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Preferential Potentiation of the Effects of Serotonin Uptake Inhibitors by 5-HT_{1A} Receptor Antagonists in the Dorsal Raphe Pathway: Role of Somatodendritic Autoreceptors

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Abstract: 5-HT_{1A} autoreceptor antagonists enhance the effects of antidepressants by preventing a negative feedback of serotonin (5-HT) at somatodendritic level. The maximal elevations of extracellular concentration of 5-HT (5-HT_{ext}) induced by the 5-HT uptake inhibitor paroxetine in forebrain were potentiated by the 5-HT_{1A} antagonist WAY-100635 (1 mg/kg s.c.) in a regionally dependent manner (striatum > frontal cortex > dorsal hippocampus). Paroxetine (3 mg/kg s.c.) decreased forebrain 5-HT_{ext} during local blockade of uptake. This reduction was greater in striatum and frontal cortex than in dorsal hippocampus and was counteracted by the local and systemic administration of WAY-100635. The perfusion of 50 μmol/L citalopram in the dorsal or median raphe nucleus reduced 5-HT_{ext} in frontal cortex or dorsal hippocampus to 40 and 65% of baseline, respectively. The reduction of cortical 5-HT_{ext} induced by perfusion of citalopram in midbrain raphe was fully reversed by WAY-100635 (1 mg/kg s.c.). Together, these data suggest that dorsal raphe neurons projecting to striatum and frontal cortex are more sensitive to self-inhibition mediated by 5-HT_{1A} autoreceptors than median raphe neurons projecting to the hippocampus. Therefore, potentiation by 5-HT_{1A} antagonists occurs preferentially in forebrain areas innervated by serotonergic neurons of the dorsal raphe nucleus. **Key Words:** 5-HT_{1A} receptor antagonist—5-Hydroxytryptamine (serotonin)—5-Hydroxytryptamine uptake inhibitors—Dorsal raphe nucleus—Median raphe nucleus—Paroxetine.
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The treatment of major depression with selective serotonin [5-hydroxytryptamine (5-HT)] reuptake inhibitors (SSRIs) is effective in less than two-thirds of patients. SSRIs selectively block the 5-HT transporter (Hyttel, 1994), and therefore their clinical actions likely derive from an enhancement of serotonergic transmission. Yet, clinical improvement requires prolonged treatments despite the capacity of SSRIs to block the 5-HT transporter rapidly. Neurochemical studies have shown that 5-HT uptake inhibitors increase the extracellular concentration of 5-HT (5-HT_{ext}) in the midbrain raphe nuclei (Adell and Artigas, 1991; Fuller, 1994; Gardier et al., 1996), which constitute the main source of forebrain serotonergic innervation (Dahlström and Fuxe, 1964). The excess 5-HT_{ext} in the midbrain raphe activates somatodendritic autoreceptors and reduces 5-HT release in forebrain (Adell and Artigas, 1991; Rutter and Auerbach, 1993; Romero et al., 1994). As a result, single treatment with clinically relevant doses of SSRIs causes little or no increase of 5-HT_{ext} in forebrain (Bel and Artigas, 1992; Invernizzi et al., 1992; Gartside et al., 1995; Malagié et al., 1996). Because the forebrain 5-HT release depends on nerve impulse, these observations are consistent with the suppression of firing evoked by antidepressants in 5-HT neurons of the dorsal raphe nucleus (Aghajanian et al., 1970; Scuvée-Moreau and Dresse, 1979; Blier and de Montigny, 1994).

It was thus hypothesized that addition of 5-HT_{1A} antagonists could enhance the antidepressant effects of SSRIs by preventing this negative feedback (Artigas, 1993). Two pilot studies using pindolol (a nonselective β-adrenoceptor/5-HT_{1A} antagonist) have reported results consistent with this hypothesis (Artigas et al., 1994*a*; Blier and Bergeron, 1995), which has received further confirmation in a controlled trial (V. Pérez et al., manuscript in preparation). (−)-Pindolol prevents the self-inhibition of dorsal raphe serotonergic neurons produced by the SSRIs and therefore potentiates their effects in various brain areas (Artigas et al., 1994*b*; Dreshfield et al., 1996; Romero et al., 1996).

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Abbreviations used: AUC, area under the curve; 5-HT, 5-hydroxytryptamine (serotonin); SSRI, selective serotonin reuptake inhibitor; WAY-100135, (*S*)-*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropanamide; WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide · 3HCl.

Serotonergic neurons of the dorsal and median raphe nuclei innervate different forebrain territories (Azmitia and Segal, 1978). The activation of 5-HT_{1A} autoreceptors by selective agonists has been reported to cause a greater reduction of activity in 5-HT neurons of the dorsal raphe nucleus (Sinton and Fallon, 1988; Blier et al., 1990) and of 5-HT synthesis and release from the corresponding axon terminals (Invernizzi et al., 1991; Casanovas and Artigas, 1996). However, such a differential sensitivity has not been found by others (Hjorth and Sharp, 1991; Hajos et al., 1995), and therefore it is not firmly established whether 5-HT release is equally inhibited by SSRIs in dorsal and median raphe tracts. Using microdialysis in awake rats, we have assessed the role of somatodendritic 5-HT_{1A} autoreceptors in the potentiation of SSRI effects by 5-HT_{1A} antagonists and the putative differential sensitivity of dorsal and median raphe neurons to self-inhibition after treatments with SSRIs.

MATERIALS AND METHODS

Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 280–320 g were used. Animals were kept in a controlled environment (12-h light–dark cycle and 22 ± 2°C room temperature). Food and water were provided ad libitum. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986).

Drugs and reagents

5-HT was from Research Biochemicals International (Natick, MA, U.S.A.). The SSRIs citalopram · HBr and paroxetine · HCl were generously provided by Lundbeck and Smith Kline Beecham, respectively. WAY-100135 {(*S*)-*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropanamide} (Cliffe et al., 1993), WAY-100635 {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide · 3HCl} (Fletcher et al., 1995), and [^{3}H]WAY-100635 (specific activity, 80 Ci/mmol) were generously supplied by Wyeth Ayerst. Other materials and reagents were from local commercial sources. Drugs were injected intraperitoneally or subcutaneously at the doses indicated (base form; 1–2 ml/kg) or dissolved in the microdialysis perfusion fluid for local applications by reverse dialysis. Neither citalopram nor WAY-100635 altered substantially the pH of the artificial CSF used to perfuse the probes.

