

# Desensitization of 5-HT<sub>1A</sub> Autoreceptors by a Low Chronic Fluoxetine Dose Effect of the Concurrent Administration of WAY-100635

Ildefonso Hervás, Ph.D., M. Teresa Vilaró, Ph.D., Luz Romero, Ph.D., M. Cecilia Scorza, M.D.,  
Guadalupe Mengod, Ph.D., and Francesc Artigas, Ph.D.

Using microdialysis, receptor autoradiography and *in situ* hybridization, we examined the effects of fluoxetine alone or with WAY-100635 on: (a) extracellular 5-HT in frontal cortex; and (b) density and sensitivity of 5-HT<sub>1A</sub> autoreceptors in rat brain. WAY-100635 (0.3 mg/kg, s.c.) doubled the increase in extracellular 5-HT produced by fluoxetine (3 mg/kg, i.p.) in frontal cortex. Two-week minipump treatments with these daily doses significantly raised extracellular 5-HT to 275 ± 33% (fluoxetine) and 245 ± 10% (fluoxetine plus WAY-100635) of controls. Fluoxetine 3 mg/kg-day desensitized dorsal raphe 5-HT<sub>1A</sub>

autoreceptors, an effect prevented by the concurrent WAY-100635 administration. However, WAY-100635 (alone or with fluoxetine) did not change 5-HT<sub>1A</sub> autoreceptor sensitivity. The density of 5-HT<sub>1A</sub> receptors and its encoding mRNA, was unaffected by these treatments. These results suggest that prolonged blockade of 5-HT<sub>1A</sub> receptors *in vivo* prevents the autoreceptor desensitization induced by fluoxetine but does not result in receptor sensitization. [Neuropsychopharmacology 24:11–20, 2001] © 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

**KEY WORDS:** 5-hydroxytryptamine uptake; 5-HT<sub>1A</sub> receptors; Dorsal raphe nucleus; Frontal cortex; Microdialysis; Selective serotonin reuptake inhibitors (SSRI)

Selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) are extensively used in the treatment of major depression. The increase in forebrain extracellular 5-HT elicited by SSRIs is limited by a negative feed-back involving raphe autoreceptors (Artigas et al. 1996). The prevention of this inhibitory mech-

anism with 5-HT<sub>1A</sub> receptor antagonists augments the neurochemical and behavioral effects of SSRIs (Gartside et al. 1995; Artigas et al. 1996; Hashimoto et al. 1997; Mitchell and Redfern 1997; Grignaschi et al. 1998; Trillat et al. 1998). At the clinical level, the β-adrenoceptor/5-HT<sub>1A</sub> receptor antagonist pindolol accelerates the antidepressant effects of SSRIs in open-label and placebo-controlled trials (Artigas et al. 1994; Blier and Bergeron 1995; Pérez et al. 1997; Zanardi et al. 1997, 1998; Bordet et al. 1998). However, its effectiveness to potentiate antidepressant response in chronically ill or treatment-resistant patients is still controversial (Maes et al. 1996, 1999; Berman et al. 1997; Moreno et al. 1997; Pérez et al. 1999). Based on this rationale, selective 5-HT<sub>1A</sub> receptor antagonists (for use with SSRIs; add-on strategy) and dual action compounds (5-HT reuptake inhibitor + 5-HT<sub>1A</sub> antagonist) are being developed for use in the treatment of major depression.

From the Department of Neurochemistry, Instituto de Investigaciones Biomédicas de Barcelona (CSIC), IDIBAPS, Barcelona, Spain. M. Cecilia Scorza permanent address: Instituto Clemente Estable, Montevideo, Uruguay.

Address correspondence to: Francesc Artigas, Department of Neurochemistry, Instituto de Investigaciones Biomédicas de Barcelona (CSIC), IDIBAPS, Rosselló, 161, 6th floor, 08036 Barcelona, Spain.

Received 13 April 2000; revised 19 May 2000; accepted 30 June 2000.

The prolonged administration of SSRIs has been reported to desensitize raphe 5-HT<sub>1A</sub> autoreceptors, as assessed by single unit recordings and brain microdialysis (Blier and de Montigny 1994; Invernizzi et al. 1994; Arborelius et al. 1995; Le Poul et al. 1995). This reduces the efficacy of the above negative feedback and increases extracellular 5-HT (Bel and Artigas 1993; Invernizzi et al. 1994; Rutter et al. 1994; Arborelius et al. 1996). Other studies, however, have failed to observe such effects even using large doses of SSRIs (Hjorth and Auerbach 1994a; Auerbach and Hjorth 1995; Bosker et al. 1995; Invernizzi et al. 1995).

To our knowledge, there are no published reports on the effects of prolonged treatments with combinations of SSRIs and 5-HT<sub>1A</sub> receptor antagonists on these experimental paradigms, except in abstract form (Dawson et al. 1998). As with pindolol, future selective 5-HT<sub>1A</sub> receptor antagonists would be administered for a limited period of time. Should prolonged blockade of 5-HT<sub>1A</sub> receptor result in receptor sensitization, the withdrawal of the antagonist would increase the efficacy of the above negative feedback and reduce the ability of the SSRI to increase extracellular 5-HT, thus increasing the possibility of a clinical relapse. Since this is crucial for the success of this therapeutic strategy, we examined the effects of two-week treatments with fluoxetine, WAY-100635 and their combination on the labeling and sensitivity of 5-HT<sub>1A</sub> autoreceptors in rat brain.

## METHODS

### Microdialysis Procedures

Male Wistar rats (280–300 g; Iffa-Credo, Lyon, France) were used. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986). A detailed description of the microdialysis procedures can be found in Adell and Artigas (1998). Briefly, anesthetized rats (pentobarbital, 60 mg/kg, i.p.) were placed in a David Kopf (Tujunga, CA) stereotaxic frame. Concentric dialysis probes (4.0 mm long) were implanted in frontal cortex and secured to the skull with anchor screws and dental cement. Dialysis membranes were made from hollow Cuprophane fibers with 252 µm OD, 220 µm ID, and 5000-dalton molecular weight cutoff (GFE09; Gambro, Lund, Sweden).

The stereotaxic coordinates (in mm; AP +3.4, DV –6.0, L –2.5) were taken from bregma and dura mater according to the rat brain atlas of Paxinos and Watson (1986). Rats were allowed to recover from anesthesia in the dialysis cages (cubic, 40 cm each side) and 20–24 h later the probes were perfused with artificial CSF (aCSF; composition: NaCl 125 mM, KCl 2.5 mM, MgCl<sub>2</sub> 1.18 mM, and CaCl<sub>2</sub> 1.26 mM; pH 6.5–7.0) at 0.25 µl/min. In some experiments, the aCSF was supplemented with 1 µM of the SSRI citalopram.

Dialysate samples of 5 µl were collected at 20-min intervals into polypropylene microcentrifuge vials. After an initial 1-h sample of dialysate was discarded, four to six fractions were collected to obtain basal values before drug administration. At the end of the experiments, rats were killed by an overdose of sodium pentobarbital and the placement of the dialysis probes was checked by perfusing Fast Green dye and examination of the probe track after cutting the brain at the appropriate level.

