants. This PS inhibition was fully antagonized by pretreatment with the 5-HT_{1A} antagonist WAY 100635, but only partially with the 5-HT_{1B} antagonist GR 127935. These data indicate that the action of the SSRI citalopram on sleep in the mouse is essentially mediated by 5-HT_{1A} receptors. Such a mechanism of action provides further support to the clinical strategy of antidepressant augmentation by 5-HT_{1A} antagonists, because the latter would also counteract the direct sleep-inhibitory side-effects of SSRIs. *Neuropsychopharmacology* (2003) **28**, 850–856, advance online publication, 5 March 2003; doi:10.1038/sj.npp.1300109

Keywords: sleep–wakefulness; knockout mice; 5-HT_{1A} receptors; 5-HT_{1B} receptors; selective serotonin reuptake inhibitor; citalopram

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed drugs in anxiodepressive disorders. Since SSRIs produce a tonic elevation of serotonin (5-hydroxytryptamine, 5-HT) levels in the extracellular space (Fuller, 1994; Malagie *et al*, 1995), their therapeutic effect is probably mediated, at least in part, by action at various levels of the serotonergic system, notably at serotonergic receptors (Barnes and Sharp, 1999). In particular, it has been proposed that the antidepressant effects of SSRIs would be related to desensitization of somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors (Blier *et al*, 1987; Artigas *et al*, 1994, 2001; Pineyro and Blier, 1999), which is induced by and participates indirectly in the increase in 5-HT concentration in the extracellular space (Gartside *et al*,

1995, 1999; Sharp *et al*, 1997; Trillat *et al*, 1998; Adell *et al*, 2001; Malagie *et al*, 2001).

Another well-known action of SSRIs is their effects on sleep and wakefulness. Indeed, in several species and notably the rat (Pastel and Fernstrom, 1987; Ursin *et al*, 1989; Lelkes *et al*, 1994; Maudhuit *et al*, 1994; Neckelmann *et al*, 1996b), the hamster (Gao *et al*, 1992), and also in humans (Van Bommel *et al*, 1993; Hendrickse *et al*, 1994), the most consistent action of SSRIs is a reduction of paradoxical sleep (PS), which is sometimes associated with an enhancement of wakefulness (W) and a secondary increase in slow wave sleep (SWS) (Maudhuit *et al*, 1994; Ursin, 2002). In the same manner, systemic treatment with selective agonists at 5-HT_{1A} and 5-HT_{1B} receptors induces an inhibition of PS and an enhancement of wakefulness, notably in rodents (Dzolic *et al*, 1992; Tissier *et al*, 1993; Bjorvatn and Ursin, 1994; Monti *et al*, 1995; Bjorvatn *et al*, 1997; Boutrel *et al*, 1999, 2002). In contrast, inactivation of 5-HT_{1A} and 5-HT_{1B} receptors (notably in mice with genetic deletions targeted at these receptors) facilitates the expression of PS (Boutrel *et al*, 1999, 2002). Altogether, these data strongly suggest that 5-HT_{1A} and/or 5-HT_{1B} receptors, because they are activated by extracellular 5-HT, might mediate the action of SSRIs on sleep and wakefulness.

*Correspondence: Dr J Adrien, Institut National de la Santé et de la Recherche Médicale, INSERM U288, CHU Pitié-Salpêtrière, 75634 Paris Cedex 13, France, Tel: +33 1 40 77 97 13, Fax: +33 1 40 77 97 90, E-mail: adrien@ext.jussieu.fr

Received 18 June 2002; revised 29 August 2002; accepted 30 October 2002

Online publication: 6 November 2002 at <http://www.acnp.org/citations/Npp110602426>

However, this hypothesis has still to be validated because (i) 5-HT_{1A} receptor antagonists were reported to be unable to prevent the effects of SSRIs on sleep or wakefulness (Bjorvatn *et al*, 1992; Neckelmann *et al*, 1996a) and (ii) to date, no study has been published with respect to the possible involvement of 5-HT_{1B} receptors in PS inhibition by SSRIs.

These considerations led us to address this question by using knockout mice that do not express 5-HT_{1A} or 5-HT_{1B} receptors (Saudou *et al*, 1994; Ramboz *et al*, 1998). We analyzed the effects of citalopram, the most selective SSRI currently available (Hyttel, 1982; Milne and Goa, 1991), on sleep-wakefulness cycles in 5-HT_{1A} and 5-HT_{1B} knockout mice and their wild-type counterparts. In addition, we investigated whether pharmacological blockade of either 5-HT_{1A} or 5-HT_{1B} receptors with selective antagonists could prevent the effects of citalopram on sleep in the mouse.

MATERIALS AND METHODS

Animals

All the procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (council directive 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service vétérinaire de la santé et de la protection animale, permissions 75-116 to MH and 75-125 to JA). All mice were of the same 129/Sv genetic background. They were produced in the laboratory from homozygous breeding of wild-type and mutant (5-HT_{1A}^{-/-} and 5-HT_{1B}^{-/-}) strains (Saudou *et al*, 1994; Ramboz *et al*, 1998; Boutrel *et al*, 1999, 2002), and housed in standard animal care facilities (see below).