Microdialysis experiments

Four different sets of experiments were performed to examine the role of somatodendritic 5-HT_{1A} autoreceptors in the control of 5-HT release during the local or systemic treatment with SSRIs. First, the effects of paroxetine, alone or in combination with WAY-100635 (1 mg/kg, s.c.), on 5-HT_{ext} were examined in four forebrain areas (striatum, frontal cortex, ventral hippocampus, and dorsal hippocampus) differentially innervated by serotonergic neurons of the dorsal and median raphe nuclei (Azmitia and Segal, 1978). Animals were randomly allocated to receive either paroxetine plus saline or paroxetine plus WAY-100635. Second, WAY-100635 (100 μmol/L) was administered through 1.5-mm-long probes located in the dorsal raphe nucleus (Ferré et al., 1994) during the systemic treatment with paroxetine to examine the involvement of somatodendritic 5-HT_{1A} autoreceptors. Pilot experiments revealed that lower concentrations of WAY-100635 (1 and 10 μmol/L) did not attenuate the reduction of cortical 5-HT release induced by application of citalopram in the dorsal raphe nucleus. A third group of experiments was performed, based on the observation that the systemic administration of an SSRI resulted in a reduction of 5-HT release in the diencephalon and hippocampus when the 5-HT transporter was blocked in these areas by the local infusion of a SSRI using reverse dialysis (Rutter and Auerbach, 1993; Hjorth and Auerbach, 1994). This reduction results from the activation of somatodendritic 5-HT_{1A} autoreceptors by the excess 5-HT_{ext} in the interstitial space outside the cell bodies produced by 5-HT uptake blockers (Adell and Artigas, 1991; Bel and Artigas, 1992; Invernizzi et al., 1992; Gartside et al., 1995; Malagié et al., 1996). WAY-100635 was then locally (in the dorsal raphe nucleus) or systemically administered to examine its effects on 5-HT_{ext} in frontal cortex. Animals were randomly implanted with probes in striatum and dorsal hippocampus. Finally, the fourth set of experiments included the local activation of somatodendritic 5-HT_{1A} receptors in the dorsal or median raphe nucleus [using 1.5-mm-long probes (Ferré et al., 1994)] or in the dorsal plus median raphe nuclei [using 4-mm-long probes, 0.5 mm away from the midline (Adell and Artigas, 1991)] by the local application of the SSRI citalopram in rats with dual probe implants (dorsal raphe–striatum; median raphe–dorsal hippocampus). The blockade of the 5-HT uptake increases 5-HT_{ext} in the midbrain raphe and reduces 5-HT release in frontal cortex (Adell and Artigas, 1991) and striatum (Romero et al., 1994, 1996). 5-HT_{1A} antagonists were then systemically administered to assess their effects on the reduction of terminal 5-HT release induced by citalopram.

Anesthetized rats (pentobarbital, 60 mg/kg i.p.) were implanted stereotaxically with 0.25-mm-o.d., concentric dialysis probes (Adell and Artigas, 1991) in the different brain areas (Table 1). Microdialysis experiments were conducted ~20 h after probe implants in conscious, freely moving animals. Animals were implanted with one or two microdialysis probes in the regions of interest, as required by the experimental model used. In one experiment, citalopram was infused into the dorsal raphe nucleus through a metal tube at 0.05 μl/min (Romero et al., 1994) instead of being delivered by reverse dialysis. Both procedures induced equivalent reductions of 5-HT release in striatum or frontal cortex. Probes were perfused with artificial CSF (125 m*M* NaCl, 2.5 m*M* KCl, 1.26 m*M* $CaCl_2$, and 1.18 m*M* $MgCl_2$) at 0.25 μl/min. Under these conditions, the in vitro recovery of the dialysis probes for 5-HT was 27 ± 2 (1.5 mm) and 42 ± 1% (4 mm). Sample collection started 60 min after the beginning of perfusion. Usually four or five fractions were collected to obtain basal values before either local infusion or systemic administration of drugs. Successive 20-min dialysate samples were collected. Because of the marked reduction of cortical and striatal 5-HT release elicited by the local infusion of citalopram in the somatodendritic area (Romero et al., 1994, 1996), probes of the animals of the fourth experimental group were perfused with artificial CSF containing 1 μmol/L citalopram. This enhanced the detectability of 5-HT and reduced the variability of the measures without affecting the experimental model, i.e., control of terminal release through the activation of somatodendritic 5-HT_{1A} by 5-HT. Similarly, cortical probes of the animals of the third

TABLE 1. *Length of microdialysis probes and stereotaxic coordinates of implants*

Region	Length (mm)	AP	DV	L	Vertical angle
Dorsal raphe nucleus	1.5	−7.8	−7.5[a]	−3.1	30°
Median raphe nucleus	1.5	−7.8	−8.9[a]	−2.0	13°
Dorsal plus median raphe nuclei	4.0	−7.8	−9.0	−0.5	0°
Dorsal hippocampus	1.5	−3.8	−4.0	−1.8	0°
Ventral hippocampus	4.0	−5.8	−8.0	−5.0	0°
Dorsal striatum	4.0	0.2	−8.0	−3.0	0°
	1.5	0.2	−6.1	−3.0	0°
Frontal cortex	4.0	3.4	−6.0	−2.5	0°

Coordinates (AP, DV, and L) are given in mm, with respect to bregma and dura mater according to the rat brain atlas of Paxinos and Watson (1986).

[a] The dorsoventral coordinate corresponds to the actual descent of the probe along a line inclined 13 or 30° with respect to the vertical.

group were perfused with 1 μmol/L citalopram, as required in this experimental model (Rutter and Auerbach, 1993; Hjorth and Auerbach, 1994).

