Control of Serotonergic Function in Medial Prefrontal Cortex by Serotonin-2A Receptors through a Glutamate-Dependent Mechanism

Raúl Martín-Ruiz,1 M. Victoria Puig,1 Pau Celada,1 David A. Shapiro,2 Bryan L. Roth,2 Guadalupe Mengod,1 and Francesc Artigas1

1Department of Neurochemistry, Institut d’Investigacions Biomèdiques de Barcelona (Consejo Superior de Investigaciones Científicas), Institut d’Investigacions Biomèdiques August Pi i Sunyer, 08036 Barcelona, Spain, and 2Departments of Biochemistry, Neurosciences and Psychiatry, Case Western Reserve University Medical School, Cleveland, Ohio 44106

We examined the in vivo effects of the hallucinogen 4-iodo-2,5-dimethoxyamphetamine (DOI). DOI suppressed the firing rate of 7 of 12 dorsal raphe (DR) serotonergic (5-HT) neurons and partially inhibited the rest (ED50 = 20 μg/kg, i.v.), an effect reversed by M100907 (5-HT2A antagonist) and picrotoxinin (GABA, antagonist). DOI (1 mg/kg, s.c.) reduced the 5-HT release in medial prefrontal cortex (mPFC) to 33 ± 8% of baseline, an effect also antagonized by M100907. However, the local application of DOI in the mPFC increased 5-HT release (164 ± 6% at 100 μM), an effect antagonized by tetrodotoxin, M100907, and BAY × 3702 (5-HT1A agonist) but not by SB242084 (5-HT2C antagonist). The 5-HT increase was also reversed by NBQX (AMPA–KA antagonist) and 1S,3S-ACPD (mGluR 2/3 agonist) but not by MK-801 (NMDA antagonist). AMPA mimicked the 5-HT elevation produced by DOI. Likewise, the electrical–chemical stimulation of thalamocortical afferents and the local inhibition of glutamate uptake increased the 5-HT release through AMPA receptors. DOI application in mPFC increased the firing rate of a subgroup of 5-HT neurons (5 of 10), indicating an enhanced output of pyramidal neurons. Dual-label fluorescence confocal microscopic studies demonstrated co-localization of 5-HT1A and 5-HT2A receptors on individual cortical pyramidal neurons.

Thus, DOI reduces the activity of ascending 5-HT neurons through a DR-based action and enhances serotonergic and glutamatergic transmission in mPFC through 5-HT2A and AMPA receptors. Because pyramidal neurons coexpress 5-HT1A and 5-HT2A receptors, DOI disrupts the balance between excitatory and inhibitory inputs and leads to an increased activity that may mediate its hallucinogenic action.

Key words: 5-hydroxytryptamine; 5-HT1A receptors; 5-HT2A receptors; AMPA; DOI; dorsal raphe nucleus; GABA; glutamate; hallucinogens; medial prefrontal cortex; microdialysis; mGluR; NMDA; single-unit recordings; thalamus
neurons in a complex manner (Hajós et al., 1998; Celada et al., 2001). Moreover, the activation of postsynaptic 5-HT<sub>1A</sub> receptors in the mPFC reduced the local release of 5-HT (Casanova et al., 1999) and the firing rate of dorsal raphe 5-HT neurons (Celada et al., 2001). These data suggest that pyramidal neurons containing 5-HT<sub>1A</sub> receptors may participate in the distal control of serotonergic activity. Because 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors may colocalize in layer V pyramidal neurons projecting to the DR, 5-HT<sub>2A</sub> receptors could be involved in this control. Therefore, in the present study we examined the actions of the hallucinogen DOI, a 5-HT<sub>2A</sub>/2C agonist, on the serotoninergic system using in vivo microdialysis and single-unit recordings. The results obtained indicate that 5-HT<sub>2A</sub> receptor activation has profound effects on serotonergic neurons, likely via an enhancement of the AMPA-mediated neurotransmission in medial prefrontal cortex.

**MATERIALS AND METHODS**

**Animals.** Male albino Wistar rats (Ifa Credo, Lyon, France) weighing 280–320 gm and kept in a controlled environment (12 hr light/dark cycle and 22 ± 2°C room temperature) with food and water provided ad libitum, were used in in vivo experiments. Animal care followed the European Union regulations (Council of E.C., 1986; 1988). Animals were sacrificed after anesthetization with pentobarbital (60 mg/kg, i.p.) and positioned in a stereotaxic apparatus. Additional doses of chloral hydrate (80 mg/kg) were administered intravenously. Body temperature was maintained according to previously described electrophysiological criteria (Wang and Aghajanian, 1977). They exhibited a regular baseline extracellular 5-HT level of 5–10 pmol/ml, with a basal 5-HT release about 10% of the peak 5-HT release elicited by an overdose of sodium pentobarbital. The placement of the dialysis probes was confirmed by perfusion of Fast green dye and visual inspection of the probe track after cutting the brain at the appropriate levels. In experiments involving the electrical or chemical stimulation of thalamic afferents to mPFC, the placement of the thalamic probes or electrodes was confirmed histologically with Neutral Red staining.