Surgery

At 2-3 months of age (body weight: 21-27 g), male wild-type and mutant 5-HT_{1A}^{-/-} and 5-HT_{1B}^{-/-} mice were implanted under sodium pentobarbital anesthesia (70-75 mg/kg i.p.) with the classical set of electrodes for polygraphic sleep monitoring, as previously described (Boutrel *et al*, 1999). In brief, EEG electrodes were inserted through the skull onto the dura over the right cerebral cortex (2 mm lateral and 4 mm posterior to bregma) and over the cerebellum (at midline, 2 mm posterior to lambda), EOG 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 super-bond cement (Limoge-Lendais *et al*, 1994), and soldered to a mini-connector secured with acrylic cement. After completion of surgery, animals were housed in individual cages (20 × 20 × 30 cm³) and maintained under standard laboratory conditions: 12-12 h light-dark cycle (light on at 07:00 am), 24 ± 1°C ambient temperature, food and water *ad libitum*. The animals were allowed at least 10 days to recover and habituate to the recording conditions.

Pharmacological Treatments

Drugs were dissolved in 0.1 ml of saline and injected intraperitoneally (i.p.) between 9:30 and 10:00 am. In case of

combined treatments, the antagonist or saline was injected 15 min prior to citalopram. For baseline data, mice were injected with saline only, in single or combined treatment, as appropriate. A washout period of at least 3 days was allowed between two consecutive treatments. One series of animals was used to analyze the effects of citalopram alone, while another one was used in experiments with combined treatments. In both series, each animal received all doses and compounds, in a counter-balanced fashion. However, some recordings were discarded from the results because of insufficient quality. Thus, the number of tests finally contributing to the data was comprised between 4 and 10.

Polygraphic Recording

Polygraphic sleep monitoring was started just after the injection(s) and continued during 8 h thereafter.

Data Analysis and Statistics

Recordings were scored manually every 15 s epoch using the classical criteria for mice (Boutrel *et al*, 2002). The effects of each treatment on the states of vigilance (W, SWS, PS) were analyzed for every 2 h period after injection, and were expressed (mean ± SEM) as minutes and as percentage of baseline (injection of saline). Statistical analyses were performed using ANOVA for the factors treatment and strain of mice. In case of significance, this ANOVA was followed by a *post hoc* Fisher test or a paired Student's *t*-test as appropriate, in order to determine statistical significance of the effect of each dose of a given compound.

Chemicals

WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide) (0.5 mg/kg i.p.) was obtained from Wyeth Research (Princeton, NJ, USA); 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) (1 mg/kg i.p.) was from Glaxo-Wellcome (Ware, UK); citalopram (1-10 mg/kg i.p.) was from Lundbeck (Copenhagen, DK).

RESULTS

Pharmacological Blockade of the Serotonin Transporter

In wild-type mice, blockade of the 5-HT transporter by citalopram induced a significant decrease in PS amounts, which lasted for 2-6 h after the injection (Figure 1). This effect was dose-dependent (ANOVA, $F_{3,21} = 22.9$ and 14.7 , $p < 0.0001$; and $F_{3,21} = 2.7$, $p = 0.06$ for the three successive 2 h periods after injection, respectively), and significant for the doses of 5 and 10 mg/kg of citalopram until 4 h ($p = 0.0005$, $n = 7$) and 6 h ($p = 0.01$, $n = 5$) after treatment, respectively. This citalopram-induced PS reduction was accounted for by an increase in PS latency (150.3 ± 7.2 and 157.4 ± 13.7 min for 5 and 10 mg/kg vs 68.8 ± 10.8 and 63.8 ± 13.5 min at baseline, respectively; means ± SEM for seven and five tests, respectively, $p < 0.01$), and a decrease in the number of episodes with no significant modification of their mean duration (54 ± 4 and 62 ± 7 s during 4 h after 5

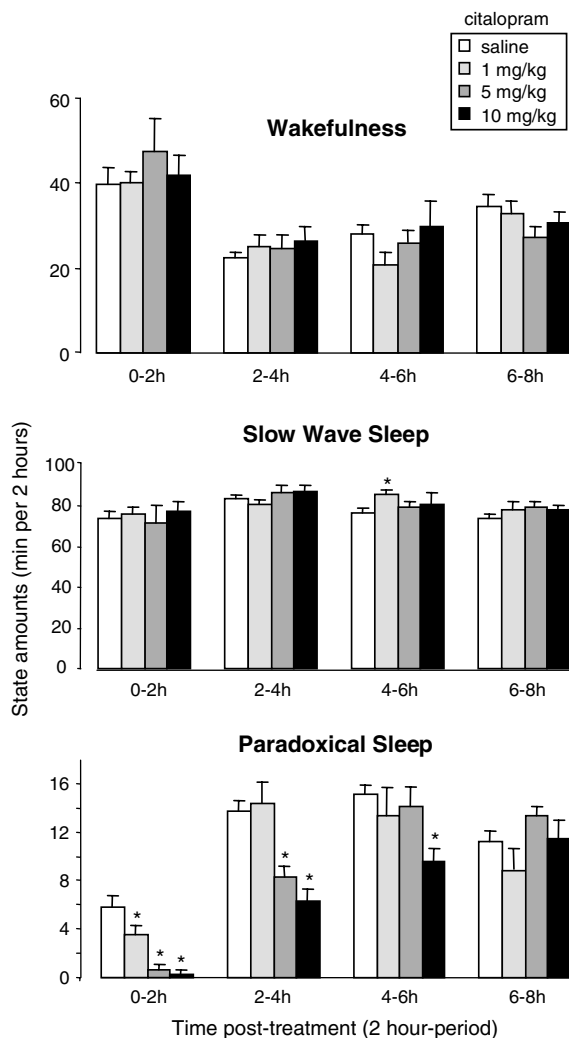


Figure 1 Effects of the SSRI citalopram at various doses on wakefulness and sleep in wild-type mice during four successive 2 h periods after injection. Data (mean \pm SEM of seven animals, five to seven tests for each dose) are expressed as min/2 h. * p < 0.05, significantly different from saline (open bars); *Post hoc* Fisher test.

and 10 mg/kg of citalopram, compared to 54 ± 6 and 57 ± 6 s after saline, respectively). After this initial decrease, PS amounts returned progressively to baseline values (Figure 1). Citalopram induced no modification of W, but a slight enhancement of SWS was observed between the 4th and the 6th hour after treatment with the dose of 1 mg/kg (Figure 1).