Chromatographic analysis

5-HT was analyzed by a modification of an HPLC method previously described (Adell and Artigas, 1991). The composition of HPLC eluant was as follows: 0.15 *M* NaH_2PO_4, 1.3 m*M* octyl sodium sulfate, 0.2 m*M* EDTA (pH 2.8 adjusted with phosphoric acid), plus 27% methanol. 5-HT was separated on a 3-μm (particle size) ODS 2 column (7.5 × 0.46 cm; Beckman) and detected amperometrically with a Hewlett Packard model 1049 detector (oxidation potential, + 0.6 V). Retention time was 3.5–4 min. The detection limit for 5-HT was 0.5–1 fmol.

In vivo labeling of 5-HT$_{1A}$ receptors by [^{3}H]WAY-100635

The extent of the diffusion of WAY-100635 in the midbrain raphe area after its local application in the dorsal raphe nucleus by reverse dialysis was determined using [^{3}H]WAY-100635. Dialysis probes (1.5 mm long) were placed in the dorsal raphe nucleus and perfused (0.25 μl/min) with artificial CSF containing 100 μmol/L WAY-100635 (plus 100 nmol/L radioactive tracer). The in vitro recovery of the dialysis probes (1.5 mm) for WAY-100635 was calculated from the radioactivity counts in equivalent volumes of the artificial CSF at the probe inlet and outlet during the infusion of [^{3}H]WAY-100635 plus WAY-100635. This was found to be 58.7 ± 4.6% (n = 4). After an infusion period of 1.2 h, the rats were decapitated, and the brains were removed and frozen. Coronal midbrain sections (14 μm thick) were cut to examine the in vivo labeling of dorsal raphe nucleus 5-HT$_{1A}$ receptors by [^{3}H]WAY-100635 (Laporte et al., 1994; Gozlan et al., 1995; Khawaja et al., 1995). Given the rostrocaudal distribution of the various neuronal groups composing the dorsal raphe nucleus, coronal sections were cut at different anteroposterior levels (approximately from −6.5 to −9.0 mm relative to bregma). Sections at hippocampal and cortical levels were also cut to examine whether 5-HT$_{1A}$ receptors in these areas (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985; Pompeiano et al., 1992) were also labeled by infusion of [^{3}H]WAY-100635. The sections were exposed to a tritium-sensitive film (Hyperfilm-^{3}H; Amersham, U.K.) for 3 weeks. The optical density of the tritium labeling in films was measured using an image analysis system (Imaging Research, St. Catharines, Ontario, Canada). The surface of areas showing a dense labeling by [^{3}H]WAY-100635 was quantified to calculate the total amount of tritium label in each section.

Data analysis

Microdialysis results are expressed as femtomoles per fraction (uncorrected for recovery) and represented as percentages of basal values (individual means of four predrug fractions) to facilitate comparisons between the different experimental groups. Statistical analysis of single drug effects has been performed using ANOVA for repeated-measures and paired *t* tests of raw data. Two-way ANOVA designs were used to examine the interaction between 5-HT$_{1A}$ antagonists and SSRIs or regional effects. ANOVA of area-under-the-curve (AUC) data was also used to examine the dose and region factors on dialysate 5-HT after the treatment with paroxetine. Autoradiographic data are expressed as femtomoles of [^{3}H]WAY-100635 per section. Data are given as mean ± SEM values. Statistical significance has been set at the 95% confidence level (two tailed).

RESULTS

Increase of 5-HT$_{ext}$ in forebrain by paroxetine

Table 2 shows the basal values of dialysate 5-HT (with and without 1 μmol/L citalopram in the perfusion fluid) in the different brain regions examined. The intraperitoneal administration of 3 mg/kg paroxetine significantly increased dialysate 5-HT levels in four forebrain areas differentially innervated by the dorsal and median raphe nuclei (striatum, frontal cortex, ventral hippocampus, and dorsal hippocampus) ($p < 0.001$ by repeated-measures ANOVA). Minimal and maximal elevations of dialysate 5-HT in the different regions were 231 and 289% of basal values (AUCs of 160 min postparoxetine) in dorsal hippocampus and striatum, respectively (Fig. 1). These elevations were not significantly different (one-way ANOVA). The increment of the paroxetine dose to 10 mg/kg did not elevate further dialysate 5-HT levels in frontal cortex ($p < 0.001$ for effect of paroxetine treatment; nonsignificant effect of the dose by two-way ANOVA). By

TABLE 2. *Basal concentration values of dialysate 5-HT*

Citalopram concentration in dialysis fluid	Region	5-HT_{ext} (fmol/fraction)
None	FC (4 mm)	2.2 ± 0.1 (41)
	STR (4 mm)	2.9 ± 0.4 (24)
	VHPC (4 mm)	3.8 ± 0.6 (10)
	DHPC (1.5 mm)	2.0 ± 0.2 (17)
	DRN (1.5 mm)	11.2 ± 4.3 (5)
	MRN (1.5 mm)	7.3 ± 1.45 (8)
	RN (4 mm)	33.0 ± 11.9 (6)
1 μmol/L	DHPC (1.5 mm)	7.5 ± 0.8 (12)
	FC (4 mm)	17.3 ± 1.5 (22)
	STR (4 mm)	17.5 ± 1.3 (17)
	STR (1.5 mm)	11.6 ± 1.6 (6)

Data are mean ± SEM values (no. of rats). DHPC, dorsal hippocampus; DRN, dorsal raphe nucleus; FC, frontal cortex; MRN, median raphe nucleus; RN, raphe nuclei (DRN + MRN); STR, dorsal striatum; VHPC, ventral hippocampus.

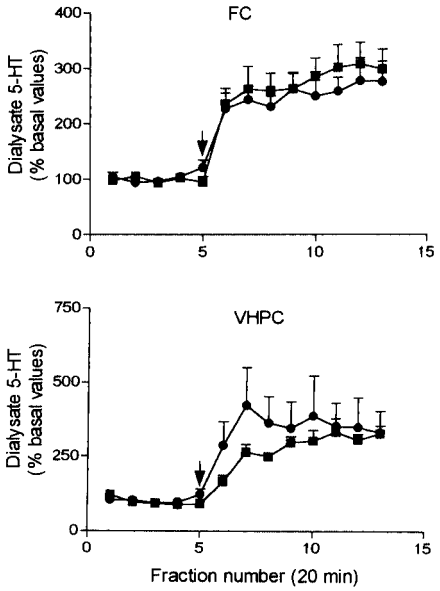

FIG. 2. Changes of dialysate 5-HT in frontal cortex (FC) and ventral hippocampus (VHPC) after two different doses of paroxetine [3 (■) and 10 mg/kg (●)]. Injection is shown by an arrow. Data are mean ± SEM (bars) values from four to six rats per group except for 10 mg/kg paroxetine in VHPC (n = 12, pooled from two different experiments). See text for statistical details.

contrast, 10 mg/kg elicited a somewhat larger increment of 5-HT_{ext} in ventral hippocampus, which was significantly different from that produced by 3 mg/kg, particularly at short times after paroxetine administration ($p < 0.001$, significant effects of the treatment and the dose factors; Fig. 2).