The concentration of 5-HT in dialysate samples was determined by HPLC, as described (Adell and Artigas, 1998). 5-HT was separated using a Beckman (San Ramon, CA) 3 µm particle size column and detected with a Hewlett Packard 1049 electrochemical detector at +0.6 V. Retention time was between 3.5 and 4 min, and the limit of detection was typically 1 fmol/sample.

**Single-unit recordings.** We examined the effect of the systemic administration of DOI on the firing activity of identified 5-HT neurons in the dorsal raphe nucleus (DR). Single-unit extracellular recordings were performed as described previously (Sawyer et al., 1985; Celada et al., 1996). Briefly, rats were anesthetized (chloral hydrate, 400 mg/kg, i.p.) and positioned in a stereotaxic apparatus. At the end of the experiments, rats were killed by an overdose of sodium pentobarbital. The placement of the dialysis probes was confirmed by perfusion of Fast green dye and visual inspection of the probe track after cutting the brain at the appropriate levels. In experiments involving the electrical or chemical stimulation of thalamic afferents to mPFC, the placement of the thalamic probes or electrodes was confirmed histologically with Neutral Red staining.

**Surgery and microdialysis procedures.** An updated description of the microdialysis procedures used can be found in Adell and Artigas (1998). Briefly, anesthetized rats (pentobarbital; 60 mg/kg, i.p.) were stereotaxically implanted with one concentric microdialysis probe equipped with a Cuprophan membrane in medial prefrontal cortex [anteroposterior (AP) +3.4, lateral (L) −0.8, dorsalventral (DV) −6.0; probe tip: 4 mm] coordinates in millimeters; Paxinos and Watson, 1986). On the next day, microdialysis experiments were performed in freely moving rats. The probes were perfused at 1.5 µl/min with artificial CSF (aCSF) (in mM: 125 NaCl, 2.5 KCl, 1.2 CaCl<sub>2</sub>, and 1.8 MgCl<sub>2</sub>) containing 1 µM citopamina. After a 1 hr stabilization period, four fractions were collected to obtain basal values before local (reverse dialysis) or systemic administration of drugs. Successive 20 min (30 µl) dialysate samples were collected. In most experiments, the partial 5-HT<sub>2A/C</sub> receptor agonist DOI was applied alone for 2 hr (six fractions). This was followed by the application of DOI in combination with other drugs for another 2 hr period. In experiments assessing this effect of t-trans-PDC on 5-HT release, the drug was perfused alone or in combination with other agents for 2 hr after collection of baseline values.

Three microdialysis experiments were conducted in chloride hydrate-anesthetized animals. In one of them, we examined the effect of the electrical stimulation of the midline thalamus projecting to mPFC, to modulate the release of 5-HT in the latter area. Microdialysis probes were implanted as above. Stimulating bipolar electrodes were used. The isolating material was peeled off −1 mm above the tip, the poles were separated 100–150 µm, and electrodes were stereotaxically placed and secured with dental cement, as for microdialysis probes. The tip of the electrodes was aimed at the top part of the centromedial thalamic nucleus (AP 3.6, L −0.7, DV −6.5). In this manner, the stimulated area was between DV −6.5 and DV −5.5, thus affecting the medio dorsal nucleus and part of the centromedial nucleus (plate 32; Paxinos and Watson, 1986). The lesion of this thalamic area has been shown to attenuate the EPSCs induced by 5-HT in layer V mPFC pyramidal neurons (Marck et al., 2001). On the next day, rats were anesthetized with chloral hydrate in single-unit recording experiments (see below). After a 2 hr stabilization period, electrical stimulation was performed (5 Hz, 1.5 mA, 0.3 msec square pulses) for 20 min with a Grass S-48 stimulator unit S-48 connected to a Grass SIU isolation unit, and dialysate fractions were collected for one more hour. In some rats, the stimulating electrode was implanted, but no current was passed.