In 5-HT_{1B} knockout mice, citalopram also produced a dose-dependent decrease in PS amounts during the first 4 h after injection (ANOVA, 0–2 h: $F_{3,19} = 4.1$, $p = 0.02$ and 2–4 h: $F_{3,19} = 6.7$, $p = 0.003$) (Figure 2). This effect was not significantly different from that in wild-type mice (ANOVA for factor strain: $F_{1,40} = 0.90$, $p = 0.35$ and $F_{1,40} = 0.004$, $p = 0.95$, for the 0–2 and 2–4 h periods after treatment, respectively) (Figure 2 vs Figure 1). It was because of an increase in PS latency (103.5 ± 12.1 for 10 mg/kg vs 48.7 ± 5.7 min after saline, $n = 4$, $p < 0.001$), and a decrease in the number of PS episodes with no modification of their mean duration (57 ± 6 and 62 ± 7 s during 4 h after 5 and 10 mg/kg of citalopram, compared to 57 ± 6 and 60 ± 4 s

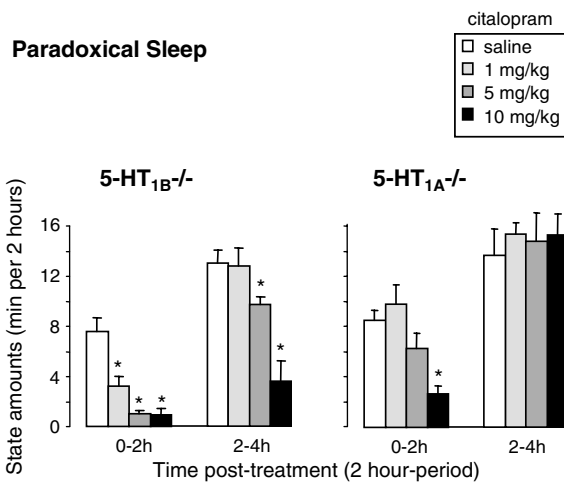


Figure 2 Effects of the SSRI citalopram at various doses on PS in 5-HT_{1B}^{-/-} (left) and 5-HT_{1A}^{-/-} (right) mice during the first and second 2 h period after injection. Data (mean \pm SEM of, respectively, eight and seven animals, four to eight tests for each dose) are expressed as min/2 h. * p < 0.05, significantly different from saline (open bars); *Post hoc* Fisher test.

after saline, respectively; means \pm SEM for eight and five tests, respectively).

In contrast, in 5-HT_{1A} knockout mice, citalopram induced a lesser decrease of PS amounts, which was observed only during the first 2 h after the injection (ANOVA: $F_{3,18} = 6.3$, $p = 0.004$), and reached statistical significance only for the highest dose tested, 10 mg/kg ($p = 0.006$, $n = 4$) (Figure 2). Like that observed in wild-type mice, this PS decrease was accounted for by an increase in PS latency (106.9 ± 15.7 vs 51.3 ± 5.4 min after saline, $n = 4$, $p < 0.001$), and by a reduced number of PS episodes with no significant modifications of their mean duration (68 ± 8 s for 10 mg/kg, vs 66 ± 11 s at baseline, $n = 4$). However, even at this highest dose, PS reduction caused by citalopram was significantly less in 5-HT_{1A}^{-/-} mutants than in both wild-type and 5-HT_{1B}^{-/-} mice (ANOVA for PS amounts across strains: $F_{2,58} = 32.0$ and 22.8 , $p < 0.0001$ during 0–2 and 2–4 h after injection, respectively) (Figure 2 vs Figure 1).

Finally, no significant modifications of W or SWS were observed after citalopram administration (1, 5, or 10 mg/kg i.p.) in 5-HT_{1B}^{-/-} and 5-HT_{1A}^{-/-} knockout mice (not shown).

Blockade of the Serotonin Transporter After Pharmacological Inactivation of 5-HT_{1A} or 5-HT_{1B} Receptors

Pretreatment with the selective 5-HT_{1A} receptor antagonist, WAY 100635 (0.5 mg/kg i.p.), prevented the PS inhibitory effect of citalopram (5 mg/kg i.p.) in wild-type mice (Table 1 and Figure 3). Similar data were obtained in 5-HT_{1B}^{-/-} mutants. In contrast, in 5-HT_{1A}^{-/-} mice, no significant differences in PS amounts were observed whether or not WAY 100635 was injected before citalopram ($p = 0.50$, $n = 5$) (Table 1 and Figure 3). A significant enhancement of SWS and concomitant decrease in W were also observed in wild-type mice only, during 2 h post-treatment (Table 1).

