Journal of Neurochemistry
Lippincott Williams & Wilkins, Philadelphia

Differential Regulation of Somatodendritic Serotonin 5-HT_{1A} 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. M. Casanovas, M. T. Vilaró, G. Mengod, and F. Artigas

Department of Neurochemistry, Instituto de Investigaciones Biomédicas de Barcelona, CSIC, Barcelona, Spain

Abstract: Single treatment with the serotonin (5-hydroxytryptamine) 5-HT_{1A} receptor agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and alnespirone (S-20499) reduces the extracellular 5-HT concentration (5-HT_{ext}) in the rat midbrain and forebrain. Given the therapeutic potential of selective 5-HT_{1A} agonists in the treatment of affective disorders, we have examined the changes in 5-HT_{1A} receptors induced by 2-week minipump administration of alnespirone (0.3 and 3 mg/kg/day) and 8-OH-DPAT (0.1 and 0.3 mg/kg/day). The treatment with alnespirone did not modify baseline 5-HT_{ext} but significantly attenuated the ability of 0.3 mg/kg s.c. alnespirone to reduce 5-HT_{ext} in the dorsal raphe nucleus (DRN) and frontal cortex. In contrast, the ability of 8-OH-DPAT (0.025 and 0.1 mg/kg s.c.) to reduce 5-HT_{ext} in both areas was unchanged by 8-OH-DPAT pretreatment. Autoradiographic analysis revealed a significant reduction of [^{3}H]8-OH-DPAT and [^{3}H]WAY-100635 {^{3}H-labeled *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide · 3HCl} binding to somatodendritic 5-HT_{1A} receptors (but not to postsynaptic 5-HT_{1A} receptors) of rats pretreated with alnespirone but not with 8-OH-DPAT. In situ hybridization analysis revealed no change of the density of the mRNA encoding the 5-HT_{1A} receptors in the DRN after either treatment. These data indicate that continuous treatment for 2 weeks with alnespirone, but not with 8-OH-DPAT, causes a functional desensitization of somatodendritic 5-HT_{1A} receptors controlling 5-HT release in the DRN and frontal cortex. **Key Words:** Serotonin 5-HT_{1A} receptors—5-Hydroxytryptamine release—Anxiety—Depression—Dorsal raphe nucleus—Frontal cortex.
J. Neurochem. **72,** 262–272 (1999).

Serotonin (5-hydroxytryptamine) 5-HT_{1A} receptor agonists exhibit anxiolytic and/or antidepressant activity in experimental models (for review, see De Vry, 1995), and some members of the azapirone family, e.g., buspirone and gepirone, are used in the treatment of affective disorders (Pecknold, 1994). Alnespirone (S-20499) is an aminochroman derivative with very high affinity for 5-HT_{1A} receptors. Alnespirone shows anxiolytic and antidepressant activity in animal models (Porsolt et al., 1992; Barrett et al., 1994). It displays full agonistic activity at somatodendritic 5-HT_{1A} receptors and inhibits forskolin-activated adenylate cyclase in hippocampal homogenates (Kidd et al., 1993). In keeping with its agonist properties at presynaptic 5-HT_{1A} receptors, it reduces the 5-HT turnover rate and 5-HT release in forebrain (Kidd et al., 1993; Casanovas et al., 1997). Alnespirone is not metabolized to 1-(2-pyrimidinyl)piperazine, a compound that antagonizes α_2-adrenoceptors (Bianchi et al., 1988), and therefore it does not directly interact with catecholamine neurons (Dugast et al., 1998).

5-HT_{1A} receptors are deeply involved in the control of the activity of ascending serotonergic neurons. The activation of somatodendritic 5-HT_{1A} receptors inhibits the firing activity of serotonergic neurons (Sprouse and Aghajanian, 1986; Blier and de Montigny, 1987) and the firing-dependent 5-HT release in projection areas (Sharp et al., 1989). This reduction appears to take place preferentially in forebrain areas innervated by serotonergic neurons of the dorsal raphe nucleus (DRN) such as frontal cortex or dorsal striatum (Casanovas and Artigas, 1996; Casanovas et al., 1997). Selective 5-HT reuptake

Received February 12, 1998; revised manuscript received July 27, 1998; accepted August 17, 1998.

Address correspondence and reprint requests to Dr. F. Artigas at Department of Neurochemistry, Instituto de Investigaciones Biomédicas de Barcelona, CSIC, Jordi Girona 18-26, E-08034 Barcelona, Spain.

Abbreviations used: AUC, area under the curve; DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine (serotonin); 5-HT_{ext}, extracellular 5-hydroxytryptamine concentration; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; PBS, phosphate-buffered saline; SSC, 150 m*M* NaCl and 15 m*M* sodium citrate; WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide · 3HCl.

inhibitors—behaving as indirect agonists at somatodendritic 5-HT$_{1A}$ receptors—also inhibit 5-HT release with the same regional pattern (Romero and Artigas, 1997).

5-HT$_{1A}$ receptors play a crucial role in the effects of antidepressant drugs. Direct and indirect agonists of the somatodendritic 5-HT$_{1A}$ receptors appear to induce receptor desensitization after chronic treatment, as measured by electrophysiological or neurochemical indexes (Blier and de Montigny, 1987; Schechter et al., 1990; for review, see Blier and de Montigny, 1994). This mechanism is thought to be involved in the delayed action of antidepressant drugs because chronic, but not acute, treatment enhances the extracellular 5-HT concentration (5-HT$_{ext}$) in some forebrain areas (for review, see Artigas et al., 1996). Hence, prevention of the 5-HT$_{1A}$-based negative feedback by concurrent administration of 5-HT$_{1A}$ receptor antagonists enhances the experimental and clinical effects of selective 5-HT reuptake inhibitors (Artigas et al., 1996; Pérez et al., 1997). However, the desensitization of 5-HT$_{1A}$ receptors after chronic selective 5-HT reuptake inhibitor or 5-HT$_{1A}$ agonist treatments has not been universally found (Bohmaker et al., 1993; Sharp et al., 1993; Hjorth and Auerbach, 1994; Invernizzi et al., 1995), a discrepancy for which no clear explanations exist.

Given the potential usefulness of alnespirone for the treatment of mood disorders, we have examined the effects of its continuous administration for 2 weeks on the density and function of 5-HT$_{1A}$ receptors using in vivo microdialysis and receptor autoradiography. A parallel comparative study using the prototypic 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) has also been conducted.

MATERIALS AND METHODS

Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 280–320 g at the time of experiments 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 before and during the experiments. Animal care followed the European Union regulations (O. J. of E. C. L358/1 18/12/1986).

Drugs and reagents

5-HT and 8-OH-DPAT were from Research Biochemicals International (Natick, MA, U.S.A.). Alnespirone and citalopram were kindly provided by the Institut de Recherches Internationales Servier (Courbevoie, Paris) and Lundbeck A/S (Valby, Copenhagen, Denmark), respectively. Other materials and reagents were from local commercial sources.

Alnespirone and 8-OH-DPAT were administered with minipumps. Two different doses of alnespirone (0.3 and 3 mg/kg/day) and 8-OH-DPAT (0.1 and 0.3 mg/kg/day) were used. When administered as single subcutaneous doses, 3 mg/kg alnespirone and 0.3 mg/kg 8-OH-DPAT produced equivalent (maximal) reductions of the 5-HT release in dorsal striatium (Casanovas et al., 1997). Drugs were dissolved in distilled water, and minipumps (Alzet 2002) were filled with these solutions. Given the weight gain of the animals, the doses used correspond to day 7 of treatment. Two different experiments were conducted to test the effects of alnespirone and 8-OH-DPAT, respectively. In each, rats were randomly assigned to receive vehicle or the lower or the higher dose of each drug. Control animals were implanted with minipumps filled with distilled water. Minipumps were implanted subcutaneously with the animal under light ether anesthesia. Rats were housed one per cage. One day before the microdialysis experiments, minipumps were removed with the animal under pentobarbital anesthesia (immediately before the implants of the microdialysis probes; see below). The minipumps were cut with an incisor blade, and it was checked that they were empty by visual inspection.