Potentiation of paroxetine effects on 5-HT_{ext} by WAY-100635

The systemic administration of WAY-100635 (1 mg/kg s.c.) to saline-pretreated rats did not modify 5-HT_{ext} in frontal cortex, striatum, or dorsal hippocampus (ANOVA for repeated measures) (Fig. 3). However, its administration caused dramatic elevations of 5-HT_{ext} in the striatum and frontal cortex of rats pretreated with 3 mg/kg i.p. paroxetine (Fig. 3; $p < 0.001$ in all cases by repeated-measures ANOVA). Dialysate 5-HT content increased to 670% of basal values in frontal cortex and to almost 1,800% in the striatum. The effect in dorsal hippocampus was less marked (see Fig. 3, lower panel). The maximal potentiation of paroxetine effects was noted 40 min after administration of WAY-100635 in all brain areas. A two-way ANOVA of the AUC values during the period of maximal potentiation (four fractions) after the injection of WAY-100635 or saline revealed significant effects of the treatment and region factors ($p < 0.001$ in both cases) and a significant interaction ($p < 0.027$) of both factors.

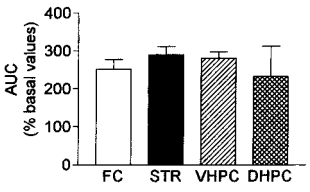

FIG. 1. Effect of systemic administration of the SSRI paroxetine (3 mg/kg, i.p.) on extracellular 5-HT in forebrain areas differentially innervated by serotonergic neurons of the dorsal or median raphe nucleus. FC, frontal cortex; STR, dorsal striatum; VHPC, ventral hippocampus; DHPC, dorsal hippocampus. Ordinates are AUCs of posttreatment values (160 min), expressed as percentages of the relative basal values in each brain area. The effects of paroxetine were statistically significant in all cases ($p < 0.001$ by ANOVA for repeated measures) without significant differences between regions (one-way ANOVA). Data are mean ± SEM (bars) values (n = 4 animals in DHPC, 6 animals in VHPC, 13 animals in STR, and 18 animals in FC) (the data for STR and FC have been pooled from two and three different experimental groups with no significant differences between them).

To assess the participation of somatodendritic 5-HT_{1A} receptors in the potentiation of the paroxetine-induced 5-HT elevations after the systemic treatment with WAY-100635, we examined the effects of its local application in the dorsal raphe nucleus (100 μmol/L for 160 min at 0.25 μl/min; total amount delivered was 2.35 nmol taking into account the yield of the dialysis membrane; 14.7 pmol/min) during the administration of paroxetine. The infusion of WAY-100635 in the dorsal raphe nucleus rapidly and significantly increased dialysate 5-HT values in frontal cortex of rats pretreated with 3 mg/kg paroxetine and remained elevated for the rest of the infusion period ($p < 0.01$ vs. paroxetine alone by Student's t test of AUCs during perfusion time; $p < 0.004$ by repeated-measures ANOVA vs. pre-WAY-100635 value; Fig. 4).

Actions of 5-HT at dorsal and median raphe 5-HT_{1A} receptors: Antagonism by WAY-100635

Somatodendritic 5-HT_{1A} receptors were activated by 5-HT after the local or systemic administration of the

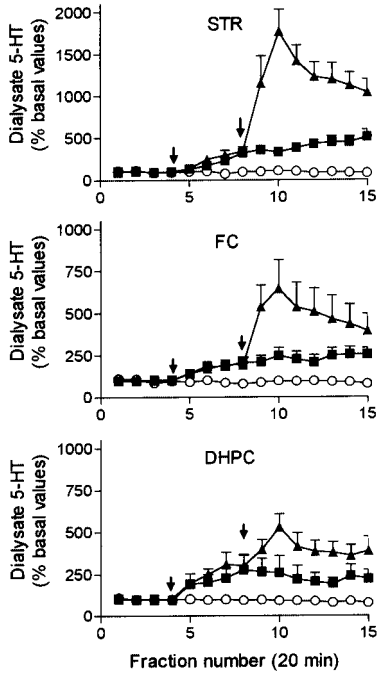

FIG. 3. Effect of administration of WAY-100635 (1 mg/kg, s.c.) or saline 80 min after administration of paroxetine (3 mg/kg i.p.) on dialysate 5-HT in dorsal striatum (STR), frontal cortex (FC), and dorsal hippocampus (DHPC). First and second arrows denote injections of paroxetine (or saline) and WAY-100635 (or saline), respectively. Data are mean ± SEM (bars) values. Experimental groups were as follows: saline + WAY-100635 (○; n = 4, 4, and 5, respectively), paroxetine + saline (■; n = 4, 6, and 4), and paroxetine + WAY-100635 (▲; n = 7, 9, and 8, respectively). Note the different scales used along the *y*-axes. See text for statistical details.

SSRIs citalopram and paroxetine, respectively. It was reasoned that the reduction of terminal 5-HT release would be attenuated by 5-HT$_{1A}$ autoreceptor antagonists. Two sets of experiments were conducted. In the first one, dialysis probes implanted in frontal cortex were perfused with artificial CSF containing 1 μmol/L citalopram, thus locally blocking 5-HT reuptake into nerve terminals. The intraperitoneal administration of

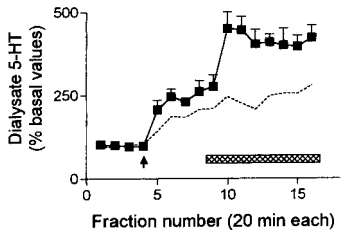

FIG. 4. Potentiation of the effect of paroxetine (3 mg/kg i.p.; arrow) on dialysate 5-HT in frontal cortex by local application of WAY-100635 in the DRN (100 μmol/L; cross-hatched horizontal bar). Data are mean ± SEM (bars) values from five rats. The dotted line indicates the effects of paroxetine alone (3 mg/kg i.p.) in a separate group of animals (n = 6).