The second experiment examined the effects of the application of bicineuline in the midline thalamus (to disinhibit afferent fibers to mPFC) on the 5-HT release in mPFC. These rats were implanted with two microdialysis probes, in mPFC (as above) and in the thalamus (coordinates: AP −3.2, L −0.5, DV −6.5; probe tip, 1.5 mm). In this location, the administration of bicineuline by reverse dialysis through the thalamic probe was also intended to affect both the centromedial and medio dorsal thalamic nuclei. The third experiment assessed the effects of the systemic administration of DOI (1 mg/kg, s.c.) on 5-HT release in the mPFC of chloral hydrate-anesthetized rats, to mimic the experimental conditions of single-unit recordings. At the end of the experiment, the rats were killed by an overdose of sodium pentobarbital. The placement of the dialysis probes was examined by perfusion of Fast green dye and visual inspection of the probe track after cutting the brain at the appropriate levels. In experiments involving the electrical or chemical stimulation of thalamic afferents to mPFC, the placement of the thalamic probes or electrodes was confirmed histologically with Neutral Red staining.

Single-unit potentials were amplified with a Neurodata IR283 (Cygnus Technology Inc., Delaware Water Gap, PA), postamplified, and filtered with a Cibertec amplifier (Madrid, Spain) and computed on-line using a DAT 1401plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK). After recording stable baseline spontaneous firing activity for at least 5 min, DOI was administered (0.025–0.4 mg/kg, i.v.; cumulative doses) every 2 min. Once a maximal effect of DOI was attained, either M100907 (100 µg/kg, i.v.; Haddjeri et al., 1999) or the GABA<sub>B</sub> receptor antagonist picrotixin (1 mg/kg, i.v.) were injected. In some neurons, 8-OD-PAT and WAY 100635, 5-HT<sub>1A</sub> receptor agonist...
and antagonist, respectively, were injected after M100907 or picrotoxinin to further confirm the serotonergic identity of the recorded cell. Only one neuron per rat was recorded.

Additional experiments were performed to examine the effects of the local application of DOI in mPFC on the firing rate of 5-HT neurons in the DR. DOI (200 μM, dissolved in aCSF) was infused through a 32 gauge stainless steel cannula (Small Parts Inc., Miami, FL) implanted in the mPFC (tip coordinates: AP +3.4, L −0.8, DV −4.5). The cannula was attached to a 10 μl Hamilton syringe by a Teflon tubing. A micro-infusion pump (Bioanalytical Systems Inc., West Lafayette, IN) was used. After recording baseline spontaneous activity for at least 5 min, 200 nl of DOI was infused over the course of 1 min. This volume has been reported to diffuse to a maximum effective diameter of 0.4–0.6 mm (Myers, 1971). In some rats, a second DOI infusion was administered 5 min later.

Data and statistical analysis. Microdialysis results are expressed as femtomoles per fraction (uncorrected for recovery) and shown in figures as percentages of basal values (individual means of four prefractional times). Statistical analysis of drug effects on dialysate 5-HT was performed using one- or two-way ANOVA for repeated measures of raw data with time as repeated factor and dose or pretreatment as independent factor. The effects of drugs or chemical stimulation on the 5-HT output were analyzed by one- or two-way ANOVA of raw data followed by post hoc Duncan test. Changes in firing rate were quantified by averaging the values in the second minute after drug injection and expressed as percentage of baseline. ED50 values were calculated with the GraphPad (San Diego, CA) Prism program. Data are expressed as the mean ± SEM. Statistical significance has been set at the 95% confidence level (two-tailed).

Cell culture and monoclonal antibody production. Stably transfected Chinese Hamster Ovary (CHO) cells expressing the 5-HT1A receptor were obtained from J. Raymond (Medical University of South Carolina, Charleston, SC). A monoclonal 5-HT1A receptor antibody was prepared as follows. Female BALB/c mice were immunized intraperitoneally with 50 μg of a synthetic peptide (NKRTPRRAALISLCL) conjugated to Limulus polyphemus hemocyanin and emulsified in Freund’s adjuvant. Immune spleens were harvested after −3 months and fused with SP2/0 myeloma cells at a ratio of four spleen cells per myeloma using phenyl-ethyleneglycol. Antibody-producing colonies were detected by ELISA as follows: immunizing peptide conjugated to BSA (10 μg/ml) was immobilized to COSTAR 9017 EIA/RIA plates in 100 μl of coating buffer (50 mM carbonate--bicarbonate, pH 9.6) and incubated overnight at room temperature. Wells were blocked with 2% milk in PBS (blocking buffer) for 1 hr, followed by addition of antibody in culture supernatant or in blocking buffer for 90 min. Antibodies were detected using a horse anti-mouse polyclonal antibody linked to horseradish peroxidase (Vector Laboratories, Inc.) or a Texas Red-labeled goat anti-mouse secondary reagent (Vector Laboratories). Cells were then incubated overnight at 4°C with constant agitation. After extensive washing with PBS, sections were incubated with a 1:20 dilution of secondary antibodies (BODIPY-FL-anti-mouse and Texas Red anti-rabbit or Texas Red anti-guinea pig) for 1 hr at room temperature. After extensive washing with PBS, sections were incubated with a 1:200 dilution of anti-5-HT1A primary antibody supernatants and shown in figures as percentages of basal values (individual means of four prefractional times). Statistical analysis of drug effects on dialysate 5-HT was performed using one- or two-way ANOVA for repeated measures of raw data with time as repeated factor and dose or pretreatment as independent factor. The effects of drugs or chemical stimulation on the 5-HT output were analyzed by one- or two-way ANOVA of raw data followed by post hoc Duncan test. Changes in firing rate were quantified by averaging the values in the second minute after drug injection and expressed as percentage of baseline. ED50 values were calculated with the GraphPad (San Diego, CA) Prism program. Data are expressed as the mean ± SEM. Statistical significance has been set at the 95% confidence level (two-tailed).