To test the functional state of 5-HT$_{1A}$ receptors, challenge doses of alnespirone (0.3 mg/kg s.c.) or 8-OH-DPAT were administered after collection of baseline dialysate values to groups of animals pretreated with saline or active drug. Given the short half-life of 8-OH-DPAT, it was possible to administer two different challenge doses (0.025 and 0.1 mg/kg s.c.) to each rat in the same experiment. The effects of challenge doses of the 5-HT$_{1A}$ agonists on 5-HT release were examined in the DRN and frontal cortex of separate groups of rats treated with either dose of alnespirone or 8-OH-DPAT and compared with those produced in controls.

Surgery and microdialysis procedures

Microdialysis procedures were performed essentially as described by Adell and Artigas (1991). Anesthetized rats (pentobarbital, 60 mg/kg i.p.) were stereotaxically implanted with one l-shaped probe in the DRN or frontal cortex. Coordinates [in mm (Paxinos and Watson, 1986)] were taken from bregma and dura mater and were as follows: AP +3.2, L −2.5, DV −6.0 for frontal cortex and AP −7.6, L 0.0 (the actual lateral coordinate at the dura mater level was −3.1 mm), DV −7.5 for DRN. In the latter area, microdialysis probes were implanted with an angle of 30° to avoid obstruction of the cerebral aqueduct. The length of membrane exposed to the brain tissue was 4 mm (o.d. = 0.25 mm) in frontal cortex and 1.5 mm in the DRN. Animals were allowed to recover from surgery for ~20 h, and then 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* $MgCl_2$) containing 1 μM citalopram at 0.25 μl/min. Sample collection started 60 min after the beginning of perfusion. Dialysate samples were collected every 20 min (5 μl). Usually five or six fractions were collected before drug administration, of which four were used to obtain the individual basal values. Challenge doses of alnespirone and 8-OH-DPAT (first injection) were administered around noon. At the end of the microdialysis experiments, the animals were killed by decapitation, and the brain was quickly removed and frozen (−20°C) for further autoradiographic and in situ hybridization analyses. The data from animals with probes outside the limits of the brain structures of interest were not included in the calculations (typically ~15–20% of those implanted in the DRN).

Chromatographic analysis

5-HT was analyzed by a modification of an HPLC method previously described (Adell and Artigas, 1991). The composition of HPLC eluent 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), and 27% methanol. 5-HT was separated in a 3-μm (partical size) ODS 2 column (7.5 × 0.46 cm; Beckman, Fullerton, CA, U.S.A.) and detected amperometrically with a Hewlett Packard model 1049 detector (oxidation potential, 0.6 V). Retention time was 3.5–4 min. The absolute detection limit

for 5-HT was 0.5–1 fmol per sample. Dialysate 5-HT values were calculated by reference to standard curves assayed daily.

Receptor autoradiography

Brain sections, 14 μm thick, were cut on a microtome-cryostat (model HM 500M; Microm), thaw-mounted onto 3-aminopropyltriethoxysilane (Sigma)-treated slides, and kept at −20°C until used. ^{3}H-labeled WAY-100635 {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide · 3HCl} (80 Ci/mmol) and [^{3}H]8-OH-DPAT (234 Ci/mmol; Amersham, U.K.) were used as ligands. Optimal preincubation time was determined in pilot experiments. In brief, sections were preincubated for different times (0, 25, 40, 75, and 100 min) and processed as described below. A preincubation time of 100 min was finally chosen (see Results). Sections were preincubated for this time at room temperature in 170 m*M* Tris-HCl (pH 7.5), 4 m*M* $CaCl_2$, and 0.01% ascorbic acid and then incubated for 1 ([^{3}H]8-OH-DPAT) or 2 h ([^{3}H]WAY-100635) at room temperature in the same buffer supplemented with 10 μM pargyline. Nonspecific binding was defined in the presence of 10 μM 5-HT. In both cases, radioligand concentration was 0.5 n*M*. Sections were washed twice (5 min each) in the same buffer at 4°C, dipped in distilled water at 4°C, and rapidly dried under cold air. Tissues were exposed to Hyperfilm-^{3}H (Amersham) together with plastic standards (^{3}H-Microscales; Amersham). Exposure time was 11 days for [^{3}H]8-OH-DPAT and 23 days for [^{3}H]WAY-100635. Quantitative analysis of the autoradiograms was performed with a computerized image analysis system (MCID, St. Catharines, Ontario, Canada). The autoradiographic analysis of sections of representative animals of all experimental groups was performed at once.

In situ hybridization histochemistry

An oligonucleotide probe complementary to the mRNA coding for the rat 5-HT_{1A} receptor [amino acids 407–422 (Albert et al., 1990)] was used. The oligonucleotide was 3′ end-labeled with terminal deoxynucleotidyltransferase (Boehringer Mannheim) and [α-^{32}P]dATP (3,000 Ci/mmol; Du Pont New England Nuclear) obtaining a final specific activity of 0.7–1.2 $\times$ 10^4 Ci/mmol. Labeled probe was purified through NACS PREPAC columns (BRL).

Before hybridization, frozen tissue sections were air-dried, fixed for 20 min in 4% paraformaldehyde in phosphate-buffered saline (1 $\times$ PBS = 2.6 m*M* KCl, 1.4 m*M* KH_2PO_4, 136 m*M* NaCl, and 8 m*M* Na_2HPO_4), washed once in 3 $\times$ PBS and twice in 1 $\times$ PBS for 5 min each, and incubated in a freshly prepared solution of predigested Pronase (Calbiochem) at a final concentration of 24 U/ml in 50 m*M* Tris-HCl (pH 7.5) and 5 m*M* EDTA for 2 min at room temperature. Proteolytic activity was stopped by immersion for 30 s in 2 mg/ml glycine in PBS. Tissues were rinsed in PBS and dehydrated through a graded series of ethanol. For hybridization, labeled probes were diluted to a final concentration of 2×10^7 cpm/ml in a solution containing 50% formamide, 4 $\times$ SSC (1 $\times$ SSC = 150 m*M* NaCl and 15 m*M* sodium citrate), 1$\times$ Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 10% dextran sulfate, 1% sarkosyl, 20 m*M* phosphate buffer (pH 7.0), 250 μg/ml yeast tRNA, and 500 μg/ml salmon sperm DNA. Tissues were covered with 80 μl of hybridization solution, overlaid with Nescofilm coverslips, and incubated overnight in humid boxes at 42°C. Sections were washed four times (45 min each) in 600 m*M* NaCl, 20 m*M* Tris-HCl (pH 7.5), and 1 m*M* EDTA at 60°C, dehydrated in graded ethanol solution, and dried. Hybridized sections were exposed to β-Max film (Amersham) for 12 days at −70°C with intensifying screens. Film optical densities over the region of the DRN were measured with the MCID computerized image analysis system.

Data and statistical analysis

Microdialysis results are expressed as femtomoles per fraction (uncorrected for recovery) or as percentages of basal values (individual means of four predrug fractions). Statistical analysis (SPSS/PC+) of drug effects on dialysate 5-HT level was performed using one- or two-way ANOVA for repeated measures of raw data with dose or region as the independent factor. ANOVA of areas under the curve (AUCs) or peak reductions was also used to examine further the influence of dose and region factors on the 5-HT output. Given the different pharmacokinetics of both drugs, fractions 6–16 were used to calculate the AUC values for alnespirone, and fractions 6–8 (0.025 mg/kg s.c.) and 14–16 (0.1 mg/kg s.c.) were used for the two challenge doses of 8-OH-DPAT (Casanovas et al., 1997). These fractions are those in which the effect of 0.025 mg/kg 8-OH-DPAT was maximal. For consistency, we used also three fractions to assess the effects of 0.1 mg/kg 8-OH-DPAT, although this dose elicited more lasting reductions of the 5-HT output. The fraction immediately after the injection of drugs was omitted owing to the elevations of 5-HT_{ext} produced by the injection stress (Adell et al., 1997; see below).

Data from radioligand autoradiography are given as femtomoles of receptor per milligram of protein. Results from in situ hybridization are expressed as film optical density. One-way ANOVA has been used to assess the effects of the treatments on 5-HT_{1A} receptor density in autoradiographic studies. Data are given as mean ± SEM values. The number of animals in each group is given in the figure legends. Statistical significance has been set at the 95% confidence level (two-tailed).

