Cerebral Cortex July 2012;22:1487–1497
doi:10.1093/cercor/bhr220
Advance Access publication September 5, 2011

5-HT_{1A} Receptor Agonists Enhance Pyramidal Cell Firing in Prefrontal Cortex Through a Preferential Action on GABA Interneurons

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5-HT_{1A} receptors (5-HT1AR) are expressed by pyramidal and γ-aminobutyric acidergic (GABAergic) neurons in medial prefrontal cortex (mPFC). Endogenous serotonin inhibits mPFC pyramidal neurons via 5-HT1AR while 5-HT1AR agonists, given systemically, paradoxically excite ventral tegmental area-projecting pyramidal neurons. This enhances mesocortical dopamine function, a process involved in the superior efficacy of atypical antipsychotic drugs on negative and cognitive symptoms of schizophrenia. Moreover, the 5-HT1AR-induced increase of pyramidal discharge may also contribute to the maintenance of activity patterns required for working memory, impaired in schizophrenia. Given the importance of these processes, we examined the neurobiological basis of pyramidal activation through 5-HT1AR using the prototypical agent 8-OH-DPAT. (±)8-OH-DPAT (7.5 μg/kg i.v.) increased discharge rate and *c-fos* expression in rat mPFC pyramidal neurons. Local blockade of $GABA_A$ inputs with gabazine (SR-95531) avoided (±)8-OH-DPAT-induced excitations of pyramidal neurons. Moreover, (±)8-OH-DPAT administration reduced the discharge rate of mPFC fast-spiking GABAergic interneurons at doses exciting pyramidal neurons. Activation of other 5-HT1AR subpopulations (raphe nuclei or hippocampus) does not appear to contribute to pyramidal excitations. Overall, the present data suggest a preferential action of (±)8-OH-DPAT on 5-HT1AR in GABAergic interneurons. This results in pyramidal disinhibition and subsequent downstream excitations of subcortical structures reciprocally connected with PFC, such as midbrain dopaminergic neurons.

Keywords: antipsychotic, dopamine, prefrontal cortex, schizophrenia, serotonergic1A receptor, ventral tegmental area

Introduction

Serotonin 5-HT_{1A} receptors (5-HT1AR) are widely expressed in mammalian brain. They are located on serotonin (5-HT)-containing neurons, where they play an autoreceptor role and postsynaptically in pyramidal and γ-aminobutyric acidergic (GABAergic) neurons of the cortex and limbic system (Pazos and Palacios 1985; Kia et al. 1996; Martinez et al. 2001; Santana et al. 2004). 5-HT inhibits neuronal discharge via pre- and postsynaptic 5-HT1AR (Andrade and Nicoll 1987; Blier and de Montigny 1987; Innis and Aghajanian 1987; Araneda and Andrade 1991; Puig et al. 2005). However, systemic administration of selective 5-HT1AR agonists inhibits 5-HT neuron activity but paradoxically increases the discharge rate of pyramidal neurons and *c-fos* expression in prefrontal cortex (PFC) (Borsini et al. 1995; Hajós et al. 1999; Díaz-Mataix et al. 2006; Lladó-Pelfort et al. 2010).

The relationship between PFC 5-HT and cognitive function is controversial. For instance, an overall increase of 5-HT release disrupts working memory (Luciana et al. 1998), although conflicting results have been reported regarding the role of 5-HT1A-R and 5-HT2A-R, probably due to the different experimental paradigms used (Williams et al. 2002; Carter et al. 2005; Fernández-Perez et al. 2005; Wingen et al. 2007). Hence, low doses of 5-HT1A-R agonists display pro-cognitive effects (Meneses and Perez-Garcia 2007), possibly due to the maintenance of activity patterns by PFC pyramidal neurons in working memory tasks (Miller and Cohen 2001; Fuster 2008). Thus, 5-HT1AR agonists improved visuospatial attention, decreased impulsivity in the 5-choice serial reaction time task (Winstanley et al. 2003) and reversed the behavioral and cognitive deficits evoked by NMDA receptor antagonists (Bubeníková-Valesová et al. 2007; Depoortère et al. 2010). Likewise, 5-HT1A-R polymorphisms have been related to attention and working memory (Beste et al. 2010).

The above observations may be relevant for the treatment of negative symptoms and cognitive deficits in schizophrenia, a devastating illness affecting ~1% of the population worldwide. Classical neuroleptics block dopamine D2 receptors and display severe motor side effects. Second generation (atypical) antipsychotic drugs (APDs)—and particularly clozapine, the prototypical APD—show lower D2 receptor occupancy and are superior to classical drugs in all symptom classes (Harvey and Keefe 2001; Woodward et al. 2005; Keefe et al. 2006; Kern et al. 2006; Sumiyoshi et al. 2006; Leucht et al. 2009; see, however, Davidson et al. 2009). This clinical superiority appears to result from the preferential targeting of several 5-HT receptors and lower blockade of dopamine D2 receptors (Bymaster et al. 1996; Arnt and Skarsfeldt 1998). In particular, APD (but not classical antipsychotics) increase cortical DA function by activating 5-HT1AR in PFC (Rollema et al. 1997; Ichikawa et al. 2001; Díaz-Mataix et al. 2005; Bortolozzi et al. 2007, 2010). This effect appears to underlie the therapeutic superiority of some APDs on nonpsychotic symptoms of schizophrenia (Sumiyoshi, Matsui, Nohara, et al. 2001; Sumiyoshi, Matsui, Yamashita, et al. 2001; Sumiyoshi et al. 2007; for review, see Meltzer and Sumiyoshi 2008 and Newman-Tancredi 2010). Likewise, tandospirone and buspirone (5-HT1AR agonists) improved cognitive deficits in schizophrenic patients (Sovner and Parnell-Sovner 1989; Sirota et al. 2001; Sumiyoshi, Matsui, Nohara, et al. 2001; Sumiyoshi, Matsui, Yamashita, et al. 2001; Sumiyoshi et al. 2007). As a result, some last generation APDs, such as aripiprazole, SLV313, or bifeprunox incorporate, among other pharmacological activities, agonist activity at 5-HT1AR (Newman-Tancredi 2010).

Given the potential therapeutic usefulness of 5-HT1AR activation in PFC, we undertook the present study to examine the cellular and network mechanisms underlying the increase in PFC pyramidal activity induced by the prototypical 5-HT1AR agonist 8-OH-DPAT.

Materials and Methods

Animals and Treatments

Male albino Wistar rats weighting 230–320 g were used (Iffa Credo, Lyon, France). Animal care followed the European Union regulations (O.J. of E.C. L358/1 18 December 1986) and was approved by the Institutional Animal Care and Use Committee. All experiments were done in chloral hydrate anesthetized animals (initial dose 400 mg/kg intraperitoneal [i.p.] followed by a maintenance dose of ~1 mg/kg/min i.p. using a perfusion pump). Stereotaxic coordinates (in mm) were taken from bregma according to the atlas of Paxinos and Watson (1998).

WAY-100635 (5-HT1AR antagonist), (±)8-OH-DPAT (5-HT1AR agonist, referred onwards as 8-OH-DPAT), R(+)-8-OH-DPAT (active enantiomer, 5-HT1AR agonist), gabazine (SR95531, $GABA_A$ antagonist), and 4-chloro-DL-phenylalanine methyl ester hydrochloride (pCPA, tryptophan hydroxylase inhibitor) were from Sigma-Aldrich (St. Louis, MO). Citalopram (serotonin reuptake inhibitor) was from H. Lundbeck A/S (Copenhagen-Valby, Denmark). Concentrated stock solutions of WAY100635, 8-OH-DPAT, and R(+)8-OH-DPAT were prepared, and aliquots were stored at −20 °C. Working solutions were prepared daily by dilution in saline and were injected i.v. (up to 1 mL/kg) through the femoral vein. Gabazine (20 mM dissolved in 0.2 M NaCl) was prepared and stored at −20 °C until the day of the experiment. It was used to fill the recording micropipette in specific experimental groups. *p*CPA was prepared daily at 150 mg/mL and injected at 300 mg/kg i.p to deplete rats of 5-HT in one experimental group. Citalopram was prepared at 10 μM in artificial CSF (aCSF) and used as the perfusion media in the microdialysis experiments.