RESULTS

Baseline 5-HT values

The basal concentration of 5-HT in dialysates from medial prefrontal cortex was 17.5 ± 0.8 fmol/fraction (n = 105) in freely moving rats and 17.0 ± 1.1 fmol/fraction (n = 24) in chloral hydrate-anesthetized rats.

Effects of the local application of DOI in medial prefrontal cortex

The local application of 1, 10, and 100 μM DOI (2 hr each) increased the 5-HT output in a concentration-dependent manner. Maximal increases at each concentration were, respectively 119 ± 20, 137 ± 16, and 209 ± 29% of baseline (n = 5) (Fig. 1A). One-way ANOVA showed a significant effect of DOI application (p < 0.00001). In subsequent experiments, we used the concentration of DOI that elicited maximal effects (100 μM).
The perfusion of 100 μM DOI (n = 7) significantly increased the 5-HT output when compared with the control group (artificial CSF; n = 7) (p < 0.02, treatment effect; p < 0.00001, time effect; p < 0.00001 time × treatment interaction) (Fig. 1B). Maximal elevation of 5-HT values were observed soon (40 min) after DOI application and remained elevated over the entire period of application. The mean increase produced by 100 μM DOI in all perfusion experiments was 164 ± 6% of baseline (average of fractions 7–10; n = 69).

The effect of DOI was completely antagonized by the coperoxion of 1 μM TTX, which reduced 5-HT values below baseline (from 185 ± 10 to 30 ± 6% of baseline; n = 4; p < 0.00001) (Fig. 2). Likewise, the 5-HT increase produced by 100 μM DOI was significantly attenuated by the coperoxion of 100 μM of the selective 5-HT2A receptor antagonist M100907 (DOI: 181 ± 8% of baseline; DOI + M100907: 119 ± 13% of baseline; n = 8; p < 0.00001) (Fig. 3A). A higher M100907 concentration (300 μM) elicited a greater reduction of the 5-HT output from 163 ± 1% to 42 ± 6% of baseline, n = 6; p < 0.00001) (Fig. 3A). In contrast, the selective 5-HT2C receptor antagonist SB 242084 did not reverse the 5-HT elevation produced by DOI and increased the 5-HT output further, from 178 ± 22% to 224 ± 25% of baseline (n = 6; p < 0.00001) (Fig. 3B).

The perfusion of the noncompetitive NMDA receptor antagonist MK-801 (300 μM) did not significantly modify the effect of DOI (n = 7) (Fig. 4A). In contrast, the application of the AMPA-KA receptor antagonist NBOX (300 μM) significantly reduced the effect of DOI (from 182 ± 15% to 122 ± 13% of baseline; n = 6; p < 0.00001) (Fig. 4B). The perfusion of the preferential mGluR2/3 agonist 1S,3S-ACPD (300 μM) modestly reversed the effect of DOI on 5-HT output only in the last two fractions (n = 5; p < 0.00001) (Fig. 4C). A higher concentration of 1S,3S-ACPD (1 mM) elicited a more marked antagonism and almost completely reversed the effect of DOI, from 190 ± 15 to 112 ± 6% of baseline (n = 5; p < 0.00001) (Fig. 4C). In contrast, the application of the selective mGluR2/3 agonist LY 379268, perfused at three different concentrations (300 μM, 1 mM, and 3 mM) failed to significantly reduce the 5-HT increase induced by the local application of DOI. The average effect of LY 379268 was 96 ± 16, 97 ± 12, and 107 ± 10% at 300 μM, 1 mM, and 3 mM, respectively (n = 4–5, data expressed as percentage of the 5-HT values during DOI alone). The effect of DOI was mimicked by the local application of AMPA (300 μM), which induced a maximal elevation of 5-HT to 188 ± 15% of baseline (p < 0.001, treatment effect; p < 0.00001, time effect; p < 0.00001, time × treatment interaction compared with controls; n = 6) (Fig. 4D).