5-HT was analyzed by a modification of a high performance liquid chromatography method previously described (updated in Adell and Artigas 1998). 5-HT was separated on a 3 µm ODS 2 column (7.5 cm × 0.46 cm; Beckman, San Ramon, CA) and detected amperometrically with a Hewlett Packard 1049 detector set at the potential of +0.6V. Retention time was 3.5–4 min. The detection limit for 5-HT was typically 0.5–1 fmol/sample. Dialysate 5-HT values were calculated by reference to standard curves run daily.

### Treatments

Four different experiments were conducted. In the first one, we examined the effects on extracellular 5-HT in frontal cortex of single doses of fluoxetine (3 mg/kg, i.p.) plus (20 min later) saline or WAY-100635 [*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl) cyclohexanecarboxamide·3HCl] (RBI, Natick, MA) (0.3 mg/kg, s.c.). A second experiment assessed the effect of two-week minipump treatments with fluoxetine (3 mg/kg-day) and fluoxetine plus WAY-100635 (0.3 mg/kg-day) on the basal extracellular 5-HT concentration. In this group, microdialysis experiments were conducted on the 14th day of treatment, with the minipump on board.

In a third experiment, the same treatments (fluoxetine 3 mg/kg-day and fluoxetine 3 mg/kg-day plus WAY-100635 0.3 mg/kg-day) were given but minipumps were removed under light anesthesia on day 14th and microdialysis experiments were conducted two days later to examine the labeling and sensitivity of 5-HT<sub>1A</sub> receptors. A complete washout of fluoxetine was expected at this time, as judged from its complete elimination in two days from the brain compartment after 21-day treatments with higher doses (10–30 mg/kg-day) (Gardier et al. 1993). In these experimental groups, dialysis probes were perfused with an artificial CSF containing 1 µM citalopram. In this experimental condition, the systemic administration of SSRIs reduces extracellular 5-HT due to the activation of somatodendritic 5-HT<sub>1A</sub> autoreceptors (see Discussion). With the same time schedule (two-week treatment plus two-day washout) and experimental conditions, a fourth experiment examined the effect of WAY-100635 (0.3 mg/kg-day) alone on the sensitivity of raphe 5-HT<sub>1A</sub> receptors. Control rats in all groups received the corresponding vehicle for the same time periods.

In chronic experiments, rats were implanted s.c. with Alzet 2002 minipumps filled to deliver vehicle (water/dimethyl sulfoxide 50/50%), fluoxetine 3 mg/kg-day (Eli Lilly and Co., Indianapolis, IN), WAY-100635 0.3 mg/kg-day or fluoxetine plus WAY-100635 for 14 days. Drugs were dissolved in vehicle. The use of dimethyl sulfoxide to dissolve fluoxetine was necessary due to the small volume of minipumps (300  $\mu$ l). Given the weight gain of the animals, the doses used correspond to the 7th day of treatment. After minipump implants, rats were kept one per cage. After removal, the minipumps were cut with an incisor blade and checked that they were empty by careful visual inspection.

### Autoradiography and *In Situ* Hybridization

We assessed the effect of the above minipump treatments on the labeling of 5-HT<sub>1A</sub> receptors using receptor autoradiography and on the mRNA encoding 5-HT<sub>1A</sub> receptors by *in situ* hybridization. These experiments were performed in rats subjected to a two-week treatment plus a two-day washout period, after the microdialysis experiments had been completed. The brains were carefully removed from the skull, frozen on dry ice and kept at  $-20^{\circ}\text{C}$  until sample processing. A detailed description of the autoradiographic and *in situ* hybridization procedures is given in Casanovas et al. (1999a). Brain sections, 14  $\mu$ m thick, were cut on a microtome-cryostat, thaw-mounted, and kept at  $-20^{\circ}\text{C}$  until used. [<sup>3</sup>H]-8-OH-DPAT (234 Ci/mmol) (Amersham, UK) was used as ligand.

Tissues were preincubated to wash out endogenous 5-HT and exogenously administered drugs that could potentially interfere with the labeling of 5-HT<sub>1A</sub> receptors. Optimal preincubation time was determined in pilot experiments using sections containing the dorsal raphe nucleus (DR) and the hippocampus (rich in postsynaptic 5-HT<sub>1A</sub> receptors) and found to be 120 min (a plateau was reached at 90 min). After preincubation, sections were incubated in the presence of [<sup>3</sup>H]-8-OH-DPAT (0.5 nM). Non-specific binding was defined in the presence of 10  $\mu$ M 5-HT. Sections were washed and dried before exposure (14 days, 4 $^{\circ}\text{C}$ ) to Hyperfilm-<sup>3</sup>H (Amersham, UK).

For *in situ* hybridization experiments, an oligonucleotide probe complementary to the mRNA coding for the rat 5-HT<sub>1A</sub> receptor (amino acids 407–422) was used (Pompeiano et al. 1992). The oligonucleotide was 3' end-labeled with terminal deoxynucleotidyltransferase (Boehringer Mannheim) and [<sup>32</sup>P] $\alpha$ -dATP (3000 Ci/mmol; DuPont New England Nuclear). Tissue sections were pretreated and hybridized as described (Casanovas et al. 1999a). Hybridized sections were exposed to  $\beta$ -max film (Amersham) for 12 days at  $-70^{\circ}\text{C}$  with intensifying screens. Quantitative image analysis was performed with the MCID computerized image analysis system (St Catharines, Ontario, Canada).

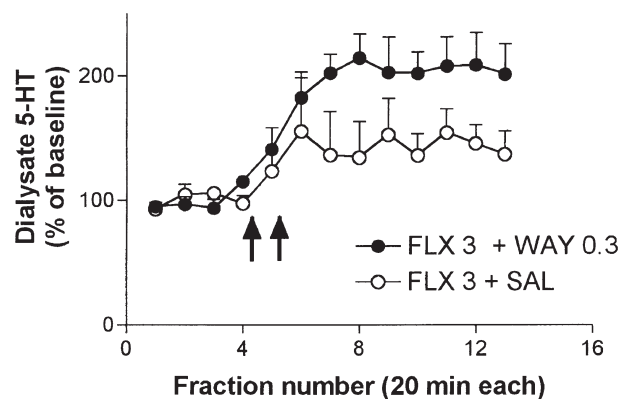
### Data Treatment

Dialysate 5-HT concentrations are expressed as fmol/fraction and represented in some figures as percentage of baseline (average of four pre-drug fractions). The statistical analysis of raw data (in fmol/fraction) was performed using two-way analysis of variance (ANOVA) for repeated measures with time and pretreatment (vehicle, fluoxetine, WAY-100635, or fluoxetine plus WAY-100635) as main effects. We analysed the effect of the independent factor (treatment group), the repeated factor (time) and the interaction between them. The latter assesses whether the change in 5-HT from pre-drug values differs between the two treatment groups. Thus, a significant *p*-value of the interaction indicates differences in the effects of two treatments on extracellular 5-HT. One-way ANOVA for independent data followed by post-hoc tests was also used. Results are expressed as mean  $\pm$  SEM. Statistical significance has been set at the 95% confidence level (two-tailed).