Table 1 Effects of the SSRI Citalopram (5 mg/kg i.p.), in Association with the 5-HT_{1A} Antagonist WAY 100635 (0.5 mg/kg i.p.) or the 5-HT_{1B} Antagonist GR 127935 (1 mg/kg i.p.), on Wakefulness and Sleep in the Three Strains of Mice

		Wild type		5-HT _{1A} ^{-/-}		5-HT _{1B} ^{-/-}	
		0–2 h	2–4 h	0–2 h	2–4 h	0–2 h	2–4 h
Wakefulness	Saline+saline	46.6 ± 3.0	29.4 ± 3.3	41.4 ± 3.9	30.3 ± 3.9	46.8 ± 2.5	31.1 ± 5.3
	Saline+citalopram	43.4 ± 3.3	25.4 ± 2.1	45.3 ± 4.9	31.7 ± 2.0	44.3 ± 5.4	31.9 ± 5.4
	WAY 100635+citalopram	32.2 ± 4.3*	22.8 ± 4.1	36.4 ± 4.4	28.5 ± 2.9	42.7 ± 8.7	28.2 ± 4.7
	GR 127935+citalopram	47.2 ± 5.0	33.4 ± 6.6	40.3 ± 5.4	31.8 ± 2.9	55.1 ± 4.4	39.1 ± 6.2
Slow wave sleep	Saline+saline	68.1 ± 2.9	77.7 ± 3.1	70.4 ± 3.4	75.1 ± 3.5	65.5 ± 2.7	75.9 ± 4.5
	Saline+citalopram	75.4 ± 3.5	86.1 ± 2.1*	68.5 ± 4.8	73.1 ± 4.5	74.2 ± 5.1	76.6 ± 5.0
	WAY 100635+citalopram	79.8 ± 4.5*	78.5 ± 3.0	75.6 ± 2.2	78.2 ± 3.1	71.1 ± 8.4	76.0 ± 5.8
	GR 127935+citalopram	70.0 ± 5.2	77.1 ± 6.2	72.7 ± 5.2	71.6 ± 3.3	62.8 ± 4.6	70.0 ± 5.2
Paradoxical sleep	Saline+saline	5.4 ± 0.4	12.8 ± 1.0	8.2 ± 0.8	14.6 ± 1.0	7.7 ± 0.5	13.1 ± 1.4
	Saline+citalopram	1.2 ± 0.3*	8.5 ± 0.8*	6.1 ± 1.0*	15.2 ± 1.2	1.4 ± 0.6*	11.5 ± 1.7
	WAY 100635+citalopram	7.9 ± 0.9*†	18.7 ± 2.1*†	8.0 ± 2.7	13.3 ± 0.6	6.2 ± 1.6 †	15.8 ± 1.4
	GR 127935+citalopram	2.8 ± 0.7*†	9.5 ± 1.0	7.0 ± 1.7	16.6 ± 3.4	2.1 ± 0.7*	10.9 ± 1.3

Results are expressed as min/2 h period (mean ± SEM for 10 mice in the wild-type and the 5-HT_{1A}^{-/-} groups, and six mice in the 5-HT_{1B}^{-/-} group; four to 10 tests for each dose, see Figure 3). **p* < 0.05, significantly different from saline+saline; Student's *t*-test. †*p* < 0.05, significantly different from treatment with saline+citalopram; Student's *t*-test. Each antagonist (or saline) was administered 15 min prior to citalopram (or saline), and vigilance states are given for two successive 2 h periods (0–2; 2–4) after the second injection.

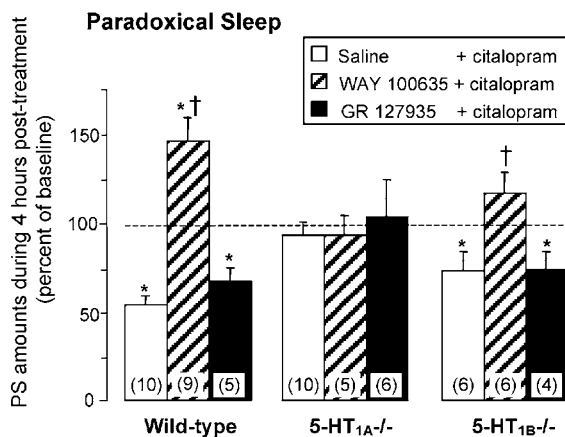


Figure 3 Effects of inactivation of 5-HT_{1A} and 5-HT_{1B} receptors on the PS inhibition induced by the SSRI citalopram (5 mg/kg i.p.) during 4 h after injection. Data (mean ± SEM of 10 mice in the wild-type and the 5-HT_{1A}^{-/-} groups, and six mice in the 5-HT_{1B}^{-/-} group) after saline and citalopram (empty bars), and WAY 100635 (0.5 mg/kg i.p., hatched bars) or GR 127935 (1 mg/kg i.p., black bars) in association with citalopram, are expressed as percentages of PS amounts in saline-treated mice. The number of tests is indicated in each bar. **p* < 0.05, significantly different from saline treatment (dashed line); Student's *t*-test. †*p* < 0.05, significantly different from treatment with saline and citalopram (solid bars); Student's *t*-test.

Pretreatment with the 5-HT_{1B} receptor antagonist, GR 127935 (1 mg/kg i.p.), did not induce any modifications of the effects of citalopram on PS amounts in any strain, except for a partial prevention of PS inhibition in wild-type mice for the first 2 h after injection of the SSRI (Table 1). This combined treatment induced during 4 h a decrease in the amounts of PS in both wild-type and 5-HT_{1B}^{-/-} mice, and no modifications in 5-HT_{1A}^{-/-} mutants, these effects being similar to those observed after injection of citalopram alone (Figure 3).