The increase in 5-HT output produced by the application of DOI was strongly antagonized by the coperoxion of the selective 5-HT1A receptor agonist BAY × 3702 (De Vry et al., 1998; Casanova et al., 2000) (30 μM; from 189 ± 30 to 60 ± 13% of baseline; n = 5; p < 0.00001) (Fig. 5).

Localization of 5-HT1A and 5-HT2A receptors on individual mPFC pyramidal neurons

The opposite effects of 5-HT1A and 5-HT2A receptor activation in mPFC on 5-HT release are in keeping with previous data on pyramidal neuron excitability (Araneda and Andrade, 1991). However, despite abundant literature on the localization of either type in cortical neurons (see introductory remarks), it is unclear whether both receptors are coexpressed in pyramidal neurons. To address this question, we prepared a monoclonal 5-HT1A receptor antibody suitable for immunochemical studies. In preliminary studies (D. A. Shapiro and B. L. Roth, unpublished observations) we observed that the monoclonal 5-HT1A receptor antibody specifically labeled 5-HT1A receptors stably expressed in CHO cells but did not visualize untransfected CHO cells. As shown in
A–F, 5-HT1A and 5-HT2A receptors were abundantly colocalized on individual mPFC pyramidal neurons. Control experiments using secondary antibodies alone showed no specific immunofluorescence (Fig. 6I). Additional studies using commercially available antibodies directed against distinct epitopes of the 5-HT1A and 5-HT2A receptors (Fig. 6G,H) also showed extensive colocalization of 5-HT1A and 5-HT2A receptors in individual cortical pyramidal neurons.

Effects of increased glutamatergic transmission in mPFC on serotonergic function

The above microdialysis results suggested that DOI increased 5-HT release in mPFC indirectly, possibly by (1) enhancing a glutamatergic tone on 5-HT nerve terminals (local effect) and/or (2) by enhancing the activity of ascending 5-HT neurons in the DR through descending excitatory inputs. We therefore examined the effects of directly increasing the glutamatergic activity in mPFC on the local 5-HT release.

We first studied whether the 5-HT-increasing effects of DOI could be mimicked by endogenous glutamate. To this end, we electrically stimulated the mediodorsal and centromedial thalamic nuclei (5 Hz, 1.5 mA, 0.3 msec) for 20 min and collected dialysate fractions from the ipsilateral mPFC. The electrical stimulation of these nuclei elevated the 5-HT output in mPFC to a maximum of 179 ± 31% of baseline (p < 0.00001; n = 6) (Fig. 7A). Sham stimulation did not alter 5-HT levels (n = 6) (Fig. 7A).

Likewise, in dual probe microdialysis experiments, the application of bicuculline (300 μM and 1 mM) by reverse dialysis in the mediodorsal and centromedial thalamic nuclei doubled the 5-HT release in mPFC (n = 8; p < 0.00007) (Fig. 7B).

Moreover, the local (in mPFC) application of the glutamate uptake inhibitor l-trans-PDC markedly increased the local 5-HT release (n = 4; p < 0.000001) (Fig. 7C). The time course of the
effect differed from that elicited by AMPA (Fig. 4D) and showed a marked tachyphylaxis. However, the 5-HT increase was fully prevented by the coapplication of 300 μM NBQX ($p < 0.00005$ vs l-trans-PDC alone) (Fig. 7C) but not by 300 μM MK-801, thus supporting the involvement of AMPA–KA receptors. The coapplication of 300 μM cyclothiazide to prevent a putative desensitization of the glutamate receptors involved in this effect failed to modify the effect of l-trans-PDC, suggesting that the transient nature of the 5-HT increase was likely attributable to presynaptic adaptive mechanisms after glutamate uptake blockade.