RESULTS

Microdialysis experiments

Baseline dialysate 5-HT values in the presence of 1 μM citalopram were 52.7 ± 3.2 and 17.5 ± 0.7 fmol per fraction in the DRN and frontal cortex, respectively (n = 46 rats each). Overall, baseline 5-HT values were not different in control and alnespirone-treated rats (Fig. 1A). A significant increment ($F_{2,20} = 5.05$, $p < 0.02$, one-way ANOVA) was observed in the frontal cortex of rats treated with 0.1 mg/kg/day 8-OH-DPAT compared with controls, yet no such difference was found for those treated with 0.3 mg/kg/day (Fig. 1B). Also, no differences in baseline 5-HT values were found in the DRN after chronic treatment with 8-OH-DPAT or alnespirone.

A challenge dose of 0.3 mg/kg alnespirone reduced the 5-HT output to 45% of baseline in frontal cortex and to 54% of baseline in the DRN of control rats (Fig. 2). This effect was significantly attenuated in both regions of rats pretreated with alnespirone (significant effect of time, $F_{15,300} = 20.68$, $p < 0.001$; time $\times$ treatment interaction, $F_{30,300} = 1.57$, $p < 0.035$ in the DRN; significant effect of time, $F_{15,300} = 34.34$, $p < 0.001$; time $\times$ treatment interaction, $F_{30,300} = 1.88$, $p < 0.005$ in frontal cortex; two-way repeated-measures ANOVA; Fig. 2). One-way ANOVA of the AUCs revealed a significant effect of the treatment ($F_{2,20} = 8.26$, $p < 0.003$ in DRN; $F_{2,20} = 3.82$,

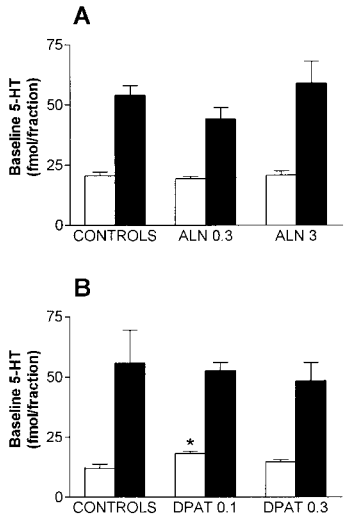

FIG. 1. Treatment with (**A**) alnespirone (ALN; 0.3 and 3 mg/kg/day) or (**B**) 8-OH-DPAT (DPAT; 0.1 and 0.3 mg/kg/day) for 2 weeks did not induce consistent changes in the baseline dialysate 5-HT values in frontal cortex (open columns) or DRN (filled columns). *A significant increment was noted in frontal cortex of the rats treated with 0.1 mg/kg/day 8-OH-DPAT (see text), but this was not accompanied by a similar change at 0.3 mg/kg/day. Data are mean ± SEM (bars) values (n = 7 or 8 rats per group).

$p < 0.04$ in frontal cortex; Fig. 2). Post hoc Tukey's test revealed significant differences between control rats and those treated with 3 mg/kg/day in both brain regions and between controls and 0.3 mg/kg/day for the DRN. The corresponding AUCs (expressed as percentages of predrug values) were 62.1 ± 4.0% for controls and 83.0 ± 3.8% for 3 mg/kg/day alnespirone. A similar 20% difference was noted in frontal cortex (55.0 ± 3.5% in controls vs. 75.0 ± 6.9% in 3 mg/kg/day alnespirone).

Given the very short half-life of 8-OH-DPAT, it was possible to administer two different challenge doses (0.025 and 0.1 mg/kg; Fig. 3). Neither reduced the 5-HT output differently in controls or 8-OH-DPAT-pretreated rats, as judged from the maximal decrease elicited. The AUCs corresponding to the three fractions with maximal decrements (6–8 for 0.025 mg/kg; 14–16 for 0.1 mg/kg) were also calculated. No significant attenuation of the effect of 0.025 or 0.1 mg/kg 8-OH-DPAT was observed in pretreated rats compared with controls (Fig. 3C and D). In contrast, there was a tendency ($p = 0.11$) toward a greater effect of 0.1 mg/kg 8-OH-DPAT in rats pretreated with 0.3 mg/kg/day.

Rats treated with 8-OH-DPAT had an increased weight gain compared with controls over the 2-week treatment period (87.1 ± 2.6, 93.6 ± 3.8, and 99.2 ± 1.9 g for controls and 0.1 and 0.3 mg/kg/day 8-OH-DPAT, respectively; $F_{2,47} = 3.74$, $p < 0.035$, one-way ANOVA).

Radioligand autoradiography and in situ hybridization

To assess whether the above differences were due to a change in receptor number, we performed an autoradiographic analysis of the brains of rats treated with both 5-HT$_{1A}$ agonists. To make sure that residual drug from the challenge dose would not interfere with the binding of the radioligands to 5-HT$_{1A}$ receptors, preliminary experiments were conducted to determine the appropriate preincubation time. Serial sections of a limited number of animals were preincubated for different intervals (0–100 min) before incubation in the presence of [^{3}H]8-

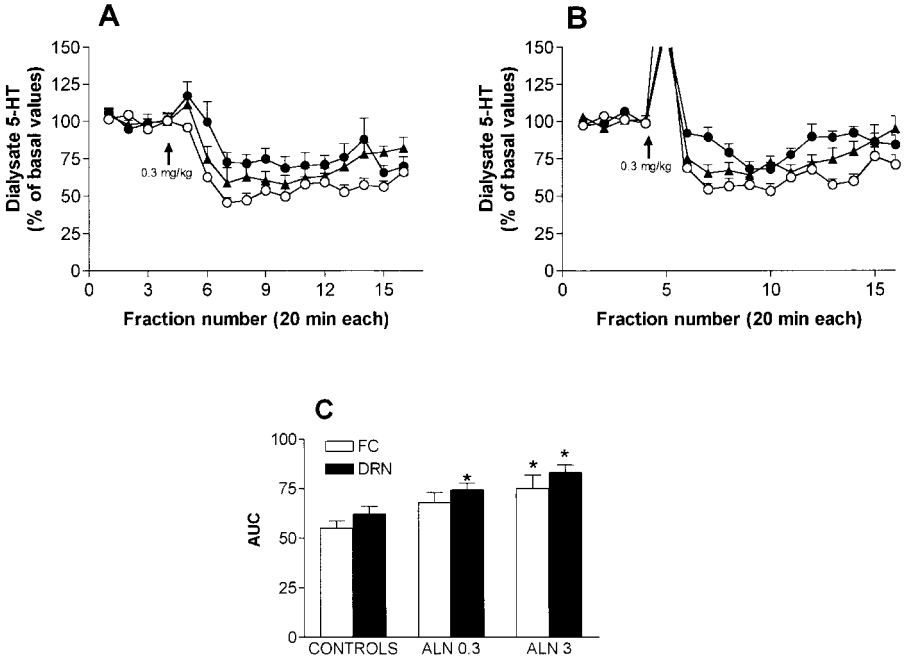

FIG. 2. Effects of a challenge dose of alnespirone (ALN; 0.3 mg/kg s.c.) on 5-HT release in animals pretreated with vehicle (○) or 0.3 (▲) or 3 (●) mg/kg/day ALN. **A** and **B:** Effects in frontal cortex (FC) and DRN, respectively. The elevation seen in the latter region is caused by subcutaneous injection of ALN. Two-way repeated-measures ANOVA revealed a significant time × treatment interaction in both areas. **C:** AUC of fractions 6–16 corresponding to both regions after ALN treatment. Asterisks denote significant differences versus controls (by one-way ANOVA). Data are mean ± SEM (bars) values (n = 7 or 8 rats per group).