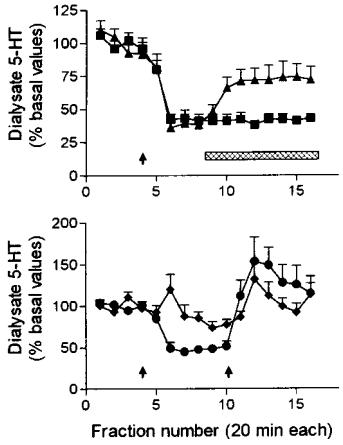

FIG. 5. Upper panel: Reduction of 5-HT release in frontal cortex induced by systemic administration of paroxetine (3 mg/kg; ■) in conditions of local blockade of the uptake by 1 μmol/L citalopram. Injection of paroxetine is shown by an arrow. The application of WAY-100635 (100 μmol/L; shown by a horizontal cross-hatched bar) partially counteracted the reduction induced by paroxetine (▲). **Lower panel:** Differential reduction of 5-HT release in dorsal striatum (●; n = 6) and dorsal hippocampus (♦; n = 4) by administration of 3 mg/kg paroxetine (first arrow) in conditions of local blockade of the uptake with 1 μmol/L citalopram. The injection of 1 mg/kg WAY-100635 (second arrow) reversed the reduction induced by paroxetine and induced an additional significant increment of 5-HT level. Data are mean ± SEM (bars) values. See text for statistical analysis.

3 mg/kg paroxetine induced a sustained reduction of cortical dialysate 5-HT to 40% of predrug values, e.g., from 16.5 ± 3.8 to 6.8 ± 1.6 fmol per fraction, that was statistically significant ($p < 0.001$; Fig. 5, upper panel). The application of WAY-100635 (100 μmol/L) in the dorsal raphe nucleus partially antagonized the paroxetine-induced reduction of dialysate 5-HT level (significant effects of WAY-100635, $p < 0.02$ by Student's *t* test of AUCs vs. paroxetine alone during perfusion time; $p < 0.001$ by repeated-measures ANOVA vs. pre-WAY-100635 values).

We further assessed the ability of paroxetine to reduce 5-HT in dialysates from dorsal striatum and dorsal hippocampus, two areas innervated in a preferential manner by serotonergic axons originating in the dorsal and median raphe nucleus, respectively. To exclude any methodological source of possible differences, the size of the microdialysis probes was the same (1.5 mm) in dorsal hippocampus and striatum. The administration of 3 mg/kg paroxetine significantly reduced 5-HT content in both areas, although to a greater extent in the striatum ($p < 0.0001$, significant effect of the treatment; $p < 0.0001$, significant treatment × region interaction; Fig. 5, lower panel). The reduction of 5-HT release was counteracted by systemic administration of 1 mg/kg WAY-100635 ($p < 0.001$ by repeated-measures ANOVA) in both regions. WAY-100635 caused 5-HT values to increase above pretreatment levels. This is likely due to additional blockade of the

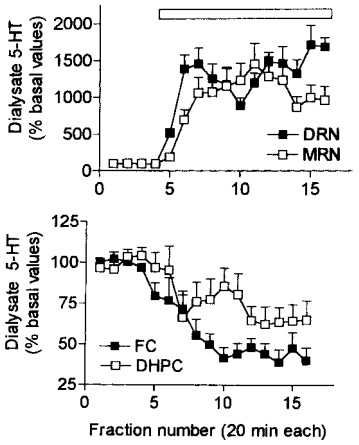

FIG. 6. Attenuation of 5-HT release in frontal cortex (FC) and dorsal hippocampus (DHPC) during infusion of citalopram (50 μmol/L; open bar) in the dorsal raphe nucleus (DRN; n = 5) and median raphe nucleus (MRN; n = 8), respectively, using 1.5-mm-long dialysis probes (see Table 1 for coordinates). Data are mean ± SEM (bars) values. **Upper panel:** Citalopram caused a rapid and sustained elevation of 5-HT_{ext} in the DRN (■) and MRN (□). **Lower panel:** The 5-HT_{ext} concentration in FC (■) and DHPC (□) was reduced during citalopram infusion in DRN and MRN, respectively, to ~40 and 65% of pretreatment values. See text for statistical details.

transporter by the systemic administration of paroxetine plus the prevention of the self-inhibition by WAY-100635.

In a second set of experiments, citalopram (50 μmol/L) was infused through 1.5-mm dialysis probes in the dorsal or median raphe of animals implanted with a second probe in frontal cortex or dorsal hippocampus, respectively. Application of citalopram in the dorsal raphe nucleus caused a rapid and sustained elevation of dialysate 5-HT, which was followed by a very marked reduction of the 5-HT output in frontal cortex ($p < 0.001$ for both regions by repeated-measures ANOVA; Fig. 6). Similarly, the application of the same citalopram concentration in the median raphe nucleus caused a very large increase in 5-HT content (maximal increase to ~15-fold of baseline) that was also accompanied by a reduction of the 5-HT output in dorsal hippocampus ($p < 0.001$ for both regions by repeated-measures ANOVA; Fig. 6, lower panel). A two-way analysis of the data also revealed a significant treatment × region interaction ($p < 0.001$).

When citalopram was infused through a vertical probe (4 mm long, 0.5 mm away from the midline) in the vicinity of both midbrain raphe nuclei, a somewhat smaller but still very marked enhancement of dialysate 5-HT in the midbrain was observed ($p < 0.001$ by repeated-measures ANOVA; Fig. 7, upper panel). This was accompanied by a decline of dialysate 5-HT values in frontal cortex to 55% of basal levels ($p < 0.001$; Fig. 7, lower panel). The injection of 1 mg/kg WAY-100635 fully reversed the attenuation of cortical 5-HT release caused by citalopram ($p < 0.001$ vs. pre-WAY-100635 values by Student's t test) and further increased dialysate 5-HT content in raphe to ~1,900% of basal levels ($p < 0.035$ vs. pre-WAY-100635 values by paired Student's t test; Fig. 7). The blockade of 5-HT_{1A} receptors by WAY-100635 was reversible, as 2 h after its administration cortical and raphe dialysate 5-HT values had almost returned to pre-WAY-100635 values, while citalopram was still being infused in the midbrain raphe.