DISCUSSION

The present data indicate that, in the mouse, blockade of the serotonin transporter by citalopram induces an inhibition of PS with no major modifications of the other states of vigilance. This action appears to be mediated mainly through 5-HT_{1A} receptor stimulation resulting from the increase in extracellular 5-HT levels caused by 5-HT reuptake inhibition.

The citalopram-induced decrease in PS that we report herein for the first time in the mouse is similar to that previously described in other rodents with various SSRIs (Pastel and Fernstrom, 1987; Ursin *et al*, 1989; Gao *et al*, 1992; Maudhuit *et al*, 1994; Neckelmann *et al*, 1996b; Ursin, 2002). In addition, mice exhibited after treatment with moderate doses of citalopram a slight secondary increase in SWS amounts (see Figure 1 and Table 1), which was also found in rats (Ursin *et al*, 1989; Maudhuit *et al*, 1994). However, in contrast to the latter studies, no modifications of wakefulness were observed herein.

The PS inhibition induced by the SSRI citalopram is most probably the consequence of the enhancement of extracellular 5-HT levels resulting from blockade of the amine reuptake (Fuller, 1994; Malagié *et al*, 1995; Fabre *et al*, 2000). Among the receptors at which 5-HT in excess could act to induce PS inhibition, the 5-HT_{1A} and 5-HT_{1B} types appeared as the most relevant candidates (Dzolic *et al*, 1992; Tissier *et al*, 1993; Bjorvatn and Ursin, 1994; Monti *et al*, 1995; Bjorvatn *et al*, 1997; Boutrel *et al*, 1999, 2002). Indeed, we can conclude from the present study that 5-HT_{1A} receptors are primarily involved in such a PS inhibition because the effect of citalopram on sleep was markedly reduced in 5-HT_{1A}^{-/-}, but globally unchanged in 5-HT_{1B}^{-/-}, mutants compared to wild-type mice. The almost complete loss of citalopram action in 5-HT_{1A}^{-/-} mutants cannot be accounted for by a downregulation of the transporter itself because only mild changes of

the latter were observed in knockout *vs* wild-type animals, notably in the brainstem (Ase *et al*, 2001), a key area for 5-HT-mediated PS regulation (Horner *et al*, 1997; Bjorvatn and Ursin, 1998). Thus, the presence of functional 5-HT_{1A} receptors seems to be a prerequisite for the effect of citalopram on sleep, at least at moderate doses. Indeed, this effect was abolished not only in 5-HT_{1A}^{-/-} mutants but also in wild-type mice after pharmacological blockade of 5-HT_{1A}, but not 5-HT_{1B} receptors.

The question can be raised of whether the citalopram-induced PS inhibition could be secondary to a decrease in core temperature produced by this drug (Goodrich, 1983; Oerther and Ahlenius, 2001). However, in the rat, citalopram-induced hypothermia can be prevented by pretreatment with either 5-HT_{1A} or 5-HT_{1B} receptor antagonists (Oerther and Ahlenius, 2001), whereas in wild-type mice, citalopram-induced PS inhibition could be antagonized by blockade of 5-HT_{1A} but not 5-HT_{1B} receptors (Figure 3, Table 1). This difference strongly suggests that the effects of citalopram on PS are probably not secondary to its effects on core temperature. However, replication on wild-type and 5-HT_{1A}^{-/-}/5-HT_{1B}^{-/-} mutant mice of the study performed by Oerther and Ahlenius (2001) in rats should provide further answer to this question.

In line with previous studies (Boutrel *et al*, 1999, 2002), the data obtained herein with the selective 5-HT_{1A} receptor antagonist WAY 100635 (Fletcher *et al*, 1996) associated with citalopram (see Table 1) confirm that this receptor type mediates a tonic inhibition of PS in the mouse (Boutrel *et al*, 2002). Indeed, as expected from such a 5-HT_{1A} receptor-mediated physiological control of PS, blockade of 5-HT_{1A} receptors by WAY 100635 promoted PS in wild-type mice, when this antagonist was administered alone (Boutrel *et al*, 2002) or in combination with citalopram (Figure 3, Table 1). Indeed, in the latter case, PS amounts could not be affected by citalopram (because of the blockade of 5-HT_{1A} receptors) and only the enhancing effect of WAY 100635 (see Boutrel *et al*, 2002) was observed. However, these data are at variance with those obtained in rats where other 5-HT_{1A} antagonists (alprenolol, NAN-190) failed to oppose the action of SSRIs on sleep (Bjorvatn *et al*, 1992; Neckelmann *et al*, 1996a). The antagonist properties of the latter ligands at various receptors in addition to 5-HT receptors (Claustre *et al*, 1991; Hodgkiss *et al*, 1992; Hamon, 1997) and/or the use of different species, might possibly explain such differences compared to the present data obtained with the highly selective 5-HT_{1A} antagonist WAY 100635.

At the doses used, citalopram after 5-HT_{1A} receptor blockade in wild-type mice induced an increase of PS during 2 h, whereas in the absence of 5-HT_{1A} receptor expression, that is, in 5-HT_{1A}^{-/-} mutants, it induced a slight PS decrease (Table 1). One probable explanation of this difference is that in the first case one deals with a clear-cut pharmacological effect of WAY 100635 (Boutrel *et al*, 2002), whereas in 5-HT_{1A}^{-/-} mutants, adaptive phenomena might have occurred, notably at the level of 5-HT receptors. Indeed, 5-HT_{1B} receptors were found to be supersensitive in 5-HT_{1A}^{-/-} mutants (Boutrel *et al*, 2002), and such a functional change might account, at least in part (see below), for the slight PS decrease observed after

citalopram treatment (Table 1). In the whole, these data further support the idea that constitutive knock out of a specific gene does not produce exactly the same effects as the pharmacological blockade of the corresponding encoded protein.