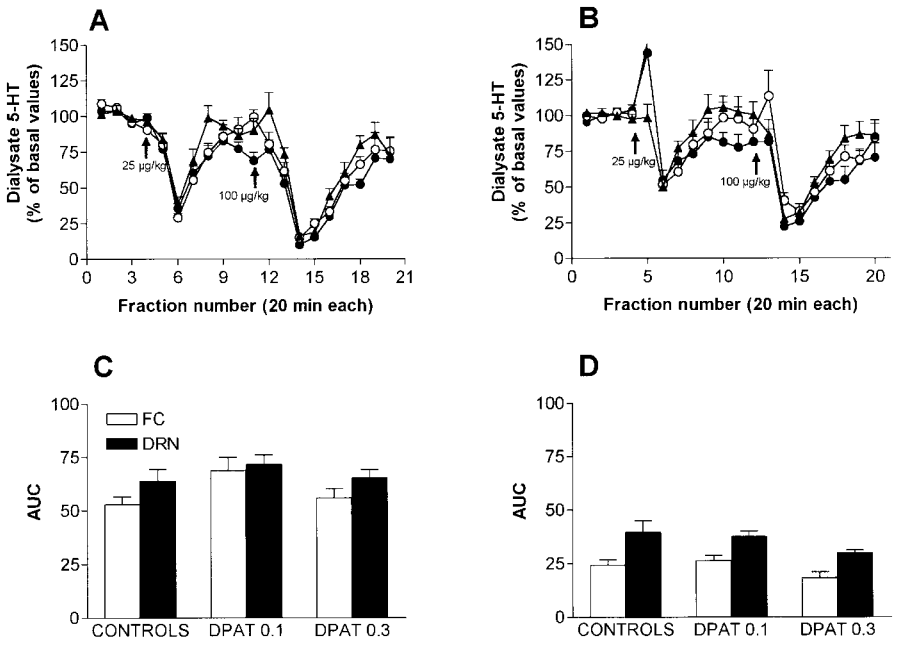

FIG. 3. Effects of two challenge doses of 8-OH-DPAT (DPAT; 0.025 and 0.1 mg/kg s.c.) on 5-HT release in animals pretreated with vehicle (○) or 0.1 (▲) or 0.3 (●) mg/kg/day DPAT. **A** and **B:** Effects in frontal cortex (FC) and DRN, respectively. The elevation seen in the latter region is caused by the subcutaneous injection of DPAT. There were no significant differences between the effects of DPAT in control or DPAT-pretreated rats. **C** and **D:** AUCs of the periods of maximal reduction of 5-HT release after subcutaneous administration of 0.025 and 0.1 mg/kg DPAT, respectively. There was a nonsignificant effect of the pretreatment (one-way ANOVA). Data are mean ± SEM (bars) values (n = 7 or 8 rats per group).

OH-DPAT. No apparent differences were noted between rats treated with 8-OH-DPAT or alnespirone. As shown in Fig. 4, specific binding increased in the DRN and hippocampus with a preincubation time up to 100 min, showing a plateau at 70–100 min. Therefore, this latter time was chosen for both radioligands.

In agreement with the antagonistic character of WAY-100635, the observed density of sites labeled by [^{3}H]WAY-100635 almost doubled that achieved with [^{3}H]8-OH-DPAT in all brain areas examined and for all treatments. A similar or even higher ratio was observed when total densities of binding sites (B_{max}) were considered (data not shown). Three different measures of labeling density were performed in the DRN, corresponding to the whole nucleus and its dorsal and ventral parts, respectively. Also, densities of receptors were measured in several postsynaptic areas, including the CA1 hippocampal subfield (strata oriens and radiatum), the dentate gyrus, the interpeduncular nucleus, and the frontoparietal cortex. The labeling of pre- and postsynaptic 5-HT$_{1A}$ receptors in several of these areas by [^{3}H]8-OH-DPAT and [^{3}H]WAY-100635 is shown, respectively, in Figs. 5 and 6.

The treatment with alnespirone induced a dose-dependent reduction of the labeling by [^{3}H]8-OH-DPAT in the DRN but not in postsynaptic areas ($F_{2,17} = 6.35$, $p < 0.01$; $F_{2,17} = 5.25$, $p < 0.02$; and $F_{2,17} = 5.75$, $p < 0.02$ in total DRN and its dorsal and ventral parts, respectively; one-way ANOVA). Post hoc Tukey's test revealed a significant reduction of the tritium labeling (~25–30%) between the control and 3 mg/kg/day alnespirone groups in all three instances (Fig. 7). The same

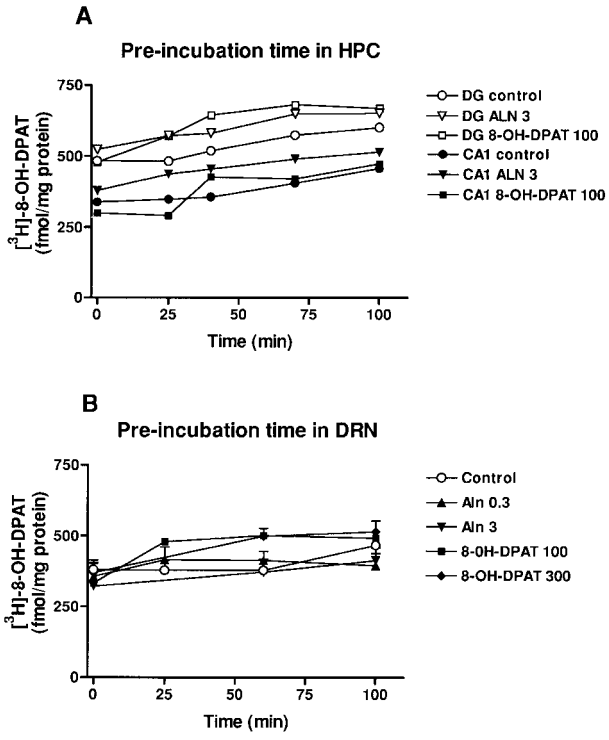

FIG. 4. Effect of preincubation time on binding of [^{3}H]8-OH-DPAT to rat brain sections for the different experimental groups: (**A**) in the hippocampal formation [HPC; area CA1 and dentate gyrus (DG) (n = 1)] and (**B**) in the DRN (n = 2 or 3 rats per group). No differences were noted between groups, and the plateau was reached at 70–100 min in most cases, so the preincubation time chosen to conduct the experiments was 100 min. ALN or Aln, alnespirone.