Effects of DOI on the firing rate of 5-HT neurons in the DR

The systemic administration of DOI to chloral hydrate-anesthetized rats induced a dose-dependent reduction of the firing rate of 5-HT neurons in the DR (Fig. 8). Two putative subgroups of neurons were identified, according to the maximal effect of DOI. In 7 of 12 neurons recorded, DOI fully suppressed the serotonergic activity at low doses. In the rest ($n = 5$), the effect of DOI on serotonergic cell firing was partial, with a

Figure 6. Colocalization of 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors in individual cortical neurons. A shows a representative mPFC section depicting individual cortical neurons expressing 5-HT$_{1A}$-like immunoreactivity; B shows the same section with 5-HT$_{2A}$-like immunoreactivity; C depicts an overlay showing colocalization of 5-HT$_{2A}$ and 5-HT$_{1A}$ receptor-like immunoreactivity in individual cortical neurons. Magnification, 400×. D shows a high-power (1000×) view of individual cortical neurons expressing 5-HT$_{1A}$-like immunoreactivity; E shows the same section with 5-HT$_{2A}$-like immunoreactivity; F depicts an overlay of 5-HT$_{1A}$ and 5-HT$_{2A}$-like immunoreactivity. G shows a low-power (400×) view of cortical neurons visualized with a guinea pig 5-HT$_{1A}$ receptor antibody, whereas H shows the same section visualized with a monoclonal 5-HT$_{2A}$ receptor antibody, with colocalization indicated by arrowheads. I shows a typical section incubated only with secondary antibodies (400×).
microdialysis experiments, the application of bicuculline (BIC; 300 μg/kg, i.v.) reversed the administration of WAY 100635 (10 μg/kg, i.v.) returned firing rate to baseline (Fig. 8D). The inhibitory effect of DOI on serotonergic cell firing was also reversed by the administration of the GABA<sub>A</sub> receptor antagonist picrotoxinin (1 mg/kg, i.v.) (Fig. 8E).

To assess whether the increased mPFC release of 5-HT induced by local DOI application was caused by the activation of pyramidal neurons projecting to the DR (thus enhancing the activity of ascending 5-HT neurons), we locally infused DOI in the mPFC while recording 5-HT neurons in the DR. In 5 of 10 serotonergic neurons in the DR, the application of DOI in mPFC (200 μM, 200 nl applied in 1 min) enhanced their firing rate (to 194 ± 45% of baseline; range, 137–371%; p < 0.02) (Fig. 9A). One additional neuron had a marginal increase (115% of baseline), and the rest were unaffected by the application of DOI in mPFC (Fig. 9B). The effects of a second dose of DOI (5 min after the first one) were examined in seven neurons. Of these, three responded with a further increase in DR firing (from 155 to 260% of baseline). The infusion of vehicle in mPFC did not affect the firing rate of DR 5-HT neurons (data not shown).

Effect of the systemic administration of DOI on 5-HT release in medial prefrontal cortex

The systemic administration of DOI (1 mg/kg, s.c.) to chloral hydrate-anesthetized rats reduced the extracellular 5-HT concentration in the mPFC to a maximal effect of 33 ± 8% of baseline 2 hr after administration (n = 4; p < 0.00001) (Fig. 10). The effect of DOI was reversed by the administration of WAY 100635 (0.5 mg/kg, s.c.) which returned 5-HT levels to above baseline (127 ± 6%; p < 0.00001) (Fig. 10).

DISCUSSION

The present results show that the stimulation of 5-HT<sub>2A</sub> receptors by the hallucinogen DOI markedly affects serotonergic transmission. Systemic DOI administration reduced the activity of DR 5-HT neurons and their release by mPFC terminals. Conversely, the selective activation of prefrontal 5-HT<sub>2A</sub> receptors raised the local 5-HT release and the firing of a subgroup of 5-HT neurons.

Despite the low density of 5-HT<sub>2A</sub> receptors in the DR and adjacent areas (Pompeiano et al., 1994; Fay and Kubin, 2000), bath application of 5-HT and DOI increased a 5-HT 2A receptor agonist 8-OH-DPAT suppressed serotonergic cell firing in both subgroups of neurons (Fig. 8C,D). The further administration of WAY 100635 (10 μg/kg, i.v.) returned firing rate to baseline (Fig. 8D). The inhibitory effect of DOI on serotonergic cell firing was also reversed by the administration of the GABA<sub>A</sub> receptor antagonist picrotoxinin (1 mg/kg, i.v.) (Fig. 8E).

mPFC (n = 4–5). l-trans-PDC (4 mM) significantly increased the 5-HT release. This effect was prevented by the coinfusion of NBQX but not by MK-801 (300 μM each). Cyclothiazide (300 μM), a drug that prevents the desensitization of AMPA receptors, did not affect the time course or potentiate the effect of l-trans-PDC, suggesting that the marked tachyphylaxis of the effect is possibly caused by a presynaptic adaptive change in glutamate synthesis and release resulting from prolonged inhibition of reuptake. /p < 0.05 versus baseline; *p < 0.05 versus l-trans-PDC alone; Duncan test post-ANOVA.
Thus, the activation by DOI of 5-HT 2A receptors on GABAergic afferents to 5-HT neurons might mediate its inhibitory effect on 5-HT neurons (Wright et al., 1990; Garratt et al., 1991; this work) inasmuch as the activation of prefrontal 5-HT2A receptors induced the opposite effect. The involvement of 5-HT2A and GABAA receptors in the DOI-induced inhibition of serotonergic cell firing is supported by the reversal induced by M100907 and picrotoxinin. Interestingly, DOI partially inhibited the firing activity of some 5-HT neurons, whereas fully suppressed other 5-HT neurons, with comparable ED50 values. This difference suggests either a partial agonist action of DOI or the involvement of two 5-HT2A receptor populations (e.g., in midbrain and mPFC) with opposed effects on the cell firing of some neurons.