In contrast to the effects reported herein on sleep regulations, it has been shown that combined treatment with 5-HT_{1A} antagonists and 5-HT reuptake inhibitors potentiated the effects of the latter drugs, at least with respect to 5-HT release in the hippocampus, food intake, and nociception (Hjorth, 1996; Trillat *et al*, 1998; Ardid *et al*, 2001). Such a facilitatory effect of 5-HT_{1A} antagonists can be interpreted as resulting from the blockade of somatodendritic 5-HT_{1A} autoreceptors from which 5-HT can trigger a negative control on serotonergic cell firing, notably after citalopram treatment (Evrard *et al*, 1999). With regard to sleep, WAY 100635 did not potentiate the action of citalopram, which confirms that the 5-HT_{1A}-mediated action of 5-HT on sleep involves 5-HT_{1A} receptors that differ from somatodendritic 5-HT_{1A} autoreceptors. Indeed, clear-cut evidence of the implication of postsynaptic 5-HT_{1A} receptors in the 5-HT-mediated inhibitory control of PS has already been reported in rats (Tissier *et al*, 1993). Further studies suggested that these receptors are located in specific brainstem nuclei (Horner *et al*, 1997; Bjorvatn and Ursin, 1998).

Interestingly, blockade of 5-HT reuptake by citalopram had almost the same effect on PS in wild-type and 5-HT_{1B}^{-/-} mutant mice. This observation is in line with recent data showing that the molecular target of citalopram, the 5-HT transporter, is not modified in the brainstem (Ase *et al*, 2001) and only slightly enhanced in the nucleus raphe dorsalis (Evrard *et al*, 1999) of 5-HT_{1B}^{-/-} mutants compared to their wild-type counterparts. We observed that PS amounts were largely reduced after citalopram treatment whether or not 5-HT_{1B} receptors were functional, that is, in 5-HT_{1B}^{-/-} mutants and in wild-type mice pretreated with either saline or the 5-HT_{1B} antagonist GR 127935 (Pauwels, 1997). Accordingly, it can be concluded that, in contrast to 5-HT_{1A} receptors, 5-HT_{1B} receptors do not play a crucial role in the inhibitory effect of citalopram on PS. Nevertheless, some modulation of this effect through the latter receptors cannot be excluded. In particular, it has to be emphasized that PS inhibition induced by treatment with saline+citalopram (5 mg/kg) was significant for only 2 h in 5-HT_{1B}^{-/-} mutants, whereas it lasted for 4 h in wild-type mice (Table 1); in addition, the 5-HT_{1B} antagonist, GR 127935, partly reversed the action of citalopram in the latter animals (Table 1, Figure 3). On the other hand, PS inhibition following treatment with 10 mg/kg of citalopram in 5-HT_{1A}^{-/-} mice (see Figure 2) might result from activation of 5-HT_{1B} receptors, inasmuch as PS in these mutant mice was found to be more sensitive to 5-HT_{1B} receptor agonists (Boutrel *et al*, 2002). Meanwhile, the fact that 5-HT_{1B}^{-/-} mutants exhibited some increase in PS amounts under baseline conditions compared to wild-type mice (see Table 1) confirms that 5-HT_{1B} receptors are involved in a tonic inhibition of PS in the mouse (Boutrel *et al*, 1999). However, overall comparison of the respective effects of 5-HT_{1B} *vs* 5-HT_{1A} receptor inactivation on PS under baseline conditions as well as after citalopram treatment (see Table 1) clearly indicates that 5-HT_{1A} much

more than 5-HT_{1B} receptors mediate the 5-HT inhibitory control of this sleep stage in the mouse.

To conclude, given that insomnia, one cardinal symptom of depression, may be exacerbated by SSRIs, notably at initiation of the treatment (Levitan *et al*, 2000; Lønborg *et al*, 2000), the present data provide further support to the therapeutic strategy consisting of the association of these antidepressants with 5-HT_{1A} antagonists (Artigas *et al*, 1994). Indeed, such combined treatments would simultaneously accelerate and/or augment the antidepressive action (Artigas *et al*, 1994, 2001; Blier and Bergeron, 1995; Stahl, 1998; Adrien, 2002), and limit the sleep-inhibitory effect of of SSRIs (Thase, 2000).

ACKNOWLEDGMENTS

This research was supported by grants from INSERM and Bristol-Myers Squibb Foundation (Unrestricted biomedical research grant program). We are grateful to pharmaceutical companies (Glaxo-Wellcome, Lundbeck, Wyeth) for generous gifts of drugs. During performance of these studies, BB was supported by an MENRT fellowship and by a grant from La Fondation pour la Recherche Médicale.