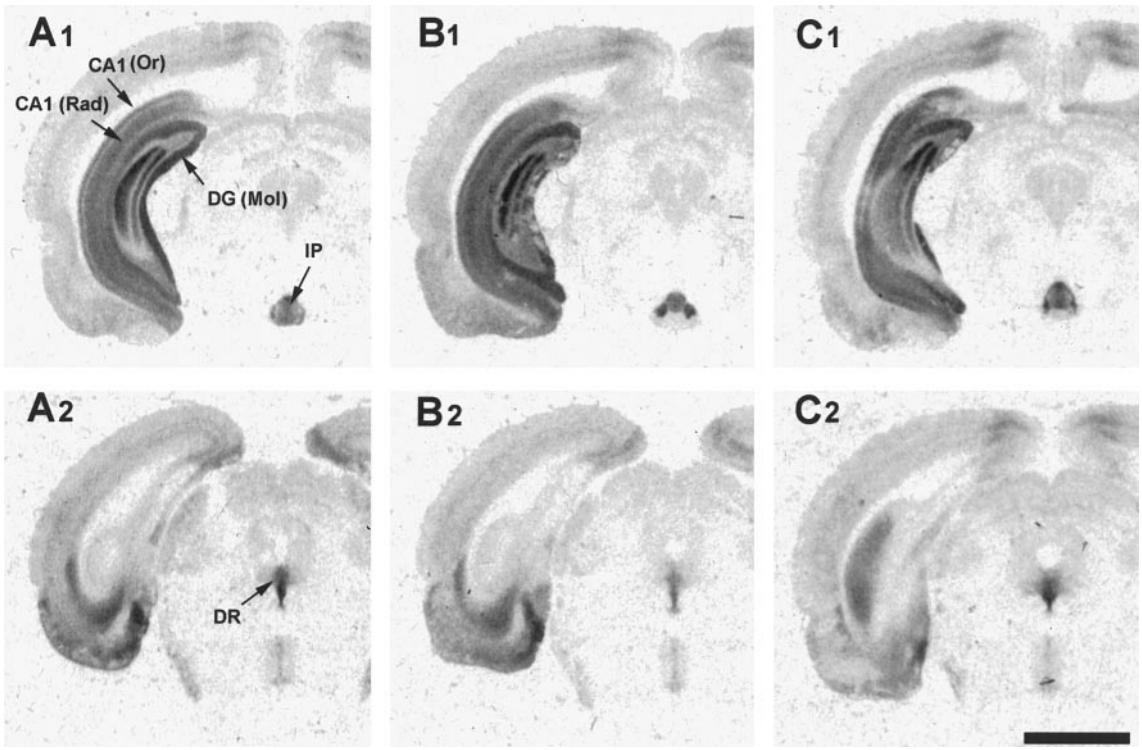

FIG. 5. Coronal sections show the autoradiographic labeling of 5-HT$_{1A}$ receptors by [^{3}H]8-OH-DPAT at two different levels in brains of rats treated for 2 weeks with (**A**) vehicle, (**B**) alnespirone (3 mg/kg/day), or (**C**) 8-OH-DPAT (0.3 mg/kg/day). CA1 (Or) and CA1 (Rad), strata oriens and radiatum, respectively, of the CA1 hippocampal subfield; DG (Mol), dentate gyrus, stratum moleculare; IP, interpeduncular nucleus; DR, DRN. Note the decreased receptor density in the DRN of the rat treated with alnespirone (B2) compared with the control rat (A2). Bar = 3 mm.

differences were noted when the tritiated 5-HT$_{1A}$ antagonist [^{3}H]WAY-100635 was used to label 5-HT$_{1A}$ receptors in adjacent sections. In this case, the corresponding values were $F_{2,17} = 5.67$, $p < 0.015$; $F_{2,17} = 4.11$, $p < 0.035$; and $F_{2,17} = 6.20$, $p < 0.01$ for the whole DRN and its dorsal and ventral parts, respectively (one-way ANOVA). No such differences were noted for postsynaptic 5-HT$_{1A}$ receptors labeled with either radioligand except for the CA1 field (stratum oriens), which showed a significantly higher labeling in rats treated with 0.3 mg/kg/day alnespirone compared with controls. Also, the labeling in the interpenduncular nucleus was higher in rats treated with 3 mg/kg/day alnespirone than with 0.3 mg/kg/day (but not vs. controls).

In animals treated with 8-OH-DPAT, one-way ANOVA revealed the existence of significant differences in [^{3}H]WAY-100635 binding to the DRN ($F_{2,17} = 4.38$, $p < 0.03$ for the whole DRN; $F_{2,17} = 5.97$, $p < 0.01$ for the ventral part), but post hoc Tukey's test indicated that these were due to a higher labeling in animals treated with 0.3 mg/kg/day 8-OH-DPAT than in those treated with 0.1 mg/kg/day (Fig. 8). No significant differences were observed when 5-HT$_{1A}$ receptors were labeled with [^{3}H]8-OH-DPAT.

The specificity of the signal obtained with the oligonucleotide probe used for in situ hybridization studies has been previously demonstrated (Pompeiano et al., 1992). On visual inspection of the autoradiograms, no apparent differences in the intensity of the hybridization signal for 5-HT$_{1A}$ receptor mRNA were observed between agonist- or vehicle-treated animals. In the region of the DRN, where significant reductions in the densities of 5-HT$_{1A}$ receptors had been observed, computer-assisted measures of film optical densities were performed. No significant difference was observed among the different treatment groups (Fig. 9). Thus, the optical density values for control, low-dose, and high-dose groups were 0.23 ± 0.01, 0.20 ± 0.01, and 0.23 ± 0.03 for alnespirone and 0.23 ± 0.01, 0.22 ± 0.02, and 0.24 ± 0.02 for 8-OH-DPAT, respectively (data from four to six rats per group).

DISCUSSION

Although some evidence has been provided in vitro (Albert et al., 1996), there is considerable debate about the capacity of 5-HT$_{1A}$ agonists to desensitize/down-regulate 5-HT$_{1A}$ receptors in vivo (for review, see De Vry, 1995). With few exceptions (Welner et al., 1989; Fanelli and McMonagle-Strucko, 1992), administration of selective agents for 2–3 weeks did not result in a reduced density of 5-HT$_{1A}$ sites in receptor binding or autoradiographic studies. Functional measures of 5-HT$_{1A}$ receptor activity have also yielded conflicting

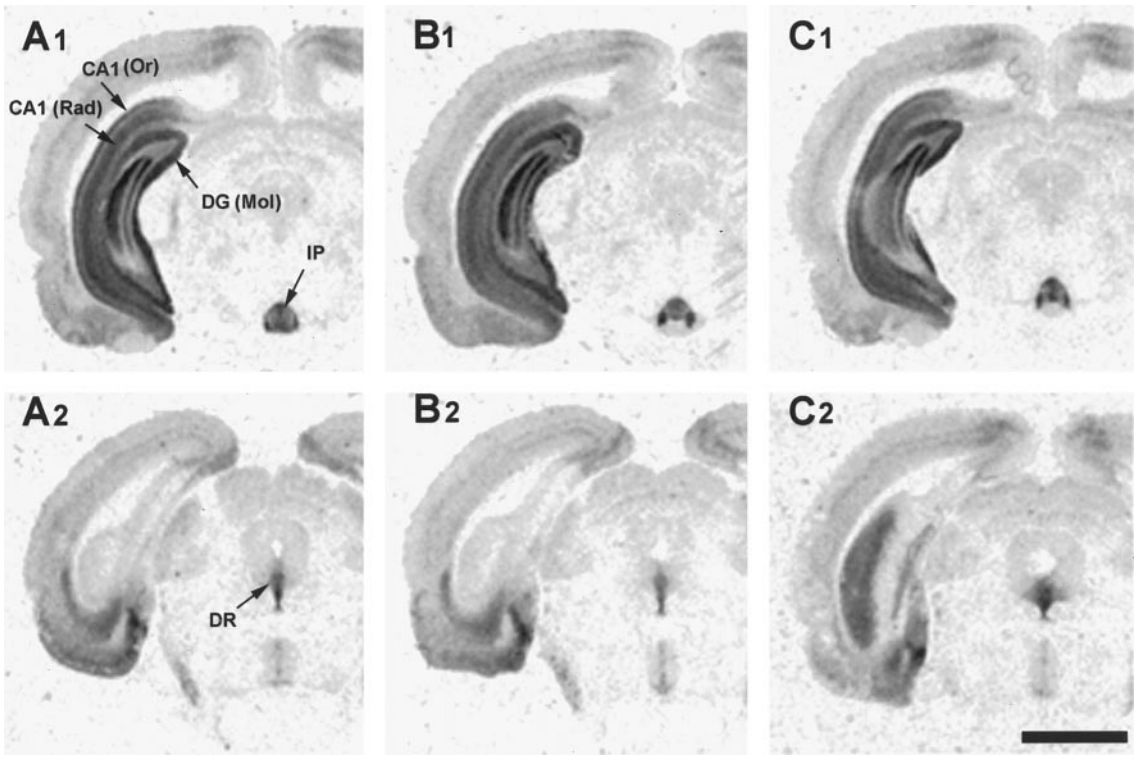

FIG. 6. Coronal sections show the autoradiographic labeling of 5-HT_{1A} receptors by [^{3}H]WAY-100635 at two different levels in brains of rats treated for 2 weeks with (**A**) vehicle, (**B**) alnespirone (3 mg/kg/day), or (**C**) 8-OH-DPAT (0.3 mg/kg/day). CA1 (Or) and CA1 (Rad), strata oriens and radiatum, respectively, of the CA1 hippocampal subfield; DG (Mol), dentate gyrus, stratum moleculare; IP, interpeduncular nucleus; DR, DRN. Note the decreased receptor density in the DRN of the rat treated with alnespirone (B2) compared with the control rat (A2). Bar = 3 mm.

results. Using electrophysiological paradigms, Blier and de Montigny (1987) found a decreased responsiveness of somatodendritic 5-HT_{1A} autoreceptors to the microiontophoretic application of 5-HT, 8-OH-DPAT, or gepirone after a 2-week treatment with the latter agent using minipumps. No such effect was observed when the challenge doses of 8-OH-DPAT or gepirone were systemically administered. Moreover, Schechter et al. (1990) found no change in 5-HT_{1A} pre- and postsynaptic receptor density but a reduced sensitivity of somatodendritic receptors as assessed electrophysiologically in vitro after a 2-week administration of ipsapirone. However, to our knowledge, no single study has so far demonstrated a down-regulation or desensitization (as measured electrophysiologically) of somatodendritic 5-HT_{1A} receptors after treatment with 8-OH-DPAT for similar intervals.