Electrophysiological Recordings

We performed single-unit extracellular recordings in anesthetized rats to analyze the responses of layer V mPFC neurons (AP +3.2 to +3.4, L −0.5 to −1, DV −1 to −4 mm) to the systemic administration of 8-OH-DPAT. Recordings were essentially performed with glass electrodes, as previously described (Puig et al. 2005; Kargieman et al. 2007; Lladó-Pelfort et al. 2010). Except in 2 experimental groups (see below), recording electrodes were filled with saline 2 M (impedances 6–12 MΩ). In some experiments, Pontamine Sky Blue was added for the identification of the recording site. 8-OH-DPAT was administered i.v. at 0.75–7.5 μg/kg (free base). In some experiments (see Results), rats received additional doses of 8-OH-DPAT (up to 60 μg/kg i.v.). The specificity of the responses induced by 8-OH-DPAT was assessed by the subsequent administration of the selective 5-HT1AR antagonist WAY100635 at 50–100 μg/kg i.v. Recordings were made in the following experimental groups.

Control and R(+)-8-OH-DPAT Experimental Groups

We recorded mPFC pyramidal neurons projecting to the ventral tegmental area (VTA), as identified by antidromic stimulation (0.4–1.5 mA, 0.2 ms, 0.9 Hz) from the VTA (AP −5.8, L −0.4, DV −8 mm), collision test (Fuller and Schlag 1976), and histological verification of the stimulation sites. R(+)8-OH-DPAT was administered at 0.375–30 μg/kg in the R(+)-8-OH-DPAT group.

Gabazine Group

Recording electrodes were filled with the selective $GABA_A$ antagonist gabazine (20 mM) dissolved in 0.2 M NaCl. This was preferred to the classical $GABA_A$ antagonist bicuculline due to the nonselective action of the latter agent (Debarbieux et al. 1998; Stocker et al. 1999). The tips of the electrodes were broken to a final resistance of 9–15 MΩ (electrode tip 5–7 μm diameter). Gabazine leaked from the recording electrode by passive diffusion to reach the recorded neuron, as shown previously for bicuculline (Steward et al. 1990; Tepper et al. 1995). mPFC pyramidal neurons were identified as above.

GABAergic Interneurons

We recorded the activity of mPFC fast-spiking putative GABAergic interneurons (AP +3.2 to +3.4, L −0.5 to −1, DV −1 to −4 mm) as identified by electrophysiological characteristics: 1) duration of the depolarization phase of the action potential (average of 10 spikes) and 2) basal discharge rate (McCormick et al. 1985; Connors and Gutnick 1990; Wilson et al. 1994; Tierney et al. 2004).

pCPA Group

To examine whether pyramidal neuron excitation in mPFC was due to the removal of an inhibitory tone in mPFC secondary to the activation of raphe $5\text{-}HT_{1A}$ autoreceptors by 8-OH-DPAT, a group of rats was subjected to 5-HT depletion with the tryptophan hydroxylase inhibitor *p*CPA prior to recording experiments. Recordings of mPFC pyramidal neurons projecting to the VTA (as above) were performed 2–4 days after pCPA (time of maximal inhibition of 5-HT synthesis after pCPA treatment; Cortés et al. 1993). 5-HT depletion was assessed by high-performance liquid chromatography (HPLC) of PFC homogenates, as described (Adell et al. 1989).

Hippocampal Neurons

We recorded the activity of pyramidal neurons in the CA1 and subiculum (AP −6.3 to −7, L −4, DV 15° −4 to −7 mm) projecting to mPFC. They were identified by antidromic stimulation (1 mA, 0.5 Hz, 0.2 ms) from the mPFC (AP +3, L −0.8, DV −3.5 mm).

In Situ Hybridization Histochemistry

The analysis of neuronal activity in PFC evoked by 8-OH-DPAT was also assessed by the expression of *c-fos* mRNA using double in situ hybridization, as described previously (Kargieman et al. 2007). Two groups of chloral hydrate anesthetized rats were administered i.v. with saline or 8-OH-DPAT (7.5 μg/kg) to mimic exactly the conditions of electrophysiological experiments and were killed 30 min later by anesthetic overdose and decapitation. The brains were rapidly removed, frozen on dry ice, and stored at −20 °C. Brain tissue sections, 14 μm thick, were cut using a microtome-cryostat (Microm HM500 OM, Walldorf, Germany), thaw mounted onto APTS (3-aminopropyltriethoxysilane, Sigma, St Louis, MO)-coated slides and kept at −20 °C until use. The oligodeoxyribonucleotide probes used were as previously described by Kargieman et al. (2007). Probes were synthesized on a 380 Applied Biosystems DNA synthesizer (Applied Biosystems, Foster City, CA).

c-fos oligonucleotide was labeled at its 3′-end with [^{33}P]-dATP (>3000 Ci/mmol; DuPont-NEN, Boston, MA) with terminal deoxynucleotidyltransferase (TdT, Calbiochem, La Jolla, CA) and purified with ProbeQuant G-50 Micro Columns (GE Healthcare UK Limited, Buckinghamshire, UK).

VGluT1 and GAD oligonucleotides were used to identify, respectively, pyramidal and GABAergic neurons and were individually labeled with Dig-11-dUTP (Boehringer Mannheim) using TdT (Roche Diagnostics GmbH, Mannheim, Germany) and purified as above. The protocols for double-label in situ hybridization were based on previously described procedures (Amargós-Bosch et al. 2004; Santana et al. 2004; Kargieman et al. 2007).

Micrography and cellular counting were performed in an Olympus BX51 Stereo Microscope equipped with an Olympus Microscope Digital Camera DP71, with the aid of Visiopharm Integrator System software (Olympus). The figures were prepared for publication using Adobe Photoshop software (Adobe Software, Mountain View, CA).

In Vivo Microdialysis

Microdialysis procedures were conducted essentially as previously described by Amargós-Boch (see updated procedure in Amargós-Bosch et al. 2004). Briefly, anesthetized rats (sodium pentobarbital, 60 mg/kg i.p.) were stereotaxically implanted with concentric microdialysis probes equipped with a Cuprophan membrane (4 mm long) in the mPFC (AP +3.2, L −0.8, DV −6.0 mm). On the following day (~20 h after probe implant), rats were anesthetized with chloral hydrate to mimic

exactly the conditions of electrophysiological recordings. Probes were perfused at 3 μL/min with aCSF containing 10 μM citalopram. After a 100-min stabilization period, dialysate samples were collected every 4 min. 8-OH-DPAT and WAY100635 were injected i.v. at the same doses than in control electrophysiological experiments. Dialysate 5-HT concentration was determined by HPLC with amperometric detection (Hewlett-Packard 1049 detector, set at +0.7 V) as described previously (Amargós-Bosch et al. 2004; Lopez-Gil et al. 2007) with a detection limit of 4 fmol/sample.

Histology

After experimental procedures were completed, animals were killed by an anesthetic overdose. The brains were quickly removed, frozen on dry ice, and kept at –80 °C before being cut in coronal sections (50 μm) with a cryostat. Brain sections were stained with neutral red, to verify the recording and stimulation sites or the correct placement of the probes.