The infusion of 50 μmol/L citalopram in the dorsal raphe nucleus caused a marked reduction of striatal 5-HT release that was not counteracted by the 5-HT_{1A} antagonist WAY-100135 at 10 mg/kg (significant effects of citalopram, $p < 0.001$ in both cases; nonsignificant effect of the pretreatment with WAY-100135; Fig. 8).

Autoradiographic analysis of distribution of [^{3}H]WAY-100635 in rat brain after local application in dorsal raphe nucleus

To examine the involvement of somatodendritic and postsynaptic 5-HT_{1A} receptors in the action of locally applied WAY-100635, we perfused 100 μmol/L WAY-100635 plus 100 nmol/L [^{3}H]WAY-100635 into the dorsal raphe nucleus, using the same experimental conditions as in previous microdialysis experiments, except for the infusion time, which was reduced to half (1 h 20 min). At this time, WAY-100635 had already exerted its full antagonistic effects (see Figs.

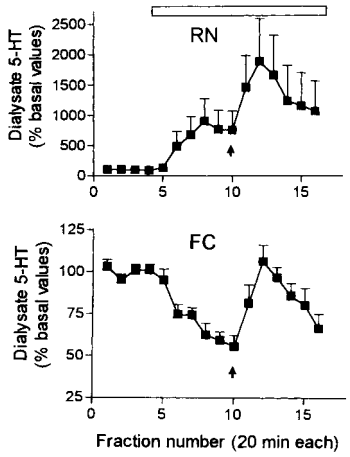

FIG. 7. Upper panel: Increase of dialysate 5-HT content in the vicinity of the dorsal and median raphe nuclei (RN) after local application of citalopram (open bar) through a vertical microdialysis probe (4 mm long, 0.25 mm o.d.; 0.5 mm away from the midline). Administration of WAY-100635 (1 mg/kg s.c.; arrow) induced an additional increase of 5-HT content up to ~1,800% of precitalopram values. **Lower panel:** Attenuation of the 5-HT release in frontal cortex (FC) by local application of citalopram in the raphe nuclei. The period of infusion is shown by an open bar. Systemic administration of WAY-100635 (1 mg/kg s.c.) completely reversed the reduction of 5-HT release induced by citalopram, although the effect was transient due to the short half-life of this compound. Data are mean ± SEM (bars) values from six rats. See text for statistical details.

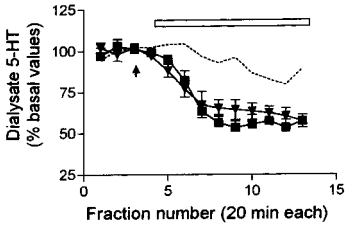

FIG. 8. Attenuation of striatal 5-HT release by application of citalopram (50 μmol/L) in the dorsal raphe nucleus (open bar). Arrow denotes the subcutaneous injection of saline (■) or 10 mg/kg WAY-100135 (▼). Two-way ANOVA did not reveal a significant effect of WAY-100135. Data are mean ± SEM (bars) values from six or seven rats. For comparison, the antagonistic effects of 15 mg/kg (−)-pindolol using the same experimental procedure are shown (dotted line). Data for pindolol are from Romero et al. (1996).

4 and 5), and therefore the infusion for additional time was not considered necessary, thus helping to minimize the amount of radioactivity used. Autoradiographic analysis showed that the radioactive label was distributed along the anteroposterior axis at the dorsal raphe level, with greater amounts in central regions (Fig. 9). Neither the neighbor median raphe nucleus nor more distant areas rich in postsynaptic 5-HT$_{1A}$ receptors, like the hippocampus or frontal cortex, showed any sign of labeling by [^{3}H]WAY-100635 (Fig. 10).

DISCUSSION

The serotonergic system is involved in many brain functions, such as motor control, cognition, process of sensory information, and mood regulation (Jacobs and Azmitia, 1992; Jacobs and Fornal, 1993; Maes and Meltzer, 1995). A relatively small number of cells in the midbrain raphe nuclei innervates all forebrain areas with a very high density of nerve terminals (Descarries et al., 1982; Oleskevich and Descarries, 1990; Jacobs and Azmitia, 1992). Serotonergic neurons are extremely sensitive to self-inhibition by 5-HT (Aghajanian et al., 1972; Haigler and Aghajanian, 1974). This process is mediated by somatodendritic 5-HT$_{1A}$ receptors coupled through G_i/G_o proteins to a K^+ channel (Aghajanian and Lakoski, 1984; Blier and de Montigny, 1987; Sprouse and Aghajanian, 1987; Innis et al., 1988). Consequently, the activation of somatodendritic 5-HT$_{1A}$ receptors results in a widespread reduction of the electric and metabolic activity in ascending serotonergic axons.

The SSRIs act as indirect 5-HT$_{1A}$ agonists owing to the excess 5-HT$_{ext}$ produced by these agents in the midbrain raphe. This activates somatodendritic 5-HT$_{1A}$ autoreceptors and limits the increment of 5-HT$_{ext}$ produced in forebrain. The present results indicate that the maximal effects of the systemic administration of paroxetine, a very potent SSRI (Thomas et al., 1987; Hyttel, 1994), in various forebrain regions are limited to a two- to threefold increment of 5-HT$_{ext}$ (see, however, the large increment of 5-HT$_{ext}$ produced in forebrain by the partial local blockade of the 5-HT transporter by 1 μmol/L citalopram in Table 2). Yet, the subsequent administration of WAY-100635, a very selective and potent 5-HT$_{1A}$ antagonist (Fletcher et al., 1995; Forster et al., 1995) counteracted the self-inhibition produced by paroxetine and markedly potentiated the increase of 5-HT$_{ext}$. In keeping with the present results, several groups have reported the potentiation of SSRI-induced forebrain increases of 5-HT$_{ext}$ by selective and nonselective 5-HT$_{1A}$ antagonists (Invernizzi et al., 1992, 1996; Hjorth, 1993; Artigas et al., 1994*b*; Gartside et al., 1995; Arborelius et al., 1996; Dreshfield et al., 1996; Romero et al., 1996). This effect is likely mediated at somatodendritic level, as the intraraphe or systemic application of 5-HT$_{1A}$ antagonists prevented the inhibition of cell firing and terminal 5-HT release elicited by the activation of somatodendritic 5-HT$_{1A}$ receptors (Blier and de Montigny, 1994; Arborelius et al., 1995; Gartside et al., 1995; Romero et al., 1996; see also Fig. 5).