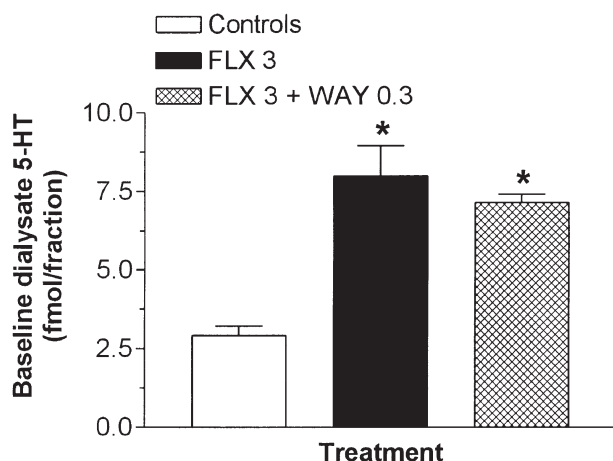
## RESULTS

### Acute Treatment with Fluoxetine and Fluoxetine + WAY-100635

Rats were treated with fluoxetine 3 mg/kg, i.p. Twenty minutes later, they received an injection of saline or WAY-100635 0.3 mg/kg, s.c. Baseline 5-HT values were  $1.5 \pm 0.1$  fmol/fraction in the fluoxetine plus saline group ( $n = 6$ ) and  $2.1 \pm 0.4$  fmol/fraction in the fluoxetine plus WAY-100635 group ( $n = 8$ ). Extracellular 5-HT in frontal cortex raised to a maximum of  $154 \pm 19\%$  of baseline in the rats treated with fluoxetine and saline and to  $213 \pm 10\%$  of baseline in rats treated with fluoxetine plus WAY-100635. Two-way repeated measures



**Figure 1.** Effect of the administration of (first arrow) 3 mg/kg fluoxetine and (second arrow) saline (open circles,  $n = 6$ ) or WAY-100635 0.3 mg/kg (filled circles;  $n = 8$ ) on extracellular 5-HT in frontal cortex. The effect of fluoxetine plus WAY-100635 was significantly greater than that of fluoxetine alone (two-way repeated measures ANOVA; see text for details).



**Figure 2.** Effect of two-week treatments with vehicle (open bar;  $n = 7$ ), fluoxetine 3 mg/kg-day (filled bar;  $n = 8$ ), or fluoxetine 3 mg/kg-day plus 0.3 mg/kg-day WAY 100635 (cross-hatched bar;  $n = 7$ ) on the basal extracellular 5-HT in frontal cortex. \* $p < .05$  vs. controls (Tukey test post-ANOVA).

ANOVA indicated a significant effect of the group ( $F_{1,12} = 7.27$ ,  $p < .02$ ), time ( $F_{12,144} = 9.37$ ,  $p < .001$ ) and time  $\times$  group interaction ( $F_{12,144} = 3.43$ ,  $p < .001$ ) (Figure 1).

#### Effect of 2-Week Treatments with Fluoxetine and Fluoxetine + WAY-100635 on Extracellular 5-HT

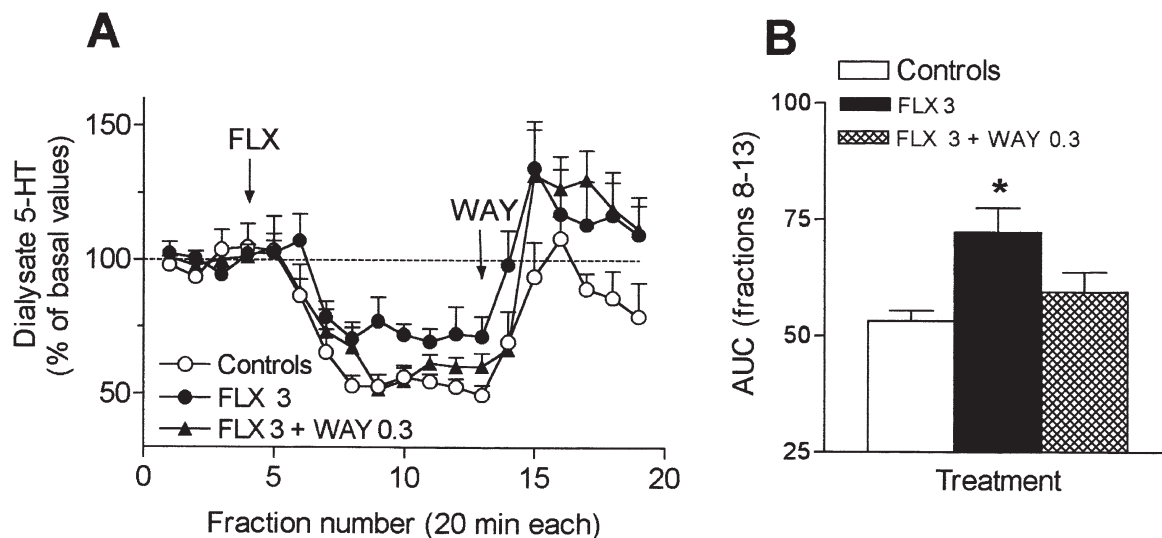
The baseline extracellular 5-HT (average of four 20-min fractions) was determined in rats treated with mini-

pumps for two weeks with vehicle, fluoxetine 3 mg/kg-day, and fluoxetine 3 mg/kg-day plus WAY-100635 0.3 mg/kg-day (no washout) and found to be  $2.9 \pm 0.3$  ( $n = 7$ ),  $8.0 \pm 1.0$  ( $n = 8$ ), and  $7.1 \pm 0.3$  fmol/fraction ( $n = 7$ ), respectively. One-way ANOVA for independent measures revealed a significant effect of the group ( $F_{2,21} = 17.49$ ,  $p < .0001$ ) with significant differences between the fluoxetine and fluoxetine plus WAY-100635 groups vs. controls (post-hoc Tukey test) (Figure 2).

#### Desensitization of 5-HT<sub>1A</sub> Receptors

After a two-day washout, the baseline 5-HT concentration (in presence of 1  $\mu$ M citalopram) in rats pretreated for two weeks with vehicle, fluoxetine 3 mg/kg-day, or fluoxetine 3 mg/kg-day plus WAY-100635 0.3 mg/kg-day was  $33.5 \pm 2.0$  fmol/fraction ( $n = 6$ ),  $24.9 \pm 2.6$  fmol/fraction ( $n = 7$ ), and  $20.3 \pm 2.6$  fmol/fraction ( $n = 7$ ), respectively. The 5-HT values in the latter group were significantly different from controls (Tukey test post one-way ANOVA).