Microdialysis studies are also controversial. Sharp et al. (1993) failed to find a reduced sensitivity of 5-HT_{1A} receptors controlling 5-HT release after 2-week treatments with 8-OH-DPAT (1 mg/kg s.c. twice daily), buspirone (0.5 and 5 mg/kg s.c. twice daily), or ipsapirone (5 mg/kg s.c. twice daily). In contrast, Kreiss and Lucki (1992, 1997) reported desensitization of DRN 5-HT_{1A} receptors after 7 and 14 days of treatment with 1

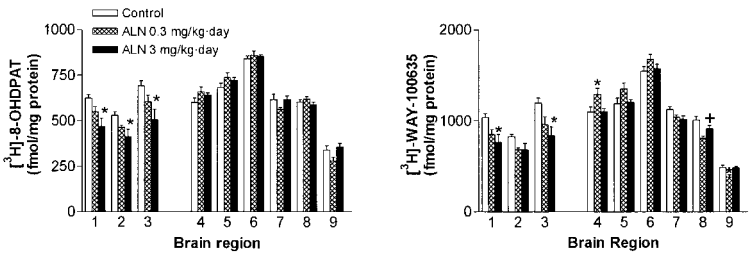

FIG. 7. 5-HT_{1A} receptor binding density in brains of rats treated with alnespirone (ALN) as assessed by quantitative autoradiography with [^{3}H]8-OH-DPAT (**left panel**) and [^{3}H]WAY-100635 (**right panel**). The numbers in the abscissa correspond to the following areas: 1, DRN (whole nucleus); 2, DRN (dorsal part); 3, DRN (ventral part); 4, CA1 (stratum oriens); 5, CA1 (stratum radiatum); 6, dentate gyrus (stratum moleculare); 7, subiculum; 8, interpeduncular nucleus; and 9, cingulate cortex. One-way ANOVA revealed the existence of a significant treatment factor for ALN in the DRN using both ligands: *significant difference versus controls; $^+$significant difference versus the lower dose (by post hoc Tukey's test).

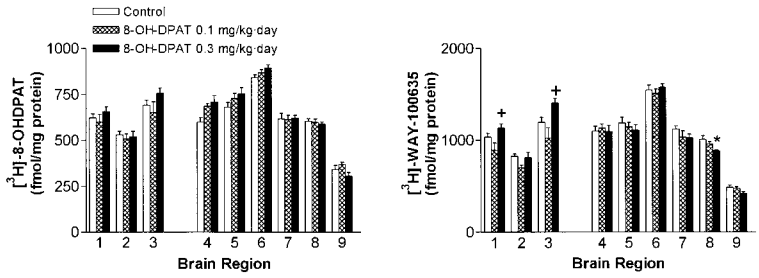

FIG. 8. 5-HT$_{1A}$ receptor binding density in brains of rats treated with 8-OH-DPAT as assessed by quantitative autoradiography with [^{3}H]8-OH-DPAT (**left panel**) and [^{3}H]WAY-100635 (**right panel**). The numbers in the abscissa correspond to the following areas: 1, DRN (whole nucleus); 2, DRN (dorsal part); 3, DRN (ventral part); 4, CA1 (stratum oriens); 5, CA1 (stratum radiatum); 6, dentate gyrus (stratum moleculare); 7, subiculum; 8, interpeduncular nucleus; and 9, cingulate cortex. *Significant difference versus controls; $^+$Significant difference versus the lower dose (by post hoc Tukey's test).

mg/kg/day s.c. 8-OH-DPAT. Both studies were conducted in chloral hydrate-anesthetized rats, and Kreiss and Lucki (1992, 1997) used a larger challenge dose [1 mg/kg/ vs. 0.025 mg/kg s.c. in the study by Sharp et al. (1993)]. The use of 1 mg/kg 8-OH-DPAT seems inappropriate to test the functional state of 5-HT$_{1A}$ receptors. In our hands, the ED$_{50}$ of 8-OH-DPAT to reduce 5-HT release in the rat brain is 0.017 mg/kg s.c. (Casanovas et al., 1997). At 1 mg/kg s.c. 8-OH-DPAT may interact with other neuronal systems, e.g., the 5-HT transporter, for which it displays a moderate affinity (Schoemaker and Langer, 1986) or with the 5-HT$_7$ receptor (Ruat et al., 1993). Furthermore, in the studies by Kreiss and Lucki (1992 1997), the reduction of striatal 5-HT release elicited by 1 mg/kg 8-OH-DPAT was severalfold less marked and had a slower time course than that observed in other studies, even using doses 10–60 times lower (Sharp et al., 1993; Casanovas et al., 1997). This discrepancy may perhaps be accounted for by methodological differences, such as a different analytical method or a different contribution of nonneuronal 5-HT pools, e.g., platelets, to dialysate 5-HT. Indeed, the marked reduction of 5-HT release observed after low 8-OH-DPAT doses in the present and previous studies from this laboratory (Adell et al., 1993; Casanovas et al., 1997) supports a neuronal origin of the 5-HT detected in DRN and forebrain dialysates in our experimental conditions.

The present study indicates that a 2-week treatment with alnespirone attenuates the ability of a further challenge dose to reduce 5-HT release in the somatodendritic and terminal regions of DRN neurons. This effect is likely accounted for by the parallel dose-dependent reduction of the density of somatodendritic 5-HT$_{1A}$ receptors. Contrary to what could be expected from the full agonistic character of 8-OH-DPAT, the continuous treatment with this agent did not reduce the density of somatodendritic or postsynaptic 5-HT$_{1A}$ receptors after treatment for the same interval. The inability of 8-OH-DPAT to desensitize/down-regulate 5-HT$_{1A}$ receptors cannot be explained by pharmacokinetic reasons, i.e., both drugs were given by minipumps to ensure a sustained occupation of 5-HT$_{1A}$ receptors, or by an insufficient dose of 8-OH-DPAT. The ED$_{50}$ values of alnespirone and 8-OH-DPAT to reduce the 5-HT release (peak effect) are 0.19 and 0.017 mg/kg s.c, respectively (Casanovas et al., 1997). Maximal reductions of 5-HT release in dorsal striatum or frontal cortex after single treatment were achieved with 3 mg/kg s.c. alnespirone and 0.3 mg/kg s.c. 8-OH-DPAT. These doses, which were the maximal daily doses used in the present study, represent 16 times the ED$_{50}$ for alnespirone and 18 times the ED$_{50}$ for 8-OH-DPAT. Therefore, they should be considered equivalent in terms of the activation of

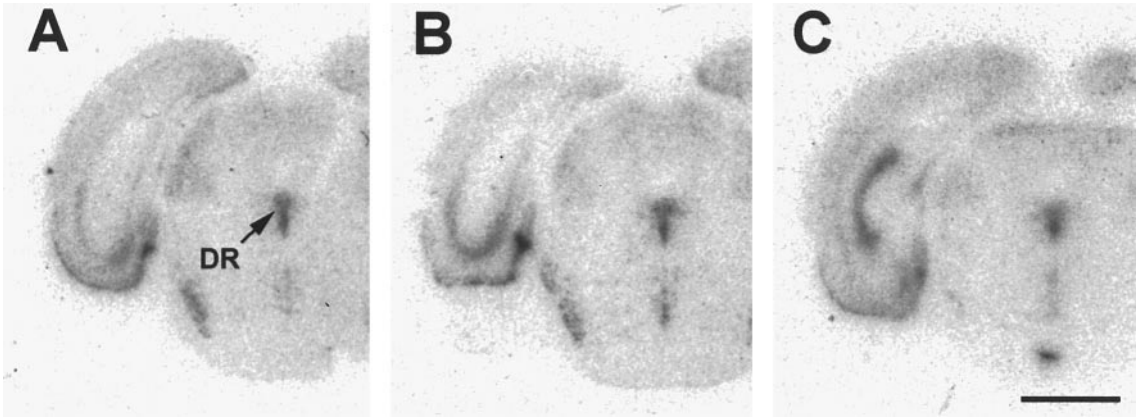

FIG. 9. Coronal sections show the distribution of 5-HT$_{1A}$ receptor mRNA in the DRN (DR) of rats treated for 2 weeks with (**A**) vehicle, (**B**) alnespirone (3 mg/kg/day), or (**C**) 8-OH-DPAT (0.3 mg/kg/day). Sections are from the same animals shown in Figs. 5 and 6. Bar = 3 mm.