Anatomical data indicate that the striatum and the frontal cortex are innervated by 5-HT neurons of the dorsal raphe nucleus in an exclusive and preferential manner, respectively (Azmitia and Segal, 1978; Imai et al., 1986). In contrast, the dorsal hippocampus is mostly innervated by 5-HT neurons of the median raphe nucleus. The preferential enhancement of 5-HT$_{ext}$ by WAY-100635 in striatum and frontal cortex agrees with previous data indicating a greater inhibition of the activity of serotonergic neurons of the dorsal raphe nucleus after systemic treatment with 5-HT$_{1A}$ agonists (Sinton and Fallon, 1988; Blier et al., 1990; Invernizzi et al., 1991; Lésourd et al., 1995; Casanovas and Artigas, 1996). It is unclear whether this is due to a differ-

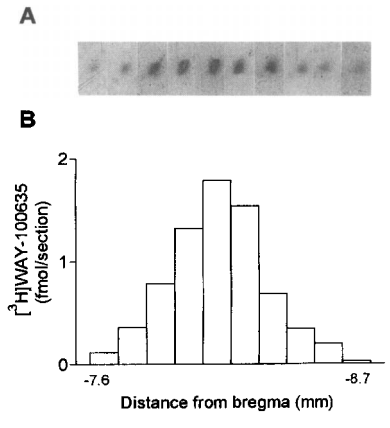

FIG. 9. A: Autoradiograms of 10 coronal sections at equally spaced anteroposterior levels of the midbrain (approximately from −7.6 to −8.7 mm with respect to bregma) show intense labeling of 5-HT$_{1A}$ receptors of the dorsal raphe nucleus by [^{3}H]WAY-100635. **B:** Bar histogram shows the amount of the radioactive tracer of the same sections. Abscissa shows the coordinates (in mm) with respect to bregma.

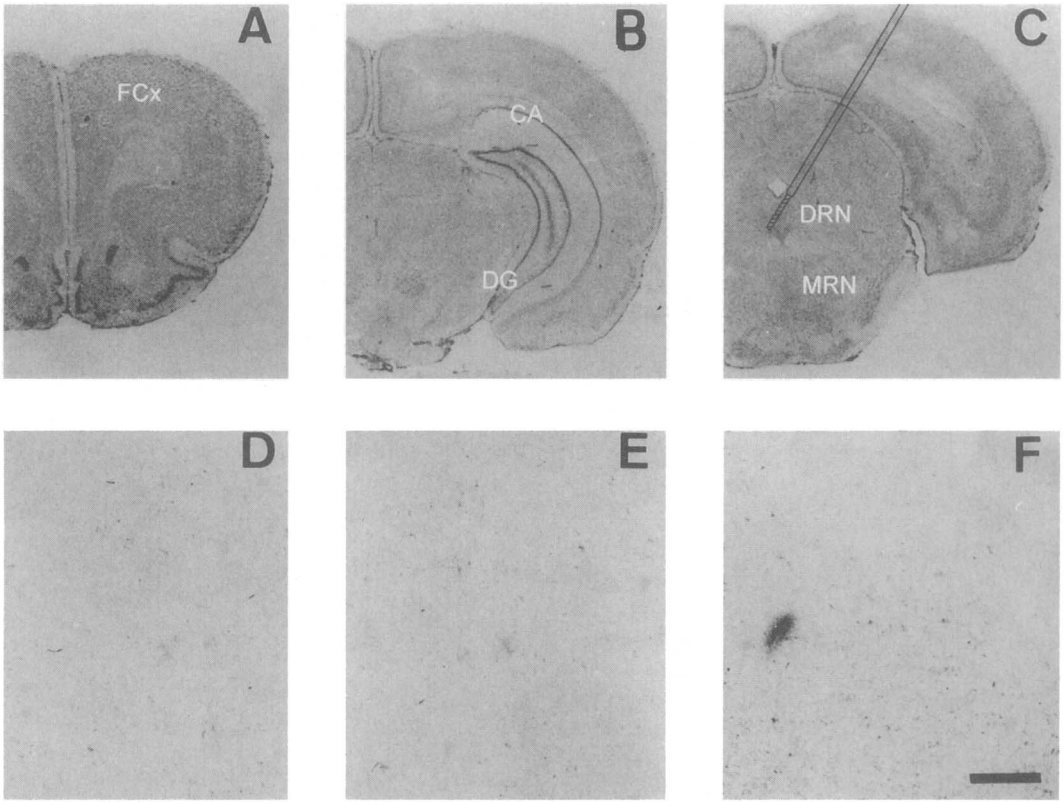

FIG. 10. A–C: Cresyl violet staining of rat brain coronal sections at frontal cortex (FCx; A), hippocampus (B), and midbrain (C) levels. Superimposed on the latter is a schematic drawing of the location of microdialysis probes in the dorsal raphe nucleus (DRN). **D–F:** Corresponding autoradiograms obtained after in vivo perfusion of [^{3}H]WAY-100635 (see Materials and Methods) in the DRN. Note the intense labeling of the DRN in F and its absence in the median raphe nucleus (MRN). Autoradiograms of cortical and hippocampal sections (D and E) showed no signs of label by the radioactive tracer. The midbrain section corresponds approximately to bregma −7.8. Bar = 2 mm. CA, Ammon's horn; DG, dentate gyrus.

ential reduction of cell firing at the cell body level by these agents or whether local factors in projection areas are involved, as recent data suggest an equal potency of paroxetine to inhibit dorsal raphe and median raphe neurons in anesthetized rats (Hajos et al., 1995). The present observations indicate that the 5-HT release by axon terminals of dorsal raphe neurons of the corticostriatal pathway is inhibited to a greater extent than that in the median raphe-hippocampal tract. This is supported by two different sets of experiments in which 5-HT$_{1A}$ receptors in the dorsal or median raphe nucleus have been locally or systemically activated by 5-HT and is in full agreement with the greater potentiation of paroxetine effects by WAY-100635 in striatum and frontal cortex (as compared with dorsal hippocampus).