The administration of 10 mg/kg, i.p. fluoxetine reduced extracellular 5-HT to 53% of baseline in control rats. The analysis by two-way ANOVA of fractions 1–13 (effect of the fluoxetine challenge) in all groups indicated the existence of a significant effect of time ( $F_{12,204} = 37.21$ ,  $p < .001$ ), group ( $F_{2,17} = 6.34$ ,  $p < .009$ ) and time  $\times$  group interaction ( $F_{24,204} = 2.58$ ,  $p < .001$ ). Likewise, one-way ANOVA of the average 5-HT values during the period of maximal effect (fractions 8–13) followed



**Figure 3.** (A) In presence of citalopram in the perfusion fluid, fluoxetine (10 mg/kg i.p., first arrow) significantly reduced extracellular 5-HT in frontal cortex of rats treated with vehicle (Controls; open circles,  $n = 6$ ), fluoxetine 3 mg/kg-day (FLX 3; filled circles,  $n = 7$ ), and fluoxetine 3 mg/kg-day plus WAY-100635 0.3 mg/kg-day (FLX 3 + WAY 0.3; filled triangles,  $n = 7$ ) (significant effect of the time and group  $\times$  time interaction; see text). The reduction in extracellular 5-HT was fully counteracted by the systemic administration of WAY-100635 (0.3 mg/kg, s.c.; second arrow), indicating the involvement of 5-HT<sub>1A</sub> autoreceptors in this effect. (B) Average reduction (fractions 8–13) of extracellular 5-HT produced by fluoxetine 10 mg/kg in the three experimental groups (vehicle, open bar; fluoxetine, filled bar; fluoxetine plus WAY-100635, crosshatched bar). \* $p < .05$  vs. controls.

by Tukey test revealed a significantly lower reduction of 5-HT in the fluoxetine group vs. the two other groups, with no differences between them (Figure 3B). The fluoxetine challenge reduced extracellular 5-HT to 53% of baseline in controls (maximal effect size: 47%). Thus, a reduction to 72% of baseline (effect size: 28%) in the fluoxetine-treated group can be equated to a 40% fall in the overall sensitivity of DR 5-HT<sub>1A</sub> autoreceptors.

The administration of WAY-100635 (0.3 mg/kg, s.c.) reversed the fluoxetine-induced decrease in extracellular 5-HT in all three groups (significant effect of time,  $F_{9,153} = 25.68$ ,  $p < .001$ ; non-significant effect of the group and of time  $\times$  group interaction; fractions 10–19) (Figure 3A).

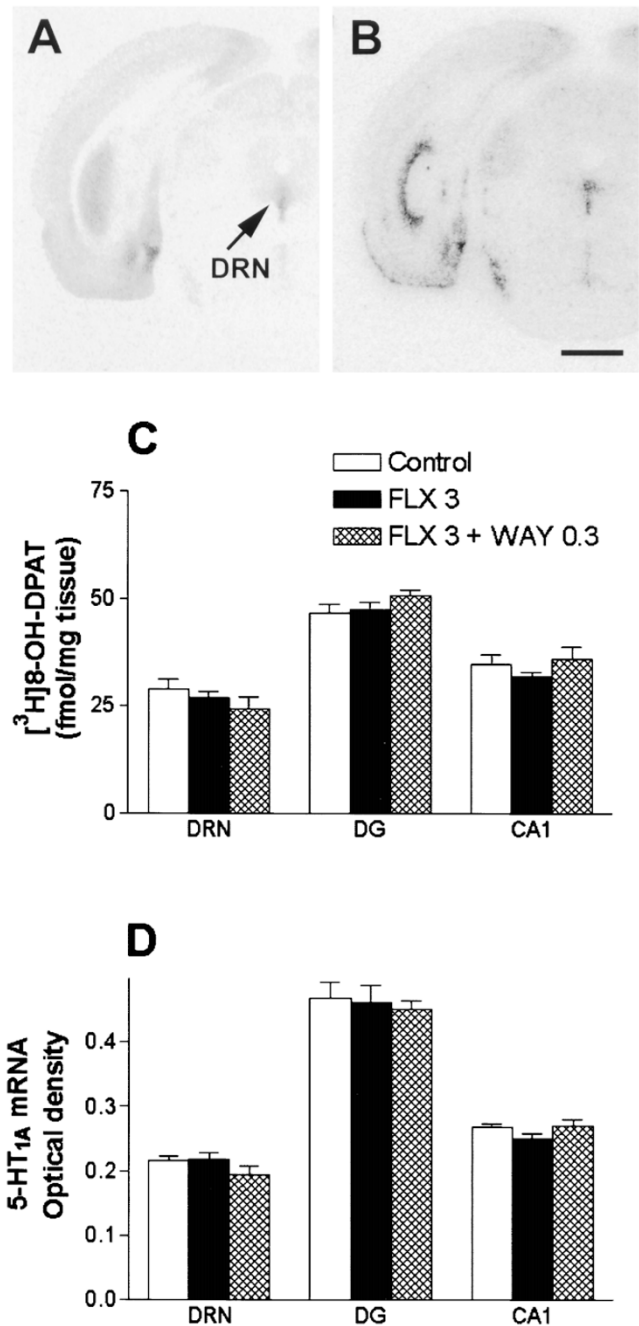
To assess whether the prolonged treatment with WAY-100635 alone could alter the sensitivity of 5-HT<sub>1A</sub> autoreceptors, we conducted an additional experiment. Two groups of rats were treated with vehicle or WAY-100635 0.3 mg/kg-day, as above ( $n = 6$  rats/group).

The administration of 10 mg/kg, i.p. fluoxetine reduced comparably extracellular 5-HT in both groups (maximal decrease to  $60 \pm 7\%$  in controls and to  $61 \pm 6\%$  of baseline in WAY 100635-treated rats). Two-way ANOVA of fractions 1–13 (effect of the fluoxetine challenge) indicated a significant effect of time ( $F_{12,96} = 3.44$ ,  $p < .001$ ) but not of the group or the time  $\times$  group interaction, indicating the absence of differences between the two groups. Likewise, the average reduction of extracellular 5-HT during the period of maximal effect (fractions 8–13) was  $62.6 \pm 5.6\%$  of baseline in controls and  $68.8 \pm 5.4\%$  in WAY-100635-treated rats (non-significant difference; Student's *t*-test). As in previous experiments, the administration of WAY-100635 completely reversed the fluoxetine-induced reduction of extracellular 5-HT.