Data and Statistical Analysis

Changes in firing rate were quantified by averaging the values in the third minute after each drug injection. Neurons were considered to be excited or inhibited when drugs induced a ±30% change of the discharge rate (Kargieman et al. 2007). Microdialysis data are given as percentage of basal values, averaged from 10 predrug fractions.

Cell counting was performed manually at the microscope with the help of the analySIS software. Only cellular profiles showing great abundance of both transcripts (dark silver grains for [^{33}P] *c-fos* mRNA over brownish cellular profiles for Dig-vGluT1 mRNA or Dig-GAD mRNA) were considered to coexpress both mRNAs. Cells were counted in 3 adjacent sections of each rat and averaged to obtain individual values. Results are given as mean ± standard error of the mean (SEM) of 3 rats.

The treatment effects were assessed by one-way repeated measures analysis of variance (ANOVA) or Student's *t*-test, as appropriate. Comparisons between groups were assessed by 2 way ANOVA or χ^2. Data are expressed as mean ± SEM. Statistical significance has been set at the 95% confidence level (2 tailed).

Results

Effects of 8-OH-DPAT on Pyramidal Neuron Firing

Systemic 8-OH-DPAT (0.75–7.5 μg/kg i.v.) administration evoked excitatory responses in 84% of the recorded mPFC pyramidal neurons (Fig. 1*A,B*). At 7.5 μg/kg i.v., the drug produced a significant increase in the firing rate of mPFC pyramidal neurons, from 2.0 ± 0.3 spikes/s to 3.4 ± 0.5 spikes/s ($F_{2,36}$ = 5.47, P < 0.01, n = 19; Fig. 1*A*). The effect of 8-OH-DPAT reached 239 ± 54% of basal firing when considering percentages of individual basal values.

In some experiments, higher doses of 8-OH-DPAT (30–60 μg/kg) were administered, which evoked a reduction of pyramidal neuron discharge (Fig. 1*C*) in 60% of the recorded neurons, from 3.4 ± 0.6 spikes/s (7.5 μg/kg i.v.) to 2.2 ± 0.7 spikes/s (60 μg/kg i.v.); ($F_{2,28}$ = 3.91, P < 0.04, n = 15). In the subset of neurons receiving all doses of 8-OH-DPAT, other minority patterns of response were also observed (Fig. 1*D*).

8-OH-DPAT-evoked excitations were significantly reversed by the subsequent administration of the 5-HT1AR antagonist WAY100635 (50 or 100 μg/kg i.v.) ($F_{2,12}$ = 11, P < 0.002, n = 7; see example in Fig. 1*B*).

To examine whether the paradoxical excitations induced by (±)8-OH-DPAT could be due to differential effects of the (+)R and (–)S enantiomers in the racemic (±)8-OH-DPAT mixture, a group of rats was treated with the active enantiomer (+)R-8-OH-DPAT (0.375–30 μg/kg i.v.). This enantiomer also produced significant excitations of mPFC pyramidal neurons at the dose of 3.75 μg/kg i.v. ($F_{2,18}$ = 5.46, P < 0.02, n = 10) to 216 ± 68% basal firing, indicating that the excitatory effect of racemic 8-OH-DPAT is predominantly mediated by the specific action of the (+)R enantiomer.

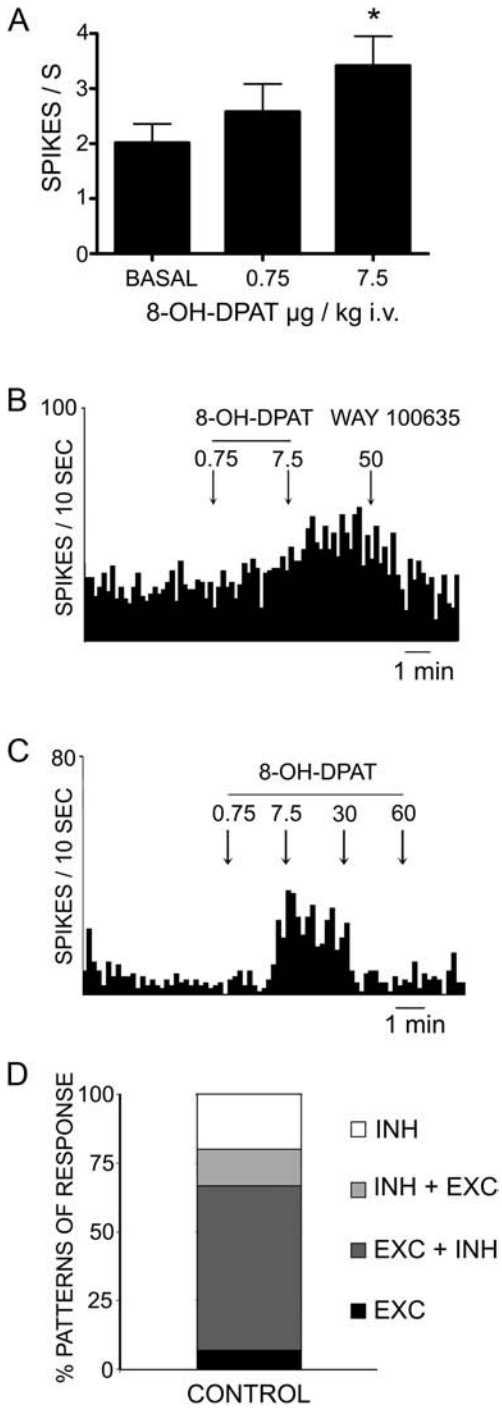

Figure 1. Effect of the administration of 8-OH-DPAT on the firing rate of mPFC pyramidal neurons. (*A*) Bar graph showing the excitation of mPFC pyramidal neurons produced by 8-OH-DPAT. *P < 0.01 versus baseline. (*B*) Representative example of a neuron excited by 8-OH-DPAT and the reversal of the effect by WAY100635. (*C*) Representative example of the most common pattern shown by mPFC pyramidal neurons when increasing doses of 8-OH-DPAT were administered. (*D*) Proportion of response patterns of mPFC pyramidal neurons after the administration of increasing doses of 8-OH-DPAT (n = 15). INH, inhibitions; INH + EXC, inhibitions followed by excitations; EXC + INH, excitations followed by inhibitions; EXC, excitations. Drug doses are given in μg/kg i.v.; injections are shown by vertical arrows.

Role of PFC GABAergic Interneurons

5-HT1AR are located in a substantial proportion of PFC GABAergic neurons in rodent brain (Santana et al. 2004). These neurons make extensive cortical networks via electrical and chemical synapses (Hestrin and Galarreta 2005). We therefore tested the hypothesis that the excitatory response of pyramidal neurons to 8-OH-DPAT could actually be a disinhibitory process produced by the activation of 5-HT1AR on GABAergic neurons and further removal of the $GABA_A$ tone on pyramidal neurons. To this end, we performed 3 sets of experiments. In the first one, to obtain an overall view of the effect of 8-OH-DPAT on pyramidal and GABAergic neurons of mPFC, we examined the changes in *c-fos* expression after the administration of 7.5 μg/kg i.v. 8-OH-DPAT. In a second set of experiments, we assessed the effect of systemic 8-OH-DPAT administration on the discharge rate of pyramidal neurons whose $GABA_A$ inputs were blocked by a local leak of the selective $GABA_A$ antagonist gabazine. Finally, in the last set of experiments, we examined the effect of systemic 8-OH-DPAT administration on the firing rate of fast-spiking putative mPFC GABAergic interneurons.