The synergistic action of WAY-100635 most likely results from the blockade of 5-HT$_{1A}$ receptors, as it prevents the suppression of cell firing induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (Forster et al., 1995). Serotonergic neurons of the dorsal raphe nucleus also express 5-HT$_{1B}$ and 5-HT$_{1D}$ subtypes (for review, see Hoyer et al., 1994), and 5-HT$_{1D}$ receptors may also behave as somatodendritic autoreceptors (Piñeyro et al., 1995). However, the affinity of WAY-100635 for 5-HT$_{1B/1D}$ receptors is much lower than that for the 5-HT$_{1A}$ receptors (Forster et al., 1995), and the cell firing of serotonergic neurons is not controlled by 5-HT$_{1B}$ receptors (Sprouse and Aghajanian, 1987, 1988). These observations argue against the involvement of other 5-HT receptor subtypes in the 5-HT-enhancing action of WAY-100635.

The infusion of citalopram in the dorsal raphe nucleus or in the vicinity of dorsal and median raphe nuclei caused a marked reduction of 5-HT release in frontal cortex, likely resulting from the activation of somatodendritic 5-HT$_{1A}$ autoreceptors, as the systemic administration of WAY-100635 fully reversed the cortical 5-HT$_{ext}$ decline and further increased the citalopram-induced elevation of 5-HT$_{ext}$ in the raphe. The latter observation clearly indicates that 5-HT release in the dorsal and median raphe nuclei is also inhibited by activation of somatodendritic 5-HT$_{1A}$ receptors during the treatment with SSRIs. It is unclear whether there is a tonic inhibition of somatodendritic 5-HT release in a physiological situation in the rat brain, because WAY-100635 did not elevate 5-HT$_{ext}$ in forebrain when administered alone. However, it moder-

ately increased the firing rate of single units in the dorsal raphe nucleus of the awake cat (Fornal et al., 1996), thus suggesting a tonic control of serotonergic cell firing by 5-HT$_{1A}$ receptors in this species during periods of behavioral activation. The absence of a corresponding increment in the forebrain 5-HT release in rats may possibly be related to changes in the 5-HT$_{1A}$ tone across the sleep–waking cycle, as the present experiments were conducted during the period of low behavioral activity, i.e., daytime.

The local application of WAY-100635 in the dorsal raphe nucleus potentiated the systemic effects of paroxetine in frontal cortex and partially counteracted the reduction of cortical 5-HT release induced by systemic paroxetine when the 5-HT uptake was locally blocked in frontal cortex. The conspicuous and selective labeling of the dorsal raphe nucleus by [^{3}H]WAY-100635 when locally infused indicated that only somatodendritic 5-HT$_{1A}$ receptors of this nucleus were involved. However, WAY-100635 seemed to be more effective at antagonizing the inhibitory effects of 5-HT at 5-HT$_{1A}$ receptors after its systemic administration, which may indicate either the involvement of postsynaptic 5-HT$_{1A}$ receptors in this effect or a more effective antagonism after systemic administration. Indeed, the activity of dorsal raphe neurons is controlled by forebrain afferents (Kalén and Wiklund, 1989; Kalén et al., 1989; Behzadi et al., 1990; Levine and Jacobs, 1992; Peyron, 1996), and it has been suggested that postsynaptic 5-HT$_{1A}$ receptors may participate in the reduction of the activity of dorsal raphe nucleus serotonergic neurons by 5-HT$_{1A}$ agonists (Blier and de Montigny, 1987; Ceci et al., 1994; Romero et al., 1994). Likewise, the hippocampal application of 5-HT$_{1A}$ agonists reduced dorsal raphe nucleus neuronal firing, but this effect may be perhaps mediated by diffusion via CSF (Jolas et al., 1995). The present data fully support that somatodendritic 5-HT$_{1A}$ receptors participate in the potentiation of paroxetine effects by WAY-100635 but cannot fully exclude the involvement of postsynaptic 5-HT$_{1A}$ receptors.

WAY-100135 has been shown to prevent the reduction of terminal 5-HT release elicited by systemic administration of 8-hydroxy-2-(di-*n*-propylaminotetralin) or by moderate increments of 5-HT$_{ext}$ in the dorsal raphe nucleus (Routledge et al., 1993; Ferré et al., 1994; Hjorth and Auerbach, 1994). However, in contrast to other mixed β-adrenoceptor/5-HT$_{1A}$ antagonists like (−)-tertatolol (Romero et al., 1994) or (−)-pindolol (Romero et al., 1996), WAY-100135 did not prevent the reduction of striatal 5-HT release induced by local application of citalopram in the dorsal raphe nucleus, a procedure causing a massive activation of 5-HT$_{1A}$ autoreceptors by 5-HT (see Figs. 6 and 7). The lesser ability of WAY-100135 to antagonize self-inhibitory effects in the dorsal raphe nucleus suggests that this compound may be far less effective than its congener WAY-100635 in potentiating the actions of SSRIs. Also, WAY-100635 increased and WAY-100135 decreased 5-HT cell firing in the awake cat (Fornal et al., 1996), a finding possibly related to the α_1-adrenolytic properties of the latter (Lanfumey et al., 1993).

In summary, the present study supports the contention that selective 5-HT$_{1A}$ autoreceptor antagonists may augment the clinical effects of SSRIs by increasing serotonergic transmission more than the latter alone. This effect takes place preferentially in forebrain areas with selective or preferential innervation from the dorsal raphe nucleus, e.g., cortex and striatum, rather than those mainly innervated by the median raphe nucleus (dorsal hippocampus). These observations are important to clarify the relative role of serotonergic transmission in motor, limbic, and cortical areas during the recovery from depression. Also, the involvement of somatodendritic 5-HT$_{1A}$ receptors in the 5-HT-enhancing effects of 5-HT$_{1A}$ antagonists has been extensively demonstrated.

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