#### Autoradiographic and *In Situ* Hybridization Experiments

Representative midbrain sections showing the DR labeled by [<sup>3</sup>H]8-OH-DPAT and by the probe complementary to the mRNA encoding the 5-HT<sub>1A</sub> receptors are displayed in Figures 4A and 4B, respectively. The pretreatment with fluoxetine 3 mg/kg-day alone or in combination with 0.3 mg/kg-day WAY-100635 did not significantly alter the labeling of 5-HT<sub>1A</sub> receptors by [<sup>3</sup>H]8-OH-DPAT binding to the DR, area CA1 or dentate gyrus (DG) (Figure 4C). Likewise, the treatment with WAY-100635 alone did not significantly modify the labeling in any of the brain regions ( $24.4 \pm 3.6$  and  $21.2 \pm 1.05$  in the DR,  $44.1 \pm 1.5$  and  $43.2 \pm 0.9$  in DG,  $31.9 \pm 1.4$  and  $30.2 \pm 1.5$  in CA1 for controls and WAY-100635-treated rats, respectively; data in fmol/mg tissue;  $n = 4$ –5 rats/group).

No significant differences were noted between any treated group and the respective controls in the density



**Figure 4.** Effects of the two-week treatment with fluoxetine 3 mg/kg-day alone (FLX 3) or in combination with WAY-100635 0.3 mg/kg-day (FLX 3 + WAY 0.3) on the density of 5-HT<sub>1A</sub> receptors and their mRNA. (A, B) Consecutive coronal sections through the DR of a control rat showing the autoradiographic labeling of 5-HT<sub>1A</sub> receptors with [<sup>3</sup>H]-8-OH-DPAT (A) and the distribution of 5-HT<sub>1A</sub> receptor mRNA (B); bar = 2mm. (C) Quantitative measurements of the labeling of 5-HT<sub>1A</sub> receptors labeled with [<sup>3</sup>H]-8-OH-DPAT in the different treatment groups ( $n = 6$ –8 rats/group). (D) Densitometric measurements of 5-HT<sub>1A</sub> receptor mRNA. Film optical density in brain regions devoid of specific hybridization signal was 0.09 ( $n = 5$ –8 rats/group).

of the mRNA encoding the 5-HT<sub>1A</sub> receptor in the DR or hippocampus (Figure 4D). Also, the treatment with WAY alone did not induce any change in the density of the mRNA neither in the DR ( $0.25 \pm 0.02$  vs.  $0.22 \pm 0.01$ ) nor in the DG ( $0.44 \pm 0.01$  vs.  $0.47 \pm 0.03$ ), or CA1 ( $0.27 \pm 0.01$  vs.  $0.27 \pm 0.01$ ) for controls and WAY-100635-treated rats, respectively (data are optical densities;  $n = 4$ – $5$  rats/group).

## DISCUSSION

Two findings derive from the present study. First, a two-week treatment with a low fluoxetine dose desensitized 5-HT<sub>1A</sub> autoreceptors. Second, the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 prevented this effect but did not sensitize nor up-regulated 5-HT<sub>1A</sub> autoreceptors when given alone or in combination with fluoxetine. These *in vivo* observations are important for the design of therapeutic strategies based on SSRI + 5-HT<sub>1A</sub> antagonist combinations. Several 5-HT<sub>1A</sub> receptor antagonists and dual action compounds are being developed. The present data suggest that withdrawal of such compounds would not result in a clinical relapse due to an exacerbation of the 5-HT<sub>1A</sub> autoreceptor-based negative feed-back that offsets the increase in 5-HT produced by SSRIs in forebrain.

With few exceptions, most studies assessing the effects of the long-term administration of SSRIs on the 5-HT system employed relatively large daily doses (e.g., 10–20 mg/kg-day) that inhibit maximally the 5-HT reuptake and markedly increase extracellular 5-HT in forebrain when given at once (Invernizzi et al. 1994, 1995; Rutter et al. 1994; Arborelius et al. 1996; Gundlah et al. 1997).

In the present study, we used a fluoxetine dose intended to mimic the early effects of a standard clinical dose (20 mg/day). In patients treated with this dose, plasma fluoxetine concentration increased steadily from 0.08  $\mu$ M at day 3 to 0.16  $\mu$ M at day 14 (Pérez et al. *in press*). In rats, 5 mg/kg, p.o. fluoxetine yielded a maximal plasma concentration of 0.15  $\mu$ M (Caccia et al. 1990). Taking into account the differences between p.o. and s.c. routes (oral bioavailability in the rat is 38%) (Caccia et al. 1990), and in absence of more pharmacokinetic data in the literature, we chose 3 mg/kg-day as a dose that could mimic the effects of fluoxetine in depressed patients. This dose elicits a moderate increase of extracellular 5-HT in frontal cortex and other forebrain areas when given at once (Hervás and Artigas 1998). Yet, due to the greater 5-HT increase in midbrain, it is sufficient to activate 5-HT<sub>1A</sub> autoreceptors, as shown by the fact that WAY-100635 potentiated its effects on extracellular 5-HT, to a level similar to that elicited by higher (10–20 mg/kg) fluoxetine doses (Malagie et al. 1995, 1996; Hervás and Artigas 1998).

In keeping with previous observations using a low fluvoxamine dose (Bel and Artigas 1993, 1996), the treatment with 3 mg/kg-day fluoxetine for two weeks significantly increased extracellular 5-HT in frontal cortex (to 275% of controls). The magnitude of the change was much greater than that produced by a single administration of the same dose (compare Figures 1 and 2). Rats treated for two weeks with fluoxetine and WAY-100635 displayed a similar increase of extracellular 5-HT (to 245% of controls). This may appear at variance with the WAY-100635-induced potentiation observed in acute experiments. It is possible that differences in 5-HT<sub>1A</sub> receptor desensitization between the two groups can explain this discrepancy (see below).

The greater baseline extracellular 5-HT in the treated groups was not observed when 1  $\mu$ M citalopram was present in the perfusion fluid. It may be that such differences are overcome by the more marked effect of citalopram on extracellular 5-HT (1  $\mu$ M citalopram increased extracellular 5-HT five-fold in frontal cortex) (Hervás et al. 2000). Furthermore, it has been shown that a washout period can abolish the increase in extracellular 5-HT elicited by chronic SSRI treatment (Arborelius et al. 1996). Since citalopram was present in the perfusion fluid from the beginning of the experiments, we could not determine which of these factors is accountable.

The systemic administration of the fluoxetine challenge (10 mg/kg, i.p.) markedly reduced extracellular 5-HT in frontal cortex with citalopram in the perfusion fluid. This agrees with previous data in the literature using this and other SSRIs (Rutter and Auerbach 1993; Auerbach et al. 1995; Romero and Artigas 1997; Hervás and Artigas 1998). It may appear paradoxical that the net effect of fluoxetine on extracellular 5-HT (increase or decrease) depends on the experimental condition used. This difference is due to the fact that SSRIs and, in general, 5-HT uptake blockers behave as indirect agonists of raphe 5-HT<sub>1A</sub> autoreceptors (see Artigas et al. 1996 for review) and reduce 5-HT release.