Unlike its systemic administration (Wright et al., 1990; Gobert and Millan, 1999; this work) the local application of DOI enhanced the 5-HT release in mPFC. We exclude a direct releasing action of DOI on serotonergic nerve endings (fenfluramine-like)
because this is insensitive to TTX (Carboni and Di Chiara, 1989). Moreover, 5-HT neurons do not express 5-HT2A receptors (Pompéiano et al., 1994; Fay and Kubin, 2000) (J. Serrats, R. Corts, and G. Mengod, unpublished observations). Some cells in the DR (not known to be serotonergic) express 5-HT2C receptors (Clemett et al., 2000). However, the lack of antagonism by SB 242084 excludes the involvement of 5-HT2C receptors in the effect of DOI.

Several lines of evidence suggest that DOI increases the 5-HT release in mPFC indirectly, through the stimulation of glutamatergic afferents from midline thalamus. The role of 5-HT2A receptors on pyramidal neurons and GABA interneurons has not been elucidated. The activation of prefrontal 5-HT2A receptors enhances the release of glutamate, which acts on pyramidal AMPA receptors and increases impulse flow in layer V pyramidal neurons projecting to the DR, thus resulting in an increased serotonergic activity and 5-HT release in mPFC. Additionally, glutamate might activate terminal AMPA–KA receptors in 5-HT terminals and increase 5-HT release in a local manner. This might imply axo-axonic contacts between glutamatergic and serotonergic axons, not yet established anatomically but for which there is functional evidence (see Discussion). The activation of inhibitory 5-HT1A receptors on pyramidal neurons counteracts the effects of DOI and decreases 5-HT release, likely by reducing DOI excitatory effects on descending inputs to the DR, because the selective activation of prefrontal 5-HT1A receptors reduces the firing rate of DR 5-HT neurons (Celada et al., 2001). The firing-suppressant action of systemic DOI administration on DR 5-HT neurons is possibly accounted for by an action on local GABAergic elements inhibiting 5-HT neurons (Liu et al., 2000). This effect would also account for the inhibition of 5-HT release observed after systemic administration of DOI.

Figure 10. The systemic administration of DOI (1 mg/kg, s.c.) markedly reduced the 5-HT output in mPFC of chloral hydrate-anesthetized rats (n = 4). The selective 5-HT2A receptor antagonist M100907 (0.5 mg/kg, s.c.) fully reversed the effect of DOI. Arrows mark injections. *p < 0.05 versus baseline; †p < 0.05 versus DOI alone; Duncan test post-ANOVA.

Figure 11. Scheme of the putative presynaptic and postsynaptic actions of DOI. In the mPFC, DOI increases 5-HT release. This effect involves the activation of prefrontal 5-HT2A receptors, possibly on glutamatergic afferents from midline thalamus. The role of 5-HT2A receptors on pyramidal neurons and GABA interneurons has not been elucidated. The activation of prefrontal 5-HT2A receptors enhances the release of glutamate, which acts on pyramidal AMPA receptors and increases impulse flow in layer V pyramidal neurons projecting to the DR, thus resulting in an increased serotonergic activity and 5-HT release in mPFC. Additionally, glutamate might activate terminal AMPA–KA receptors in 5-HT terminals and increase 5-HT release in a local manner. This might imply axo-axonic contacts between glutamatergic and serotonergic axons, not yet established anatomically but for which there is functional evidence (see Discussion). The activation of inhibitory 5-HT1A receptors on pyramidal neurons counteracts the effects of DOI and decreases 5-HT release, likely by reducing DOI excitatory effects on descending inputs to the DR, because the selective activation of prefrontal 5-HT1A receptors reduces the firing rate of DR 5-HT neurons (Celada et al., 2001). The firing-suppressant action of systemic DOI administration on DR 5-HT neurons is possibly accounted for by an action on local GABAergic elements inhibiting 5-HT neurons (Liu et al., 2000). This effect would also account for the inhibition of 5-HT release observed after systemic administration of DOI.
transmitter–synaptic pool (Timmerman and Westerink, 1997) and depend to a large extent on a cysteine–glutamate exchanger (Baker and Kalivas, 2000). Our own data agree with this view, because extracellular glutamate was largely insensitive to manipulations known to alter synaptic transmission (M.V. Puig, A. Adell, P. Celada, and F. Artigas, unpublished observations).