Induction of c-fos in mPFC by 8-OH-DPAT

The i.v. administration of 7.5 μg/kg 8-OH-DPAT to chloral hydrate anesthetized rats (as performed in electrophysiological experiments) induced a marked increase in the expression of the immediate early gene *c-fos* (Fig. 2). The expression of *c-fos* was notably more marked in all subdivisions of the mPFC where extracellular recordings were made as well as in orbitofrontal cortex. Double in situ hybridization experiments revealed that the increase in *c-fos* expression occurred in glutamatergic (vGluT1-positive) but not GABAergic (GAD-positive) neurons (Fig. 2*B–D*). Cell counting revealed that 7.5 μg/kg i.v. 8-OH-DPAT induced the expression of *c-fos* in nearly

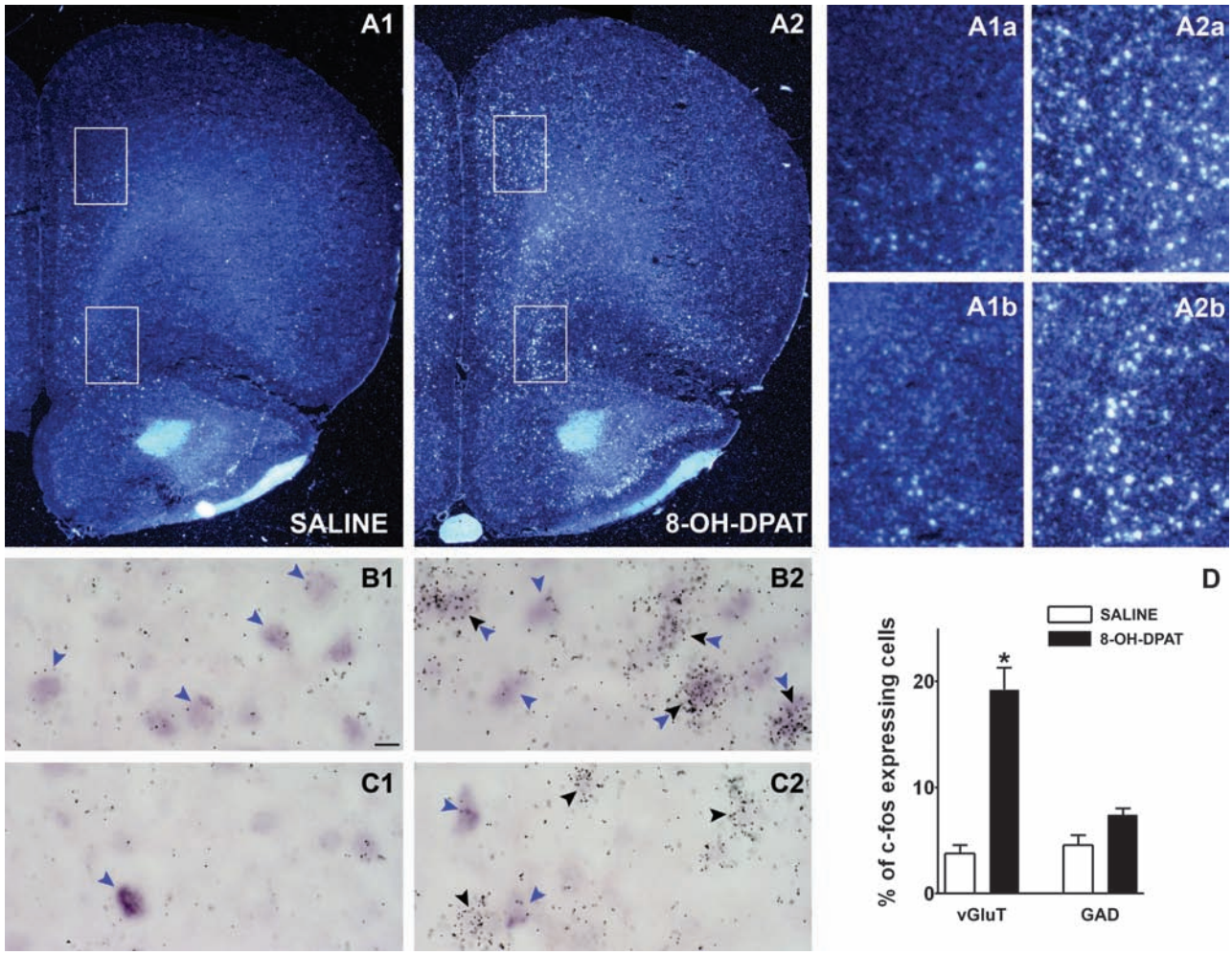

Figure 2. Effects of the i.v. administration of 7.5 μg/kg of 8-OH-DPAT on *c-fos* expression in PFC. (*A*) Macroscopic dark-field images from emulsion-dipped coronal sections at the level of PFC (AP +3.2 mm) from control (*A*1) and treated (*A*2) rats showing the localization of cells expressing *c-fos* mRNA. Note the 8-OH-DPAT-induced expression of *c-fos* in various areas of the PFC, notably in its medial part (magnification at *A*1a, control; *A*2a, treated), where the extracellular recordings were made, and its orbital part (magnification at *A*1b, control; *A*2b, treated). (*B*) High-magnification photomicrographs showing the detection in mPFC (prelimbic area) of *c-fos* mRNA using ^{33}P-labeled oligonucleotides (silver grains) in pyramidal cells, visualized by hybridization with digoxigenin-labeled oligonucleotides complementary to vGluT1 mRNA (dark precipitates). Note the increase in the number of *c-fos* positive cells induced by 8-OH-DPAT treatment. (*B*1: saline; *B*2: 8-OH-DPAT). Blue arrowheads mark some cells positive for vGluT1 mRNA, black arrowheads mark some cells positive for *c-fos* mRNA. Double-labeled cells are marked by both arrowheads. (*C*) High-magnification photomicrographs showing the expression of *c-fos* mRNA (silver grains) in GABAergic cells of the prelimbic PFC, visualized by GAD mRNA (dark precipitate). Note the increase in the number of *c-fos* positive cells not expressing GAD mRNA in the 8-OH-DPAT group (*C*2) (black arrowheads). Note also the presence of *c-fos* in a non-GABAergic (likely pyramidal) neuron adjacent to a GABAergic neuron not expressing *c-fos* in the lower left corner of the image. (*D*) Bar graphs showing drug effects on the percentage of pyramidal (vGluT1-positive) and GABAergic neurons (GAD-positive) expressing *c-fos* mRNA. Bars show mean ± SEM of 3 rats/group. $^*P < 0.001$ versus saline (scale bar: 10 μm).

20% of pyramidal cells in the prelimbic subdivision of PFC without any significant effect on the expression in GABAergic (GAD-positive) neurons (Fig. 2*D*). Two-way ANOVA of *c-fos* data revealed a significant effect of treatment ($F_{1,8}$ = 51.98, P < 0.0001), cellular phenotype ($F_{1,8}$ = 19.08, P < 0.003), and a significant treatment × phenotype interaction ($F_{1,8}$ = 24.18, P < 0.002, n = 3 for each group) with significant post hoc differences between saline and 8-OH-DPAT in the vGluT1-positive neurons and no significant differences between saline and 8-OH-DPAT in GAD-positive neurons.

Effects of Local Blockade of GABAA Inputs on Responses of Pyramidal Neuron to 8-OH-DPAT Administration

The local blockade of $GABA_A$ inputs by gabazine onto the recorded pyramidal neurons increased the basal firing rate of mPFC pyramidal neurons (control 2.0 ± 0.3 spikes/s, n = 30; gabazine 4.4 ± 0.7 spikes/s, n = 16; P < 0.001, Student's t-test) and avoided the excitatory effect of 8-OH-DPAT (control n = 19, gabazine n = 14; gabazine effect $F_{1,31}$ = 5.66, P < 0.03; 8-OH-DPAT effect $F_{2,62}$ = 2.98, P = 0.06; 8-OH-DPAT × gabazine interaction $F_{2,62}$ = 3.29, P < 0.05; Fig. 3*A*). Moreover, the presence of gabazine also induced a change in the response patterns of mPFC pyramidal neurons, being pyramidal neurons less responsive to the drug (control n = 15, gabazine n = 14; χ^2 P < 0.01; Fig. 3*B-F*). Figure 3*B,C* illustrate the individual responses of the recorded neurons to 8-OH-DPAT administration in control conditions and in presence of a gabazine leak.