In normal conditions (i.e., without an SSRI in the perfusion fluid), this effect is more than compensated by the inhibition of 5-HT reuptake in nerve terminals and, therefore, they increase extracellular 5-HT at moderate and high doses. However, in conditions of local inhibition of reuptake in forebrain, the local (in raphe) or systemic administration of selective and non-selective 5-HT reuptake inhibitors results in a reduction of 5-HT release in forebrain (Adell and Artigas 1991; Rutter and Auerbach 1993; Auerbach et al. 1995; Romero and Artigas 1997). This effect involves the activation of raphe 5-HT<sub>1A</sub> receptors, as it is antagonized by WAY-100635 and non-selective 5-HT<sub>1A</sub> antagonists (Hjorth and Auerbach 1994b; Romero et al. 1994; Auerbach et al. 1995; Romero and Artigas 1997; Hervás and Artigas 1998). Therefore, the reduction in extracellular 5-HT in frontal

cortex during local blockade of the 5-HT uptake can be used as a functional index of the sensitivity of raphe 5-HT<sub>1A</sub> autoreceptors. The use of SSRIs to probe the sensitivity of 5-HT<sub>1A</sub> autoreceptors is preferable to that of direct 5-HT<sub>1A</sub> agonists (e.g., 8-OH-DPAT) since these may also decrease extracellular 5-HT in frontal cortex through the activation of postsynaptic 5-HT<sub>1A</sub> receptors (Casanovas et al. 1999b).

The decrease in 5-HT produced by the fluoxetine challenge in rats pretreated with fluoxetine 3 mg/kg·day was significantly less marked than in control rats. The *in vivo* release of 5-HT in frontal cortex depends on the activity of DR neurones (McQuade and Sharp 1997). Thus, the present results suggest that the fluoxetine pretreatment desensitized 5-HT<sub>1A</sub> autoreceptors in the DR. The magnitude of the change may appear moderate, but is similar to that produced by a maximal daily dose of a selective 5-HT<sub>1A</sub> receptor agonist given for the same time period (Casanovas et al. 1999a). The fact that the desensitization is not complete (i.e., the fluoxetine challenge could still significantly reduce extracellular 5-HT in fluoxetine-pretreated rats) agrees with other studies showing that 5-HT<sub>1A</sub> autoreceptor antagonists can increase the activity of DR 5-HT cells and augment the effect of the chronic treatment with a high citalopram dose (20 mg/kg·day) on cortical extracellular 5-HT (Arborelius et al. 1996; Gundlach et al. 1997).

The presence of WAY-100635 in the minipumps prevented the desensitization of 5-HT<sub>1A</sub> receptors produced by fluoxetine pretreatment, as shown by the identical reduction of extracellular 5-HT in controls and in the group treated with the combination. Likewise, the pretreatment with the same daily dose of WAY-100635 alone did not alter the ability of the fluoxetine challenge to reduce extracellular 5-HT compared with the respective controls. Both observations suggest that the continuous blockade of 5-HT<sub>1A</sub> receptors by WAY-100635, alone or with fluoxetine, did not result in receptor sensitization. The differences in sensitivity of 5-HT<sub>1A</sub> receptors between the fluoxetine and fluoxetine plus WAY-100635 groups suggest that the increase in extracellular 5-HT observed at the 14th day of treatment (no washout) may have a different origin. Hence, the 5-HT elevation can be tentatively ascribed to 5-HT<sub>1A</sub> autoreceptor desensitization (fluoxetine alone) and to the presence of the 5-HT<sub>1A</sub> antagonist (fluoxetine plus WAY-100635).

The change in 5-HT<sub>1A</sub> autoreceptor sensitivity in the fluoxetine-pretreated group was not accompanied by a decrease in the labeling of 5-HT<sub>1A</sub> receptors or their encoding mRNA in the DR. Also, postsynaptic 5-HT<sub>1A</sub> receptors in hippocampus were unaffected. It should be emphasized that both observations (change in receptor sensitivity and unchanged density) have been obtained in the same animals. Previous work with SSRIs and se-

lective 5-HT<sub>1A</sub> agonists is inconclusive in regards to the ability of these agents to down-regulate the density of raphe 5-HT<sub>1A</sub> autoreceptors after chronic (2–3 weeks) treatments (Welner et al. 1989; Hensler et al. 1991; Fanelli and McMonagle-Strucko 1992; Casanovas et al. 1999a; Le Poul et al. 1999), whereas there is convincing evidence on their ability to induce a functional desensitization (Blieher and de Montigny 1987, 1994; Hensler et al. 1991; Invernizzi et al. 1994; Casanovas et al. 1999a; see however Sharp et al. 1993).

Overall, our observations accord with the unchanged receptor density observed in some of the above rat studies. They are also in agreement with a recent report indicating that prolonged treatment with SSRIs did not change the labeling of pre- and postsynaptic 5-HT<sub>1A</sub> receptors in depressed patients, as assessed by positron emission tomography with [<sup>11</sup>C]WAY-100635 (Sargent et al. 2000). On account of the present observations, the unchanged receptor density in the latter study does not preclude the existence of a functional receptor desensitization. The mismatch between receptor desensitization and unchanged receptor density may be tentatively explained by a reduction in midbrain G<sub>i</sub> and G<sub>o</sub> proteins that would diminish the efficacy of the coupling to the effector system (Li et al. 1996). Also, it is unclear whether receptor autoradiography can visualize internalized receptors in addition to those on the membrane.

In keeping with the above functional studies, the prolonged treatment with WAY-100635 alone or in combination with fluoxetine did not up-regulate the density of 5-HT<sub>1A</sub> receptors and its encoding mRNA. These *in vivo* observations may appear at variance with the widely held concept of receptor sensitization/up-regulation by antagonists. To our knowledge, this is the first study assessing the *in vivo* effects of the prolonged blockade of 5-HT<sub>1A</sub> receptors on their sensitivity/labeling in rat brain, and therefore the present conclusions must await further confirmation. Likewise, few *in vitro* data are also available. Exposure to WAY-100635 of a stable cell line transfected with human 5-HT<sub>1A</sub> receptors resulted in a paradoxical receptor down-regulation (Smith et al. 1998) despite the fact that WAY-100635 has no intrinsic activity (Newman-Tancredi et al. 1998). This observation, although puzzling, is indicative that WAY-100635 does not up-regulate or sensitize 5-HT<sub>1A</sub> autoreceptors *in vitro*. Yet, given the different experimental conditions between both studies, any similarity may be coincidental.

In summary, the present results show that a two week treatment with a low fluoxetine dose markedly increases extracellular 5-HT in frontal cortex and desensitizes 5-HT<sub>1A</sub> autoreceptors in the DR. This change occurs without a parallel modification of the 5-HT<sub>1A</sub> receptor protein/mRNA. WAY-100635 enhances the effect of fluoxetine after single treatment and prevents

the desensitization induced by chronic fluoxetine but does not sensitize or up-regulate 5-HT<sub>1A</sub> autoreceptors.

### ACKNOWLEDGMENTS

This work was supported by Lilly S.A. and the Fondo de Investigación Sanitaria (FIS 989/0697). I.H. was recipient of a fellowship from the Generalitat de Catalunya (CIRIT). The Department of Neurochemistry is a *Grup de Recerca de Qualitat* (1997-SGR23) from the Generalitat de Catalunya. Thanks are given to Leticia Campa for excellent technical assistance and to the pharmaceutical companies by their kind supply of drugs.