5-HT_{1A} receptors controlling 5-HT release. Because no data are available on the receptor reserve for alnespirone in the DRN, it is unclear whether the above doses are also equivalent in terms of receptor occupancy. In any case, it appears that the daily doses of 8-OH-DPAT used were sufficient to activate somatodendritic 5-HT_{1A} receptors as treated rats displayed a dose-dependent weight gain increase, an effect that may be attributed to the hyperphagic action of 8-OH-DPAT (Hutson et al., 1986).

The down-regulation of 5-HT_{1A} receptor density observed in the DRN after chronic alnespirone treatment was not paralleled by a decrease of 5-HT_{1A} receptor mRNA abundance in this nucleus. This fact suggests that the regulatory events leading to long-term decreased numbers of receptors do not involve reduced levels of gene transcription, but are more likely to take place at the level of the receptor protein synthesis and/or degradation. Although in several neurotransmitter systems short-term agonist-induced down-regulation of receptor number is often accompanied by decreased levels of receptor mRNA (for review, see Collins et al., 1991), a situation similar to the one found in the present study has also been reported. Thus, agonist exposure for 72 h of choroid plexus epithelial cells results in loss of 5-HT_{2C} receptor binding sites without alterations in the levels of 5-HT_{2C} receptor mRNA (Barker and Sanders-Bush, 1993). We are not aware, however, of any study addressing the effect of prolonged (weeks) 5-HT agonist treatment on the levels of both receptor number and mRNA abundance.

The unchanged density/efficacy of postsynaptic 5-HT_{1A} receptors after sustained alnespirone and 8-OH-DPAT administration is also common to other 5-HT_{1A} agonists (Blier and de Montigny, 1987; Schechter et al., 1990; Fanelli and McMonagle-Strucko, 1992; for review, see De Vry, 1995) and may be ascribed to the different characteristics of pre- and postsynaptic 5-HT_{1A} receptors. Thus, they appear to be identical from molecular and biochemical points of view (Albert et al., 1990; Radja et al., 1992). However, whereas postsynaptic hippocampal 5-HT_{1A} receptors are negatively coupled to adenylyl cyclase (De Vivo and Maayani, 1986) and to a potassium channel via pertussis toxin-sensitive G proteins (Andrade et al., 1986), only the latter effector system appears to be present in DRN (Clarke et al., 1996). Differences in the ability to evoke an agonistic response have also been reported, with selective 5-HT_{1A} agonists displaying a higher agonistic character at DRN receptors compared with hippocampus (Sprouse and Aghajanian, 1988). Finally, the larger receptor reserve in the DRN (Meller et al., 1990; Cox et al., 1993) may also be accountable. Hence, the sustained interaction of selective agonists with 5-HT_{1A} receptors may result in a differential regulation of the protein at pre- and postsynaptic sites.

However, despite these differences, it seemed surprising that alnespirone down-regulated 5-HT_{1A} receptors in the DRN, whereas 8-OH-DPAT did not. In a striking similarity with the present results, Bohmaker et al. (1993) reported the lack of desensitization of the 5-HT_{1A} receptors controlling 5-HT synthesis after a 2-week treatment with 8-OH-DPAT (either twice daily or with minipumps; 0.4 and 4 mg/kg/day). In contrast, the treatment with gepirone or ipsapirone reduced the ability of a further challenge dose to reduce 5-HT synthesis. The interpretation of these differences is difficult, because all these agents behave as full agonists at somatodendritic 5-HT_{1A} receptors and suppress the serotonergic cell firing rate (Sprouse and Aghajanian, 1988; Kidd et al., 1993). Nonetheless, behavioral differences between selective 5-HT_{1A} agonists have been reported (Scott et al., 1994). Bohmaker et al. (1993) speculated that the differences between 8-OH-DPAT and azapirones after chronic treatment might be due to a complex control of serotonergic neurons by pre-and postsynaptic 5-HT_{1A} receptors. Evidence for this possibility comes from electrophysiological (Blier and de Montigny, 1987) and neurochemical (Romero et al., 1994) studies. Under this assumption, the effects of a challenge dose of alnespirone on 5-HT release would be mainly due to the activation of somatodendritic receptors, whereas those of 8-OH-DPAT would also involve postsynaptic 5-HT_{1A} receptors because the latter agent is considered to have a greater agonist character at postsynaptic sites (Sprouse and Aghajanian, 1988). However, the data of the present study do not support these arguments (Bohmaker et al., 1993) because no consistent changes in density in postsynaptic areas were observed after 8-OH-DPAT treatment.

In summary, the present data support the proposal that the ability of a challenge dose of alnespirone to reduce 5-HT release is diminished by a 2-week pretreatment. This is most likely accounted for by a reduction of the density of somatodendritic 5-HT_{1A} receptors, as indicated by autoradiographic experiments. The failure of 8-OH-DPAT to desensitize/down-regulate 5-HT_{1A} receptors at doses equivalent in terms of the activation of 5-HT_{1A} receptors controlling 5-HT release is unclear and deserves further investigation.

Acknowledgment: Work supported by grants from the Institut de Recherches Internationales Servier, the Fondo de Investigación Sanitaria (grant 98/0697), and the CICYT (grant SAF96-0336). We acknowledge the skillful technical assistance of Leticia Campa. Thanks are given to the pharmaceutical companies for their kind supply of drugs.

REFERENCES

Adell A. and 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 Schmiedebergs Arch. Pharmacol.* **343,** 237–244.

Adell A., Carceller A., and Artigas F. (1993) In vivo brain dialysis study of the somatodendritic release of serotonin in the raphe nuclei of the rat: effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin. *J. Neurochem.* **60,** 1673–1681.

Adell A., Casanovas J. M., and Artigas F. (1997) Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. *Neuropharmacology* **36,** 735–741.

Albert P. R., Zhou Q. Y., Van Tol H. H. M., Bunzow J. R., and Civelli O. (1990) Cloning, functional expression and messenger RNA tissue distribution of the rat 5-hydroxytryptamine$_{1A}$ receptor gene. *J. Biol. Chem.* **265,** 5825–5832.

Albert P. R., Lembo P., Storring J. M., Charest A., and Saucier C. (1996) The 5-HT$_{1A}$ receptor: signaling, desensitization, and gene transcription. *Neuropsychopharmacology* **14,** 19–25.

Andrade R., Malenka R. C., and Nicoll R. A. (1986) A G protein couples serotonin and GABA$_B$ receptors to the same channel in hippocampus. *Science* **234,** 1261–1265.

Artigas F., Romero L., de Montigny C., and Blier P. (1996) Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT$_{1A}$ antagonists. *Trends Neurosci.* **19,** 378–383.

Barker E. L. and Sanders-Bush E. (1993) 5-Hydroxytrypamine$_{1C}$ receptor density and mRNA levels in choroid plexus epithelial cells after treatment with mianserin and (−)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane. *Mol. Pharmacol.* **44,** 725–730.

Barrett J. E., Gamble E. H., Zhang L., and Guardiola-Lemaitre B. (1994) Anticonflict and discriminative stimulus effects in the pigeon of a new methoxy-chroman 5-HT(1A) agonist, (+)S 20244 and its enantiomers (+)S 20499 and (−)S 20500. *Psychopharmacology (Berl.)* **116,** 73–78.

Bianchi G., Caccia S., Della Vedova F., and Garattini S. (1988) The alpha 2-adrenoceptor antagonist activity of ipsapirone and gepirone is mediated by their common metabolite 1-(2-pyrimidinyl)-piperazine (PmP). *Eur. J. Pharmacol.* **151,** 365–371.