Effect of 8-OH-DPAT on Fast-Spiking GABAergic Interneurons

Fast-spiking putative mPFC GABAergic interneurons were identified by their electrophysiological characteristics (see Materials and Methods). GABAergic interneurons showed shorter action potentials than pyramidal neurons, as described (Tierney et al. 2004) (0.39 ± 0.03 ms vs. 0.78 ± 0.05 ms, n = 20 and 30, respectively; Student's t-test P < 0.0001; Fig. 4*A*) and higher basal firing rates than pyramidal neurons (9.8 ± 1.6 spikes/s vs. 2.0 ± 0.3 spikes/s, n = 20 and 30, respectively; Student's t-test P < 0.0001; Fig. 4*B*). Despite some overlap in one or other variable, both neuronal subsets were grouped in 2 clearly different clusters (Fig. 4*C*).

8-OH-DPAT reduced the firing rate of fast-spiking mPFC GABAergic interneurons at 7.5 μg/kg i.v. ($F_{2,38}$ = 4.78, P < 0.02, n = 20; see example in Fig. 5*A*). The comparison of the dose-response effects of 8-OH-DPAT for pyramidal and GABAergic neurons revealed a significant effect of neuronal phenotype ($F_{1,37}$ = 18.86, P < 0.0001; pyramidal neurons, n = 19; GABAergic neurons, n = 20) as well as a significant interaction between 8-OH-DPAT and neuronal phenotype ($F_{2,74}$ = 8.44, P < 0.0005; Fig. 5*B*). In some cases, a pyramidal and a putative GABAergic neuron were simultaneously recorded, as in the

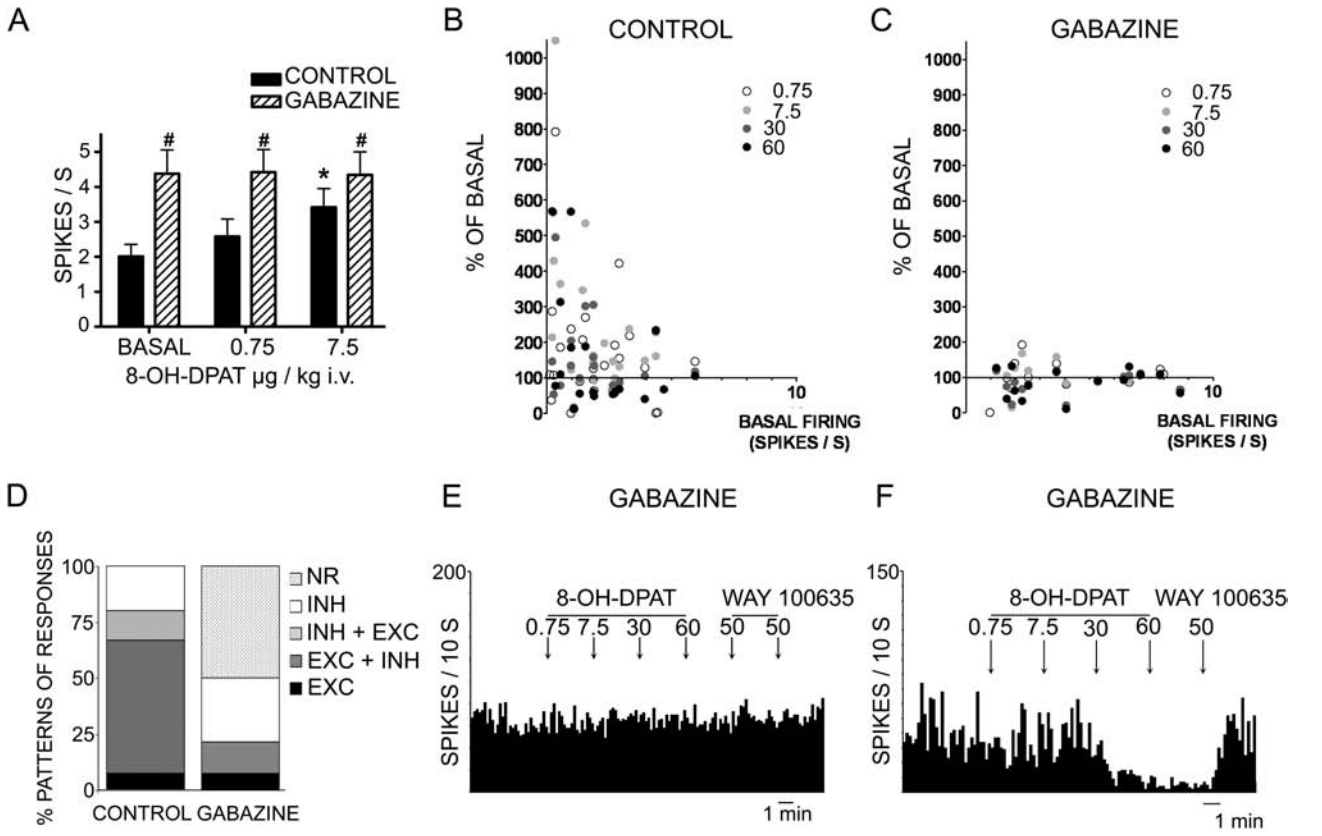

Figure 3. Effects of the local application of gabazine ($GABA_A$ antagonist) through the recording electrode on the responses of mPFC pyramidal neurons to 8-OH-DPAT administration. (*A*) Bar diagram showing the change in the firing rate of pyramidal neurons produced by 8-OH-DPAT in control conditions and during blockade of $GABA_A$ inputs with gabazine. *P < 0.01 versus basal; #P < 0.03 versus control. *B* and *C* plot the change in firing rate (percentage of baseline) induced by 8-OH-DPAT versus the predrug firing rate in control conditions (*B*) and in presence of gabazine (*C*). (*D*) Proportions of response patterns produced by 8-OH-DPAT on mPFC pyramidal neurons in the control situation (n = 15) and after the local blockade of $GABA_A$ inputs by gabazine (n = 14). NR, no response; INH, inhibitions; INH + EXC, inhibitions followed by excitations; EXC + INH, excitations followed by inhibitions; EXC, excitations. (*E*) and (*F*) show examples of the 2 most common patterns of response produced by increasing doses of 8-OH-DPAT on mPFC pyramidal neurons after the local blockade of $GABA_A$ inputs: lack of response and inhibition (50% and 29% of responses, respectively). The inhibition is reversed by the subsequent administration of WAY100635. Drug doses are given in μg/kg i.v.