### REFERENCES

- Adell A, Artigas F (1991): Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An in vivo microdialysis study. *Naunyn-Schmied Arch Pharmacol* 343:237–244
- Adell A, Artigas F (1998): A microdialysis study of the in vivo release of 5-HT in the median raphe nucleus of the rat. *Br J Pharmacol* 125:1361–1367
- Arborelius L, Nomikos GG, Grillner P, Hertel P, Hook BB, Hacksell U, Svensson TH (1995): 5-HT<sub>1A</sub> receptor antagonists increase the activity of serotonergic cells in the dorsal raphe nucleus in rats treated acutely or chronically with citalopram. *Naunyn-Schmied Arch Pharmacol* 352:157–165
- Arborelius L, Nomikos GG, Hertel P, Salmi P, Grillner P, Hook BB, Hacksell U, Svensson TH (1996): The 5-HT<sub>1A</sub> receptor antagonist (S)-UH-301 augments the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. *Naunyn-Schmied Arch Pharmacol* 353:630–640
- 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
- Artigas F, Romero L, de Montigny C, Blier P (1996): Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT<sub>1A</sub> antagonists. *Trends Neurosci* 19:378–383
- Auerbach SB, Hjorth S (1995): Effect of chronic administration of the selective serotonin (5-HT) uptake inhibitor citalopram on extracellular 5-HT and apparent autoreceptor sensitivity in rat forebrain in vivo. *Naunyn-Schmied Arch Pharmacol* 352:597–606
- Auerbach SB, Lundberg JF, Hjorth S (1995): Differential inhibition of serotonin release by 5-HT and NA reuptake blockers after systemic administration. *Neuropharmacology* 34:89–96
- Bel N, Artigas F (1993): Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. *Synapse* 15:243–245
- Bel N, Artigas F (1996): Reduction of serotonergic function in rat brain by tryptophan depletion. Effects in control and fluvoxamine-treated rats. *J Neurochem* 67:669–676
- Berman RM, Darnell AM, Miller HL, Anand A, Charney DS (1997): Effect of pindolol in hastening response to fluoxetine in the treatment of major depression: A double-blind, placebo-controlled trial. *Am J Psychiatry* 154:37–43
- Blier P, Bergeron R (1995): Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J Clin Psychopharmacol* 15:217–222
- Blier P, de Montigny C (1987): Modification of 5-HT neuron properties by sustained administration of the 5-HT<sub>1A</sub> agonist gepirone: Electrophysiological studies in the rat brain. *Synapse* 1:470–480
- Blier P, de Montigny C (1994): Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* 15:220–226
- Bordet R, Thomas P, Dupuis B, Réseau de Recherche et d'Experimentation Psychopharmacologique (1998): Effect of pindolol on onset of action of paroxetine in the treatment of major depression: Intermediate analysis of a double-blind, placebo-controlled trial. *Am J Psychiatry* 155:1346–1351
- Bosker FJ, Vanesseveldt KE, Klompmaakers AA, Westenberg HGM (1995): Chronic treatment with fluvoxamine by osmotic minipumps fails to induce persistent functional changes in central 5-HT(1A) and 5-HT(1B) receptors, as measured by in vivo microdialysis in dorsal hippocampus of conscious rats. *Psychopharmacology* 117:358–363
- Caccia S, Cappi M, Fracasso C, Garattini S (1990): Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. *Psychopharmacology* 100:509–514
- Casanovas JM, Vilaro MT, Mengod G, Artigas F (1999a): Differential regulation of somatodendritic serotonin 5-HT<sub>1A</sub> receptors by 2-week treatments with the selective agonists alnespirone (S-20499) and 8-hydroxy-2-(di-n-propylamino)tetralin: Microdialysis and autoradiographic studies in rat brain. *J Neurochem* 72:262–272
- Casanovas JM, Hervás I, Artigas F (1999b): Postsynaptic 5-HT<sub>1A</sub> receptors control 5-HT release in the rat medial prefrontal cortex. *Neuroreport* 10:1441–1445
- Dawson LA, Nguyen HQ, Smith DL, Schechter LE (1998): Effects of chronic fluoxetine treatment, in the presence and absence of 5-HT<sub>1A</sub> receptor blockade, on extracellular 5-HT. A microdialysis study. *Soc Neurosci Abs* 24:1109
- Fanelli RJ, McMonagle-Strucko K (1992): Alteration of 5-HT<sub>1A</sub> receptor binding sites following chronic treatment with ipsapirone measured by quantitative autoradiography. *Synapse* 12:75–81
- Gardier AM, Le Poul E, Trouvin JH, Chanut E, Desalles MC, Jacquot C (1993): Changes in dopamine metabolism in rat forebrain regions after cessation of long-term fluoxetine treatment relationship with brain concentrations of fluoxetine and norfluoxetine. *Life Sci* 54:51–56
- Gartside SE, Umbers V, Hajos M, Sharp T (1995): Interaction between a selective 5-HT<sub>1A</sub> receptor antagonist and an SSRI in vivo: Effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol* 115:1064–1070
- Grignaschi G, Invernizzi RW, Fanelli E, Fracasso C, Caccia S, Samanin R (1998): Citalopram-induced hypophagia is enhanced by blockade of 5-HT<sub>1A</sub> receptors: Role of 5-HT<sub>2C</sub> receptors. *Br J Pharmacol* 124:1781–1787