REFERENCES

- Adell A, Celada P, Artigas F (2001). The role of 5-HT_{1B} receptors in the regulation of serotonin cell firing and release in the rat brain. *J Neurochem* 79: 172–182.
- Adrien J (2002). Neurobiological basis for the relation between sleep and depression. *Sleep Med Rev* 6: 341–352.
- Ardid D, Alloui A, Brousse G, Jourdan D, Picard P, Dubray C *et al* (2001). Potentiation of the antinociceptive effect of clomipramine by a 5-HT_{1A} antagonist in neuropathic pain in rats. *Br J Pharmacol* 132: 1118–1126.
- Artigas F, Celada P, Laruelle M, Adell A (2001). How does pindolol improve antidepressant action. *Trends Pharmacol Sci* 22: 224–228.
- Artigas F, Perez V, Alvarez E (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch Gen Psychiatry* 51: 248–251.
- Ase AR, Reader TA, Hen R, Riad M, Descarries L (2001). Regional changes in density of serotonin transporter in the brain of 5-HT_{1A} and 5-HT_{1B} knockout mice, and of serotonin innervation in the 5-HT_{1B} knockout. *J Neurochem* 78: 619–630.
- Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* 38: 1083–1152.
- Bjorvatn B, Fagerkard S, Eid T, Ursin R (1997). Sleep/waking effects of selective 5-HT_{1A} receptor agonist given systemically as well as perfused in the dorsal raphe nucleus in rats. *Brain Res* 70: 81–88.
- Bjorvatn B, Neckelmann D, Ursin R (1992). The 5-HT_{1A} antagonist (–)-alprenolol fails to modify sleep or zimeldine-induced sleep-waking effects in rats. *Pharmacol Biochem Behav* 42: 49–56.
- Bjorvatn B, Ursin R (1994). Effects of the selective 5-HT_{1B} agonist, CGS 12066B, on the sleep/waking stages and EEG power spectrum in rats. *J Sleep Res* 3: 97–105.
- Bjorvatn B, Ursin R (1998). Changes in sleep and wakefulness following 5-HT_{1A} ligands given systemically and locally in different brain regions. *Rev Neurosci* 9: 265–273.
- Blier P, Bergeron R (1995). Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J Clin Pharmacol* 15: 217–222.
- Blier P, de Montigny C, Chaput Y (1987). Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J Clin Psychopharmacol* 7: 24S–35S.
- Boutrel B, Franc B, Hen R, Hamon M, Adrien J (1999). Key role of 5-HT_{1B} receptors in the regulation of paradoxical sleep as evidenced in 5-HT_{1B} knock-out mice. *J Neurosci* 19: 3204–3212.
- Boutrel B, Monaca C, Hen R, Hamon M, Adrien J (2002). Involvement of 5-HT_{1A} receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT_{1A} knock-out mice. *J Neurosci* 22: 4686–4692.
- Claustre Y, Rouquier L, Serrani Z, Benavides J, Scatton B (1991). Effects of the putative 5-HT_{1A} receptor antagonist NAN-190 on rat brain serotonergic transmission. *Eur J Pharmacol* 204: 71–77.
- Dzoljic MR, Ukponmwan OE, Saxena PR (1992). 5-HT₁-like receptor agonists enhance wakefulness. *Neuropharmacology* 31: 623–633.
- Evrard A, Laporte AM, Chastanet M, Hen R, Hamon M, Adrien J (1999). 5-HT_{1A} and 5-HT_{1B} receptors control the firing of serotonergic neurones in the dorsal raphe nucleus of the mouse. *Eur J Neurosci* 11: 3823–3831.
- Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP *et al* (2000). Altered expression and functions of serotonin 5-HT_{1A} and 5-HT_{1B} receptors in knock-out mice lacking the 5-HT transporter. *Eur J Neurosci* 12: 2299–2310.
- Fletcher A, Forster EA, Bill DJ, Brown G, Cliffe IA, Hartley JE *et al* (1996). Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. *Behav Brain Res* 73: 337–353.
- Fuller RW (1994). Minireview: uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. *Life Sci* 55: 163–167.
- Gao B, Duncan WC, Wehr TA (1992). Fluoxetine decreases brain temperature and REM sleep in Syrian hamsters. *Psychopharmacology* 106: 321–329.
- Gartside SE, Clifford EM, Cowen PJ, Sharp T (1999). Effects of (–)-tertatolol, (–)-penbutolol and (+/–)-pindolol in combination with paroxetine on presynaptic 5-HT function: an *in vivo* microdialysis and electrophysiological study. *Br J Pharmacol* 127: 145–152.
- Gartside SE, Umbers V, Hajos M, Sharp T (1995). Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI *in vivo*: effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol* 115: 1064–1070.
- Goodrich C (1983). Thermoregulatory effects of the specific uptake inhibitor citalopram in maturing mice. *Gen Pharmacol* 14: 525–527.
- Hamon M (1997). The main features of central 5-HT_{1A} receptors. In: Baumgarten HG, Göthert M (eds). *Serotonergic Neurons and 5-HT Receptors in the CNS, Handbook of Experimental Pharmacology*, Vol. 129. Springer-Verlag: Berlin. pp 239–268.
- Hendrickse WA, Roffwarg HP, Grannemann BD, Orsulak PPJ, Armitage R, Cain JW *et al* (1994). The effects of fluoxetine on the polysomnogram of depressed outpatients: a pilot study. *Neuropsychopharmacology* 10: 85–91.
- Hjorth S (1996). Pindolol, but not buspirone, potentiates the citalopram-induced rise in extracellular 5-hydroxytryptamine. *Eur J Pharmacol* 303: 183–186.
- Hodgkiss JP, Dawson IM, Kelly JS (1992). An intracellular study of the action of NAN-190 on neurons in the dorsal raphe nucleus of the rat. *Brain Res* 576: 157–161.
- Horner RL, Sanford LD, Annis D, Pack AI, Morrison AR (1997). Serotonin at the laterodorsal tegmental nucleus suppresses rapid-eye-movement sleep in freely behaving rats. *J Neurosci* 17: 7541–7552.
- Hyttel J (1982). Citalopram—pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. *Prog Neuropsychopharmacol Biol Psychiatry* 6: 277–295.