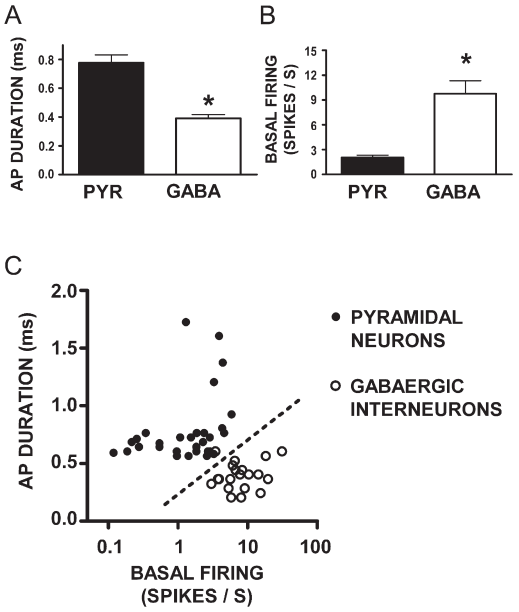

Figure 4. Characterization of fast-spiking GABAergic interneurons in mPFC. (*A*) Fast-spiking interneurons show shorter action potential duration compared with mPFC pyramidal neurons. $^{*}P < 0.001$. (*B*) Fast-spiking interneurons show significantly higher firing rates than mPFC pyramidal neurons. $^{*}P < 0.001$. (*C*) Plotting the action potential duration versus the basal firing rate creates 2 separate clusters grouping pyramidal neurons on one side and fast-spiking GABAergic interneurons on the other. The duration of the action potentials corresponds to the depolarization phase (average of 10 spikes) for both neuronal types.

example depicted in Figure 5*C*, which shows an opposite effect of 8-OH-DPAT on the discharge rate of pyramidal and GABAergic neurons.

Additional doses of 8-OH-DPAT (30 and 60 μg/kg i.v.) did not modify the effect of 7.5 μg/kg i.v. 8-OH-DPAT on mPFC GABAergic interneurons. Firing rate changed from 10.0 ± 1.9 in baseline to 9.5 ± 1.8 (0.75 μg/kg i.v.), 7.3 ± 1.5 (7.5 μg/kg i.v), 6.9 ± 1.3 (30 μg/kg i.v), and 6.7 ± 1.5 spikes/s (60 μg/kg i.v) ($F_{4,60}$ = 5.00, $P < 0.002$, n = 16; nonsignificant differences between the dose of 7.5 and 30 or 60 μg/kg i.v.; post hoc Newman–Keuls).

The inhibitions induced by 8-OH-DPAT on putative GABAergic interneurons were reversed by the subsequent administration of 50–100 μg/kg i.v. of the 5-HT1AR antagonist WAY100635 ($F_{2,20}$ = 15.51, $P < 0.0001$, n = 11; significant differences between basal and 8-OH-DPAT and between 8-OH-DPAT and WAY100635; $P < 0.001$, post hoc Newman–Keuls test).

Involvement of other 5-HT$_{1A}$ Receptor Subsets

Role of 5-HT$_{1A}$ Autoreceptors

Previous observations using microiontophoretic application of 5-HT1AR agonists indicated a preferential sensitivity of 5-HT1AR located on DR serotonergic neurons compared with those in the hippocampal formation (Sprouse and Aghajanian 1988). We therefore examined whether the excitatory effect of low 8-OH-DPAT doses on mPFC pyramidal neurons could be due to a secondary excitation after the removal of a putative inhibitory serotonergic tone on mPFC pyramidal neurons. To this end, we performed 2 different sets of experiments. In one of them, we examined the effect of 8-OH-DPAT on 5-HT release in mPFC using exactly the same experimental conditions than

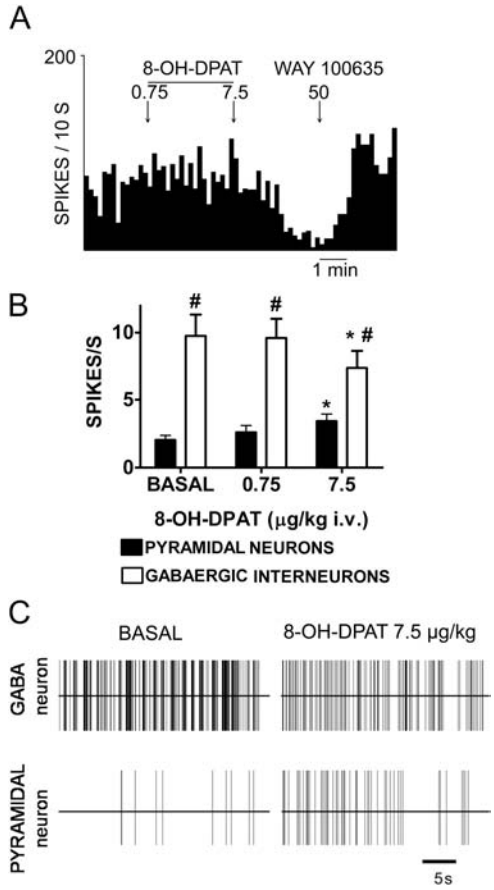

Figure 5. (*A*) Representative example of the effect of the 5-HT1AR agonist 8-OH-DPAT (injections shown by vertical arrows) on the activity of fast-spiking mPFC GABAergic interneurons and its reversal by the subsequent administration of WAY-100635. (*B*) Bar graph showing the opposite and simultaneous effect of 8-OH-DPAT on the activity of mPFC pyramidal neurons (control group) and fast-spiking GABAergic interneurons. $^{*}P < 0.05$ versus baseline; $^{\#}P < 0.0001$ versus pyramidal neurons. (*C*) Dual recording of a pyramidal neuron and a putative GABAergic interneuron showing the temporal coincidence of the inhibition of the mPFC GABAergic interneuron and the excitation of the pyramidal neuron. Action potential duration and basal firing rate: 0.84 ms and 0.16 spikes/s versus 0.24 ms and 8.17 spikes/s for pyramidal neuron and GABAergic interneuron, respectively. Drug doses are given in μg/kg i.v.

in electrophysiological recordings (i.e., i.v. drug administration to chloral hydrate anesthetized rats) and rapid sampling of dialysates. In a second set of experiments, we examined the effect of 8-OH-DPAT on mPFC pyramidal neurons of rats depleted of 5-HT with the 5-HT synthesis inhibitor *p*CPA.

Baseline 5-HT concentration in dialysate samples of anesthetized rats was 20.5 ± 6.7 fmol/4-min fraction (n = 4). The administration of 7.5 μg/kg i.v. of 8-OH-DPAT reduced the 5-HT output to 47 ± 6% of baseline. Higher doses of 8-OH-DPAT further reduced 5-HT release to 32 ± 7% (30 μg/kg i.v.) and 27 ± 4% of baseline (60 μg/kg i.v.). WAY100635 (50 μg/kg i.v.) significantly reversed the 8-OH-DPAT-induced decrease of the extracellular 5-HT concentration in the mPFC (Fig. 6*A*).

In 5-HT-depleted rats (*p*CPA; 300 mg/kg i.p. 2–4 days before recordings), the tissue concentration of 5-HT in mPFC was ~5% of control rats (0.13 ± 0.01 nmol/g vs. 2.51 ± 0.12 nmol/g; n = 10 and 9, respectively; $P < 0.00001$, Student's t-test). 5HT depletion by *p*CPA did not alter the capacity of low 8-OH-DPAT doses (up to 7.5 μg/kg i.v.) to increase pyramidal discharge ($F_{2,18}$ = 4.09, $P < 0.04$, n = 10; Fig. 6*B*).

Hippocampal 5-HT$_{1A}$ Receptors

Given the high density of 5-HT1AR in the hippocampus (Pompeiano et al. 1992) and the existence of a dense projection from the hippocampal formation to the prelimbic subdivision of the mPFC (Jay et al. 1989), we examined whether activation of hippocampal 5-HT1AR could contribute to the observed effects of 8-OH-DPAT in mPFC.

Hippocampal pyramidal neurons projecting to the mPFC, identified by antidromic activation from the mPFC, were silent in chloral hydrate anesthetized rats. The administration of 8-OH-DPAT did not alter their discharge rate (n = 7). A representative example of an antidromically identified hippocampal neuron projecting to mPFC is shown in Figure 6*C*.