Blier P. and de Montigny C. (1987) Modification of 5-HT neuron properties by sustained administration of the 5-HT$_{1A}$ agonist gepirone: electrophysiological studies in the rat brain. *Synapse* **1,** 470–480.

Blier P. and de Montigny C. (1994) Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.* **15,** 220–226.

Bohmaker K., Eison A. S., Yocca F. D., and Meller E. (1993) Comparative effects of chronic 8-OH-DPAT, gepirone and its ipsapirone treatment on the sensitivity of somatodendritic 5-HT$_{1A}$ autoreceptors. *Neuropharmacology* **32,** 527–534.

Casanovas J. M. and Artigas F. (1996) Differential effects of ipsapirone on 5-HT release in the dorsal and median raphe neuronal pathways. *J. Neurochem.* **67,** 1945–1952.

Casanovas J. M., Lésourd M., and Artigas F. (1997) The effect of the selective 5-HT$_{1A}$ agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. *Br. J. Pharmacol.* **122,** 733–741.

Clarke W. P., Yocca F. D., and Maayani S. (1996) Lack of 5-hydroxytryptamine (1A)-mediated inhibition of adenylyl cyclase in dorsal raphe of male and female rats. *J. Pharmacol. Exp. Ther.* **277,** 1259–1266.

Collins S., Caron M. G., and Lefkowitz R. L. (1991) Regulation of adrenergic receptor responsiveness through modulation of receptor gene expression. *Annu. Rev. Physiol.* **53,** 497–508.

Cox R. F., Meller E., and Waszczak B. L. (1993) Electrophysiological evidence for a large receptor reserve for inhibition of dorsal raphe neuronal firing by 5-HT(1A) agonists. *Synapse* **14,** 297–304.

De Vivo M. and Maayani S. (1986) Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. *J. Pharmacol. Exp. Ther.* **238,** 248–253.

De Vry J. (1995) 5-HT$_{1A}$ receptor agonists: recent developments and controversial issues. *Psychopharmacology (Berl.)* **121,** 1–26.

Dugast C., Soulière F., Schmitt P., Casanovas J. M., Fattaccini C. M., Mocaër E., Lésourd M., Renaud B., Artigas F., Hamon M., and Chouvet G. (1998) Is the potent 5-HT$_{1A}$ receptor agonist, alnespirone (S-20499), affecting dopaminergic systems in the rat brain? *Eur. J. Pharmacol.* **350,** 171–180.

Fanelli R. J. and McMonagle-Strucko K. (1992) Alteration of 5-HT$_{1A}$ receptor binding sites following chronic treatment with ipsapirone measured by quantitative autoradiography. *Synapse* **12,** 75–81.

Hjorth S. and Auerbach S. B. (1994) Lack of 5-HT(1A) autoreceptor desensitization following chronic citalopram treatment, as determined by *in vivo* microdialysis. *Neuropharmacology* **33,** 331–334.

Hutson P. H., Dourish C. T., and Curzon G. (1986) Neurochemical and behavioural evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors. *Eur. J. Pharmacol.* **129,** 347–352.

Invernizzi R., Bramante M., and Samanin R. (1995) Extracellular concentrations of serotonin in the dorsal hippocampus after acute and chronic treatment with citalopram. *Brain Res.* **696,** 62–66.

Kidd E. J., Hajdahmane S., Jolas T., Lanfumey L., Fattaccini C. M., Guardiola-Lemaitre B., Gozlan H., and Hamon M. (1993) New methoxy-chroman derivatives, 4(*N*-(5-methoxy-chroman-3-yl) *N*-propylamino)butyl-8-azaspiro-(4,5)-decane-7,9-dione [(+/−)S 20244] and its enantiomers, (+)S 20499 and (−)S 20500, with potent agonist properties at central 5-hydroxytryptamine$_{1A}$ receptors. *J. Pharmacol. Exp. Ther.* **264,** 863–872.

Kreiss D. S. and Lucki I. (1992) Densensitization of 5-HT$_{1A}$ autoreceptors by chronic administration of 8-OH-DPAT. *Neuropharmacology* **31,** 1073–1076.

Kreiss D. S. and Lucki I. (1997) Chronic administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT differentially desensitizes 5-HT$_{1A}$ autoreceptors of the dorsal and median raphe nucleus. *Synapse* **25,** 107–116.

Meller E., Goldstein M., and Bohmaker K. (1990) Receptor reserve for 5-hydroxytryptamine-mediated inhibition of serotonin synthesis: a possible relationship to anxiolytic properties of 5-hydroxytryptamine agonists. *Mol. Pharmacol.* **37,** 231–237.

Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates.* Academic Press, Sydney.

Pecknold J. C. (1994) Serotonin 5-HT$_{1A}$ agonists—a comparative review. *CNS Drugs* **2,** 234–251.

Pérez V., Gilaberte I., Faries D., Alvarez E., and Artigas F. (1997) Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. *Lancet* **349,** 1594–1597.

Pompeiano M., Palacios J. M., and Mengod G. (1992) Distribution and cellular localization of mRNA coding for 5-HT$_{1A}$ receptor in the rat brain: correlation with receptor binding. *J. Neurosci.* **12,** 440–453.

Porsolt R. D., Lenegre A., Caignard D. H., Pfeiffer B., Mocaer E., and Guardiola-Lemaitre B. (1992). Psychopharmacological profile of a new chroman derivative with 5-HT$_{1A}$ agonist properties S 20499 (+). *Drug Dev. Res.* **27,** 389–402.

Radja F., Daval G., Hamon M., and Vergé D. (1992) Pharmacological and physicochemical properties of pre- versus postsynaptic 5-hydroxytryptamine$_{1A}$ receptor binding sites in the rat brain: a quantitative autoradiographic study. *J. Neurochem.* **58,** 1338–1346.

Romero L. and Artigas F. (1997) Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT$_{1A}$ receptor antagonists in the dorsal raphe pathway. Role of somatodendritic autoreceptors. *J. Neurochem.* **68,** 2593–2603.

Romero L., Celada P., and 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.

Ruat M., Traiffort E., Leurs R., Tardivel-Lacombe J., Diaz J., Arrang J. M., and Schwartz J. C. (1993) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT$_7$) activating cAMP formation. *Proc. Natl. Acad. Sci. USA* **90,** 8547–8551.

Schechter L. E., Bolanos F. J., Gozlan H., Lanfumey L., Haj-Dahmane S., Laporte A. M., Fattaccini C. M., and Hamon M. (1990) Alterations of central serotoninergic and dopaminergic neurotransmission in rats chronically treated with ipsapirone: biochemical and electrophysiological studies. *J. Pharmacol. Exp. Ther.* **255,** 1335–1347.

Schoemaker H. and Langer S. Z. (1986) [^{3}H]8-OH-DPAT labels the serotonin transporter in the rat striatum. *Eur. J. Pharmacol.* **124,** 371–373.

Scott P. A., Chou J. M., Tang H., and Frazer A. (1994) Differential induction of 5-HT_{1A} mediated responses *in vivo* by three chemically dissimilar 5-HT_{1A} agonists. *J. Pharmacol. Exp. Ther.* **270,** 198–208.

Sharp T., Bramwell S. R., and Grahame-Smith D. G. (1989) 5-HT_1 agonists reduce 5-hydroxytryptamine release in rat hippocampus *in vivo* as determined by brain microdialysis. *Br. J. Pharmacol.* **96,** 283–290.

Sharp T., McQuade R., Bramwell S., and Hjorth S. (1993) Effect of acute and repeated administration of 5-HT_{1A} receptor agonists on 5-HT release in rat brain in vivo. *Naunyn Schmiedebergs Arch. Pharmacol.* **348,** 339–346.

Sprouse J. S. and Aghajanian G. K. (1986) (−)-Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT_{1A} selective agonists. *Eur. J. Pharmacol.* **128,** 295–298.

Sprouse J. S. and Aghajanian G. K. (1988) Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology* **27,** 707–715.

Welner S. A., de Montigny C., Descoches J., Desjardins P., and Suranyi-Cadotte B. E. (1989) Autoradiographic quantification of serotonin$_{1A}$ receptors in rat brain following antidepressant drug treatment. *Synapse* **4,** 347–352.