- Lelkes Z, Obal F, Alföldi P, Erdős A, Rubicsek G, Benedek G (1994). Effects of acute and chronic treatment with trazodone, an antidepressant, on the sleep-wake activity in rats. *Pharmacol Res* 30: 105–115.
- Levitan RD, Shen JH, Jinfal R, Driver HS, Kennedy SH, Shapiro CM (2000). Preliminary randomized double-blind placebo-controlled trial of tryptophan combined with fluoxetine to treat major depressive disorder: antidepressant and hypnotic effects. *J Psychiatry Neurosci* 25: 337–346.
- Limoge-Lendais I, Robert C, Degrange M, Goldberg M, Stinus L, Limoge A (1994). Study on superbond adhesion to the skull for chronic electrode implantation in the rat. *Neurosci Protocol* 70: 1–11.
- Londborg PD, Smith WT, Glaudin V, Painter JR (2000). Short-term cotherapy with clonazepam and fluoxetine: anxiety, sleep disturbance and core symptoms of depression. *J Affect Disord* 61: 73–79.
- Malagié I, Trillat AC, Bourin M, Jacquot C, Hen R, Gardier AM (2001). 5-HT_{1B} autoreceptors limit the effects of selective serotonin re-uptake inhibitors in mouse hippocampus and frontal cortex. *J Neurochem* 76: 865–871.
- Malagié I, Trillat AC, Jacquot C, Gardier AM (1995). Effects of acute fluoxetine on extracellular serotonin levels in the raphe: an *in vivo* microdialysis study. *Eur J Pharmacol* 286: 213–217.
- Maudhuit C, Jolas T, Lainey E, Hamon M, Adrien J (1994). Effects of acute and chronic treatment with amoxapine and cericlamine on the sleep-wakefulness cycle in the rat. *Neuropharmacology* 33: 1017–1025.
- Milne RJ, Goa KL (1991). Citalopram—a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in depressive illness. *Drugs* 41: 450–477.
- Monti JM, Monti D, Jantos H, Ponzoni A (1995). Effects of selective activation of the 5-HT_{1B} receptor with CP-94253 on sleep and wakefulness in the rat. *Neuropharmacology* 34: 1647–1651.
- Neckelmann D, Bjorkum AA, Bjorvatn B, Ursin R (1996a). Sleep and EEG power spectrum effects of the 5-HT_{1A} antagonist NAN-190 alone or in combination with citalopram. *Behav Brain Res* 75: 159–168.
- Neckelmann D, Bjorvatn B, Bjorkum AA, Ursin R (1996b). Citalopram: differential sleep/wake and EEG power spectrum effects after single dose and chronic administration. *Behav Brain Res* 79: 183–192.
- Oerther S, Ahlenius S (2001). Involvement of 5-HT_{1A} and 5-HT_{1B} receptors for citalopram-induced hypothermia in the rat. *Psychopharmacology* 154: 429–434.
- Pastel RH, Fernstrom JD (1987). Short-term effects of fluoxetine and trifluoromethyl-phenylpiperazine on electroencephalographic sleep in the rat. *Brain Res* 436: 92–102.
- Pauwels PJ (1997). 5-HT_{1B/D} receptor antagonists. *Gen Pharmacol* 29: 293–303.
- Pineyro G, Blier P (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol Rev* 51: 533–591.
- Ramboz S, Oosting R, Ait Amara D, Kung HF, Blier P, Mendelsohn M et al (1998). Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci USA* 95: 14476–14481.
- Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L et al (1994). Enhanced aggressive behavior in mice lacking 5-HT_{1B} receptor. *Science* 265: 1875–1878.
- Sharp T, Umbers V, Gartside SE (1997). Effect of a selective 5-HT reuptake inhibitor in combination with 5-HT_{1A} and 5-HT_{1B} receptor antagonists on extracellular 5-HT in rat frontal cortex *in vivo*. *Br J Pharmacol* 121: 941–946.
- Stahl SM (1998). Mechanisms of action of serotonin selective reuptake inhibitors. *J Affect Disord* 51: 215–235.
- Thase ME (2000). Treatment issues related to sleep and depression. *J Clin Psychiatry* 61(Suppl. 11): 46–50.
- Tissier M, Lainey E, Fattaccini CM, Hamon M, Adrien J (1993). Effects of ipsapirone, a 5-HT_{1A} agonist, on sleep/wakefulness cycles: probable post-synaptic action. *J Sleep Res* 2: 103–109.
- Trillat AC, Malagié I, Mathe-Allainmat M, Anmella MC, Jacquot C, Langlois M et al (1998). Synergistic neurochemical and behavioral effects of fluoxetine and 5-HT_{1A} receptor antagonists. *Eur J Pharmacol* 357: 179–184.
- Ursin R (2002). Serotonin and sleep. *Sleep Med Rev* 6: 55–67.
- Ursin R, Bjorvatn B, Sommerfelt L, Underland G (1989). Increased waking as well as increased synchronization following administration of selective 5-HT uptake inhibitors to rats. *Behav Brain Res* 34: 117–130.
- Van Bommel AL, Beersma DG, Van Den Hoofdakker RH (1993). Changes in EEG power density of NREM sleep in depressed patients during treatment with citalopram. *J Sleep Res* 2: 156–162.