Discussion

The present study shows that the 5-HT1AR agonist 8-OH-DPAT increases the firing activity of mPFC pyramidal neurons projecting to VTA. This effect likely reflects a pyramidal disinhibition after the preferential activation of 5-HT1AR in local GABAergic interneurons by 8-OH-DPAT. These effects do not appear to be due to nonspecific actions of (±)8-OH-DPAT. Likewise, other neuronal structures where 5-HT1AR are abundantly expressed, such as the hippocampus or in the raphe nuclei, do not appear to be involved.

Although pyramidal neurons were identified by antidromic stimulation from VTA, the similar localization of PFC neurons projecting to several midbrain structures (Gabbott et al. 2005) and their branching to innervate dopaminergic and serotonergic nuclei (Vázquez-Borsetti et al. 2011) suggests a widespread action on midbrain-projecting pyramidal neurons. Moreover, *c-fos* studies indicate the additional activation of pyramidal neurons in upper cortical layers projecting to other brain areas (Gabbott et al. 2005).

Recent studies have shown the possibility of agonist-directed trafficking at 5-HT1AR (Valdizán et al. 2009; Newman-Tancredi 2010). Hence, since 8-OH-DPAT is a racemate, the excitation produced by the administration of an agonist of the 5-HT1A inhibitory receptor could be due to actions of R(−)8-OH-DPAT enantiomer on different signaling pathways. However, given the similarity of the actions of (±)8-OH-DPAT and R(+)8-OH-DPAT, our results suggest that most effects of the racemate mixture are due to the interaction of (+)8-OH-DPAT with 5-HT1A-R.

Although 8-OH-DPAT interacts with 5-HT$_7$ receptors (Ruat et al. 1993), its affinity is lower than for 5-HT1AR (http://pdsp.med.unc.edu/pdsp.php). Moreover, the excitatory effect of 8-OH-DPAT was antagonized by WAY-100635, supporting the exclusive involvement of 5-HT1A-R.

The present observations indicate that the excitatory actions of 8-OH-DPAT on mPFC pyramidal neurons are based on local network properties. The mPFC contains a high density of 5-HT1AR, located in pyramidal and GABAergic neurons (Santana et al. 2004), thus raising the possibility that local cellular interactions can account for the excitatory effect of low doses of 8-OH-DPAT. This hypothesis was also driven by previous neurochemical observations with the selective 5-HT1AR agonist BAY × 3702, which increased dopamine release in mPFC after local application at low concentrations and reduced it at high concentrations, with the effect of low concentrations being suppressed by local GABA$_A$ blockade (Díaz-Mataix et al. 2005). Indeed, GABAergic inputs onto GABA$_A$ receptors have been shown to have a major impact on the function of brain networks and may play an important role in schizophrenia (González-Burgos and Lewis 2008).

The present results give support to the involvement of local GABAergic interneurons in 8-OH-DPAT-induced pyramidal excitations. The local blockade of GABA$_A$ inputs with a gabazine leak markedly changed the response of pyramidal neurons to 8-OH-DPAT, mainly avoiding its excitatory effect and reducing the percentage of neurons responding to the drug. This was

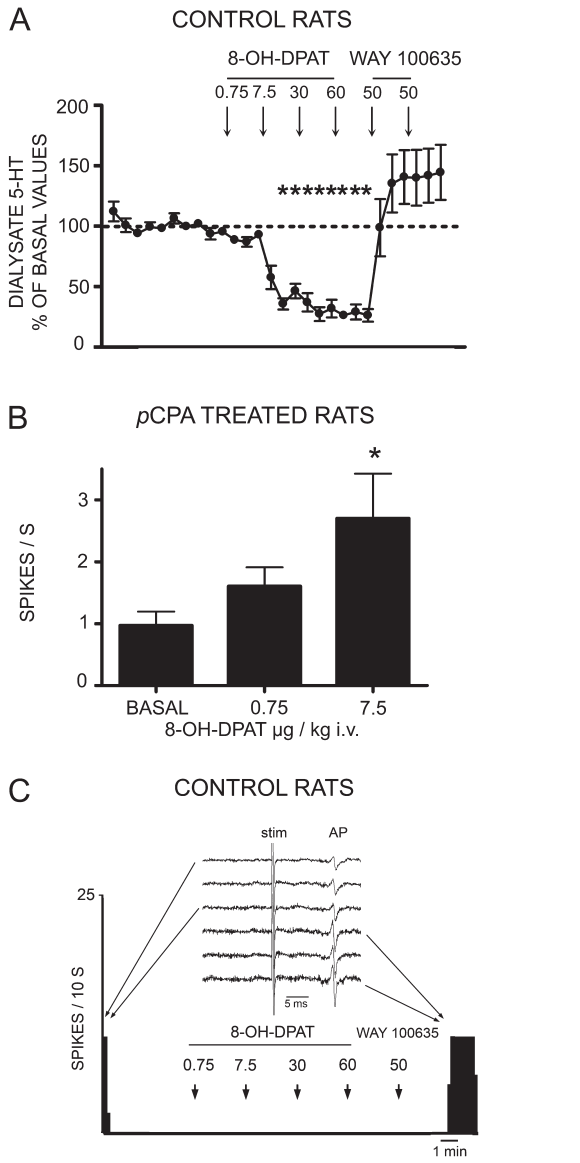

Figure 6. (*A*) Effect of the intravenous administration of 8-OH-DPAT on extracellular 5-HT in the mPFC of control anesthetized rats. 8-OH-DPAT significantly decreased 5-HT release in mPFC. This decrease was reversed by the subsequent administration of WAY100635. $F_{24,48} = 10.52$, $P < 0.00001$, $n = 4$, $^*P < 0.001$ versus baseline. (*B*) Bar graph showing the excitation on mPFC pyramidal neurons after the intravenous administration of 8-OH-DPAT in *p*CPA-treated rats. $^*P < 0.04$. (*C*) Effect of the systemic administration of 8-OH-DPAT on the firing rate of a CA1/Sub hippocampal neuron projecting to mPFC. Note the absence of spontaneous discharges in chloral hydrate anesthetized rats. The administration of 8-OH-DPAT or WAY 100635 did not change the firing rate of the neuron. The neuron was identified by antidromic stimulation (stim) before recording basal condition and after the administration of the drugs as shown in the traces above the integrated firing rate histogram. AP, antidromic potentials after stimulation from mPFC. Drug doses are given in µg/kg i.v.

accompanied by an increase of the baseline pyramidal discharge, as expected from the partial blockade of local $GABA_A$ inputs and the corresponding shift in the balance between excitatory and inhibitory inputs. Changes induced by gabazine in the response in front of 8-OH-DPAT cannot be due to an upper firing threshold, since mPFC pyramidal neurons projecting to VTA can discharge at much higher rates in the present experimental conditions (Puig et al. 2003; Kargieman et al. 2007).

In agreement with a previous study (Hajós et al. 1999), we found an overall increase of *c-fos* expression in mPFC after systemic 8-OH-DPAT administration. The use of double in situ hybridization techniques allowed us to identify that only pyramidal neurons, but not GABAergic neurons, expressed *c-fos* in response to 8-OH-DPAT administration. The data from the present *c-fos* studies further support the view that 8-OH-DPAT selectively activates PFC pyramidal neurons, in agreement with the above electrophysiological observations. Hence, both sets of experiments were carried out in the same conditions (i.v. administration to anesthetized rats) and the expression of *c-fos* occurs in parallel with an increased neuronal activity (Dragunow and Faull 1989; Konkle and Bielajew 2004).