- Gundlach C, Hjorth S, Auerbach SB (1997): Autoreceptor antagonists enhance the effect of the reuptake inhibitor citalopram on extracellular 5-HT: This effect persists after repeated citalopram treatment. *Neuropharmacology* 36:475–482
- Hashimoto S, Inoue T, Koyama T (1997): Effects of the co-administration of 5-HT<sub>1A</sub> receptor antagonists with an SSRI in conditioned fear stress-induced freezing behavior. *Pharmacol Biochem Behav* 58:471–475
- Hensler JG, Covachich A, Frazer A (1991): A quantitative autoradiographic study of serotonin<sub>1A</sub> receptor regulation. Effect of 5,7-dihydroxytryptamine and antidepressant treatments. *Neuropsychopharmacology* 4:131–144
- Hervás I, Artigas F (1998): Effect of fluoxetine on extracellular 5-hydroxytryptamine in rat brain. Role of 5-HT autoreceptors. *Eur J Pharmacol* 358:9–18
- Hervás I, Queiroz C, Adell A, Artigas F (2000): Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. *Br J Pharmacol* 130:160–166
- Hjorth S, Auerbach SB (1994a): Lack of 5-HT<sub>1A</sub> autoreceptor desensitization following chronic citalopram treatment, as determined by in vivo microdialysis. *Neuropharmacology* 33:331–334
- Hjorth S, Auerbach SB (1994b): Further evidence for the importance of 5-HT<sub>1A</sub> autoreceptors in the action of selective serotonin reuptake inhibitors. *Eur J Pharmacol* 260:251–255
- Invernizzi R, Bramante M, Samanin R (1994): Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: Role of presynaptic 5-HT<sub>1A</sub> receptors. *Eur J Pharmacol* 260:243–246
- Invernizzi R, Bramante M, Samanin R (1995): Extracellular concentrations of serotonin in the dorsal hippocampus after acute and chronic treatment with citalopram. *Brain Res* 696:62–66
- Le Poul E, Laaris N, Doucet E, Fattaccini CM, Mocaër E, Hamon M, Lanfumey L (1999): Chronic alnespirone-induced desensitization of somatodendritic 5-HT<sub>1A</sub> autoreceptors in the rat dorsal raphe nucleus. *Eur J Pharmacol* 365:165–173
- Le Poul E, Laaris N, Doucet E, Laporte AM, Hamon M, Lanfumey L (1995): Early desensitization of somatodendritic 5-HT<sub>1A</sub> autoreceptors in rats treated with fluoxetine or paroxetine. *Naunyn-Schmied Arch Pharmacol* 352:141–148
- Li Q, Muma NA, Van de Kar LD (1996): Chronic fluoxetine induces a gradual desensitization of 5-HT<sub>1A</sub> receptors: Reductions in hypothalamic and midbrain G(i) and G(o) proteins and in neuroendocrine responses to a 5-HT<sub>1A</sub> agonist. *J Pharmacol Exp Ther* 279:1035–1042
- Maes M, Vandoolaeghe E, Desnyder R (1996): Efficacy of treatment with trazodone in combination with pindolol or fluoxetine in major depression. *J Affective Disord* 41:201–210
- Maes M, Libbrecht I, van Hunsel F, Campens D, Meltzer HY (1999): Pindolol and mianserin augment the antidepressant activity of fluoxetine in hospitalized major depressed patients, including those with treatment resistance. *J Clin Psychopharmacol* 19:177–182
- Malagié I, Trillat A-C, 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
- Malagié I, Trillat AC, Douvier E, Anmella MC, Dessalles MC, Jacquot C, Gardier AM (1996): Regional differences in the effect of the combined treatment of WAY 100635 and fluoxetine: An in vivo microdialysis study. *Naunyn-Schmied Arch Pharmacol* 354:785–790
- McQuade R, Sharp T (1997): Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *J Neurochem* 69:791–796
- Mitchell PJ, Redfern PH (1997): Potentiation of the time-dependent, antidepressant-induced changes in the agonistic behaviour of resident rats by the 5-HT<sub>1A</sub> receptor antagonist, WAY-100635. *Behav Pharmacol* 8:585–606
- Moreno FA, Gelenberg AJ, Bachar K, Delgado PL (1997): Pindolol augmentation of treatment-resistant depressed patients. *J Clin Psychiatry* 58:437–439
- Newman-Tancredi A, Chaput C, Gavaudan S, Verrièle L, Millan MJ (1998): Agonist and antagonist actions of (-)pindolol at recombinant, human serotonin<sub>1A</sub> (5-HT<sub>1A</sub>) receptors. *Neuropsychopharmacology* 18:395–398
- Paxinos G, Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*. Sydney, Academic Press
- Pérez V, Gilaberte I, Faries D, Alvarez E, Artigas F (1997): Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. *Lancet* 349:1594–1597
- Pérez V, Soler J, Puigdemont D, Alvarez E, Grup de Recerca en Trastorns Afectius, Artigas F (1999): A double-blind, randomized, placebo-controlled trial of pindolol augmentation in depressive patients resistant to serotonin reuptake inhibitors. *Arch Gen Psychiatry* 56:375–379
- Pérez V, Puigdemont D, Gilaberte I, Alvarez E, Grup de Recerca en Trastorns Afectius, Artigas F (2000): Augmentation of fluoxetine antidepressant action by pindolol: Analysis of clinical, pharmacokinetic and methodological factors. *J Clin Psychopharmacol* (In press)
- Pompeiano M, Palacios JM, Mengod G (1992): Distribution and cellular localization of mRNA coding for 5-HT<sub>1A</sub> receptor in the rat brain: Correlation with receptor binding. *J Neurosci* 12:440–453
- Romero L, Artigas F (1997): Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT<sub>1A</sub> receptor antagonists in the dorsal raphe pathway: Role of somatodendritic autoreceptors. *J Neurochem* 68:2593–2603
- Romero L, Celada P, Artigas F (1994): Reduction of in vivo striatal 5-hydroxytryptamine release by 8-OH-DPAT after inactivation of Gi/Go proteins in dorsal raphe nucleus. *Eur J Pharmacol* 265:103–106
- Rutter JJ, Auerbach SB (1993): Acute uptake inhibition increases extracellular serotonin in the rat forebrain. *J Pharmacol Exp Ther* 265:1319–1324
- Rutter JJ, Gundlach C, Auerbach SB (1994): Increase in extracellular serotonin produced by uptake inhibitors is enhanced after chronic treatment with fluoxetine. *Neurosci Lett* 171:183–186
- Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C,

- Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000): Brain serotonin<sub>1A</sub> receptor binding measured by positron emission tomography with [<sup>11</sup>C]WAY-100635: Effects of depression and antidepressant treatment. *Arch Gen Psychiatry* 57:174–180
- Sharp T, McQuade R, Bramwell S, Hjorth S (1993): Effect of acute and repeated administration of 5-HT<sub>1A</sub> receptor agonists on 5-HT release in rat brain in vivo. *Naunyn-Schmied Arch Pharmacol* 348:339–346
- Smith D, Nawoschik SP, Dunlop J, Schechter LE (1998): The 5-HT<sub>1A</sub> receptor antagonist WAY-100635 induces a downregulation of 5-HT<sub>1A</sub> receptor sites in a human 5-HT<sub>1A</sub> clonal cell line. *Soc Neurosci Abs* 24:1108
- Trillat AC, Malagie I, Mathe-Allainmat M, Anmella MC, Jaquot C, Langlois M, Gardier AM (1998): Synergistic neurochemical and behavioral effects of fluoxetine and 5-HT<sub>1A</sub> receptor antagonists. *Eur J Pharmacol* 357:179–184
- Welner SA, de Montigny C, Desroches J, Desjardins P, Suranyi-Cadotte BE (1989): Autoradiographic quantification of serotonin<sub>1A</sub> receptors in rat brain following antidepressant drug treatment. *Synapse* 4:347–352
- Zanardi R, Artigas F, Franchini L, Sforzini L, Gasperini M, Smeraldi E, Pérez J (1997): How long should pindolol be associated with paroxetine to improve the antidepressant response? *J Clin Psychopharmacol* 17:446–450
- Zanardi R, Franchini L, Gasperini M, Lucca A, Smeraldi E, Pérez J (1998): Faster onset of action of fluvoxamine in combination with pindolol in the treatment of delusional depression: A controlled study. *J Clin Psychopharmacol* 18:441–446