Finally, further evidence supporting the involvement of GABAergic interneurons in the excitatory effect of 8-OH-DPAT on pyramidal neurons is the reduction of cell firing of mPFC fast-spiking putative interneurons at 8-OH-DPAT doses that excite pyramidal neurons. Fast-spiking interneurons have been described as parvalbumin-expressing cells, making synapses on the cell body, basal dendrites, and axon hillock of pyramidal neurons (De Felipe et al. 2001). The special location and synaptic connectivity of these neurons allows them to exert a tight control of pyramidal neuron activity.

Therefore, the observed inhibition of putative fast-spiking GABAergic interneurons by low doses of 8-OH-DPAT could translate into a disinhibition of pyramidal neurons. 5-HT1AR are expressed by approximately 25% of GABAergic neurons and 50–60% of pyramidal neurons in mPFC (Santana et al. 2004). The former proportion may seem low to explain the mainly excitatory effects of 8-OH-DPAT on pyramidal neurons. However, cortical GABAergic interneurons are connected by electrical and chemical synapses (Galarreta and Hestrin 1999, 2001; Hestrin and Galarreta 2005; Fukuda 2007). These GABAergic networks occur between different classes of interneurons (Simon et al. 2005), one of which are parvalbumin-positive fast-spiking interneurons (Galarreta and Hestrin 2002; Hestrin and Galarreta 2005) and can extend for hundreds of microns in the neocortex (Fukuda 2007). Actually, it has been estimated that a single interneuron can contact more than 200 pyramidal neurons (Cobb et al. 1995), therefore magnifying the effects on single GABAergic interneurons. Furthermore, immunohistochemical studies reported the presence of 5-HT1AR immunoreactivity in parvalbumin- and calbindin-positive GABAergic interneurons (Aznar et al. 2003). Thus, the activation of 5-HT1AR in a limited population of GABA cells in a cortical GABA network can result in the hyperpolarization of the whole network and the subsequent disinhibition of many pyramidal neurons. Similar findings have been reported for the noncompetitive NMDA receptor antagonist MK-801. This agent evoked moderate inhibitions of mPFC GABA interneurons and marked excitations of pyramidal neurons (Homayoun and Moghaddam 2007).

The reasons for this preferential action on GABAergic interneurons remain to be elucidated. The recent demonstration that 5-HT1AR can be coupled to different G proteins in different brain areas (Mannoury la Cour et al. 2006; Newman-Tancredi et al. 2009; Valdizán et al. 2009; Newman-Tancredi 2010) opens the possibility that agonist-directed trafficking can also occur in different cell types. Moreover, since 5-HT1AR are coupled to GiRK channels (Andrade and Nicoll 1987) among other signaling pathways, their activation may have a greater impact on fast-spiking interneurons, discharging at higher rates than pyramidal neurons. These possibilities remain to be examined in full.

8-OH-DPAT actions on other 5-HT1AR regional subsets may not be fully discarded, although they seem to play a minor role. First, 8-OH-DPAT inhibits the activity of dorsal raphe serotonergic neurons via 5-HT1A-R with an ED50 of 0.8 μg/kg i.v. in our experimental conditions (Romero et al. 2003; Lladó-Pelfort L, Celada P, Artigas F, unpublished observations) and similar ED50 values have been reported elsewhere (Hajós et al. 1999). Thus, the excitatory effect of 8-OH-DPAT could potentially be due to the loss of an inhibitory serotonergic tone on mPFC pyramidal neurons. However, the present results do not support this view. Hence, although 8-OH-DPAT reduced 5-HT release at 7.5 μg/kg i.v., it excited pyramidal neurons at the same dose in *p*CPA-treated rats, lacking 5-HT, which indicates that 8-OH-DPAT-induced excitations were not produced by the reduction of an inhibitory serotonergic tone in mPFC.

The hippocampal formation is the brain area with the highest density of 5-HT1AR (Pazos and Palacios 1985; Pompeiano et al. 1992) and hippocampal–mPFC projections exert a phasic control on mPFC pyramidal and GABAergic neurons (Dégenètais et al. 2003; Tierney et al. 2004). However, in our experimental conditions, mPFC-projecting hippocampal neurons were silent and unresponsive to 8-OH-DPAT, which also rules out hippocampal 5-HT1AR as a source of the 8-OH-DPAT-induced changes in mPFC.

Moreover, since both serotonergic and hippocampal axons contact with pyramidal and GABAergic neurons (Smiley and Goldman-Rakic 1996; Jansson et al. 2001; Dégenètais et al. 2003), an effect of 8-OH-DPAT on 5-HT1AR outside the PFC should translate into a common action on both neuronal types, unlike what the present electrophysiological and histological observations indicate.

In addition to the main excitatory effect, 8-OH-DPAT also inhibited some pyramidal neurons, an effect likely accountable by the direct action on pyramidal neurons expressing 5-HT1A-R (Kia et al. 1996; Santana et al. 2004) not subjected to the above disinhibitory mechanism. This inhibitory response is consistent with previous observations showing that microiontophoretic application of high doses of 5-HT1AR agonists inhibits cortical putative pyramidal neurons (Araneda and Andrade 1991; Ashby et al. 1994; Rueter and Blier 1999). Likewise, the excitation produced by low 8-OH-DPAT doses was reversed by higher doses, which also agrees with these previous studies.

Like 8-OH-DPAT, other 5-HT1AR agonists, given systemically, increase the discharge rate of pyramidal neurons in mPFC (Hajós et al. 1999; Díaz-Mataix et al. 2006; Lladó-Pelfort et al. 2010; Lladó-Pelfort et al. unpublished observations; this study), which suggests a common mechanism of action, possibly involving GABAergic interneurons.

The present observations may be relevant for the treatment of negative symptoms and cognitive deficits in schizophrenia, which are poorly treated by current APDs. An increase of

pyramidal neuron activity in mPFC may balance monoamine neurotransmission in this area and facilitate the activity of mPFC in tasks, such as working memory, which require persistent activity of pyramidal neurons (Williams and Goldman-Rakic 1995; Fuster 2008; Robbins and Arnsten 2009). Moreover, although 8-OH-DPAT is not available for human use, a novel 5-HT1AR agonist (F15599) with a preferential excitatory activity on mPFC pyramidal neurons at low doses (Lladó-Pelfort et al. 2010) shows clear procognitive properties (Depoortère et al. 2010).

In summary, the present results support the view that 5-HT1AR agonists exert their activation of mPFC pyramidal neurons by reducing $GABA_A$-mediated inputs. This action likely involves the activation of 5-HT1AR in local GABAergic networks. Given the role of mPFC 5-HT1AR in the control of the ascending dopaminergic pathways, the present results may help to identify new targets for the development of drugs acting on negative symptoms and cognitive deficits in schizophrenia and related disorders.

Funding

Fondo de Investigaciones Sanitarias (FIS) (grants FIS PI060264, FIS PI09/1245) from the Ministry of Science and Innovation (Instituto de Salud Carlos III); Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM; JAE fellowship from CSIC (to L.L.-P.); Researcher Stabilization Program of the Health Department of the Generalitat de Catalunya (to P.C.). We also acknowledge support from the Innovative Medicines Initiative Joint Undertaking (IMI) under Grant Agreement N° 115008 (NEWMEDS). IMI is a public–private partnership between the European Union and the European Federation of Pharmaceutical Industries and Associations.

Notes

We thank Leticia Campa, Mónica Gutiérrez, Noemí Jurado, and Verónica Paz for skillfull technical assistance. Regarding the potential conflict of interest, we declare that over the past 3 years Dr F. Artigas has received consulting and educational honoraria from Eli Lilly, Sanofi-Aventis, Lundbeck, Pierre Fabre, and Elan. The rest of authors declare that except for income received from our primary employers, no financial support or compensation has been received from any individual or corporate entity for research or professional service, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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