The present results accord with previous in vitro work in mPFC slices showing that the activation of local 5-HT2A receptors increases spontaneous EPSCs in layer V pyramidal neurons by an AMPA-dependent mechanism (Aghajanian and Marek, 1997, 1999b, 2000; Marek et al., 2000). However, none of these previous studies reported overt depolarization in pyramidal neurons by 5-HT or DOI. In contrast, we observed that the stimulation of 5-HT2A receptors in mPFC by DOI increased the firing of identified 5-HT neurons in the DR. Likewise, in pilot experiments, systemic DOI administration activated projection neurons in the mPFC (Puig, Celada, and Artigas, unpublished observations). This strongly suggests that DOI increases impulse flow in pyramidal neurons, including those projecting to the DR. The discrepancy between the present results and those of Aghajanian and Marek (2000) may be attributable to methodological differences, perhaps reflecting the loss of active glutamatergic inputs in the slice preparation used by these authors.

The cellular source or sources of glutamate and the precise localization or localizations of 5-HT2A receptors accounting for this effect are debated. 5-HT2A receptors occur in three different populations in frontal cortex: pyramidal neurons (major 5-HT2A−containing neurons), GABAergic interneurons, and axon terminals (Willins et al., 1997; Jakab and Goldman-Rakic, 1998, 2000; Xu and Pandey, 2000). Layer V contains the somata of pyramidal neurons and dense bundles of apical dendrites, highly enriched in 5-HT2A receptors (Willins et al., 1997; Jakab and Goldman-Rakic, 1998; Xu and Pandey, 2000) (Fig. 6). GABA interneurons are the main target of 5-HT terminals in monkey prefrontal cortex (Smiley and Goldman-Rakic, 1996) and DOI increases extracellular GABA in rat brain (Abi-Saab et al., 1999). However, 5-HT2A receptors on thalamic afferents have been suggested to mediate DOI effects. Hence, μ-opioid agonists and lesions of the medial thalamus attenuated the 5-HT-induced EPSCs in layer V pyramidal neurons (Marek and Aghajanian, 1998; Marek et al., 2001), which suggests a presynaptic site of action. Moreover, the systemic administration of DOI increased Fos expression in superficial layer V and above, an effect also dependent on the integrity of thalamic inputs onto mPFC (Scruogs et al., 2000). However, most Fos-expressing neurons were nonpyramidal, whereas 5-HT induces spontaneous EPSCs in layer V pyramidal neurons by activation of 5-HT2A receptors (Lambe et al., 2000), which makes uncertain the relationship between both observations. The present data suggest that glutamate from thalamic afferents to mPFC enhances 5-HT release, although it remains to be determined whether this is the glutamate source involved in the local DOI effects.

Indeed, a preferential action of DOI on terminal 5-HT2A receptors would leave the dense population of pyramidal 5-HT2A receptors without a significant role. Interestingly, the DOI-induced increase in 5-HT release was potently counteracted by co-persorption of the selective 5-HT1A receptor agonist BAY × 3702. 5-HT1A and 5-HT2A receptors colocalize on cortical pyramidal neurons (Fig. 6A–J) and have opposite effects on their excitability (Araneda and Andrade, 1991). The apparent absence of 5-HT1A receptors on 5-HT terminals (Pompeiano et al., 1992; Kia et al., 1996) excludes a presynaptic action of BAY × 3702. Pyramidal neurons in mPFC project to and control the activity of DR 5-HT neurons (see introductory remarks). Thus, DOI and BAY × 3702 may modulate the release of 5-HT in mPFC by increasing and decreasing, respectively, the activity of descending excitatory pathways to DR neurons projecting in turn to the mPFC (Fig. 11). Thus, the application of DOI and 8-OH-DPAT in mPFC increased and decreased, respectively, the firing rate of DR 5-HT cells (Celada et al., 2001; this work). However, the present study cannot determine whether the effect of DOI on pyramidal activity is direct (via pyramidal 5-HT2A receptors) or indirect (via terminal 5-HT2A receptors, increase in glutamate and activation of AMPA receptors on pyramidal neurons) (Fig. 11). Additionally, the activation of terminal AMPA receptors increases transmitter release in vivo and in vitro (Ohta et al., 1994; Whitting et al., 1994; Maione et al., 1997, Tao et al., 1997; Lockhart et al., 2000). This raises the possibility that a second (local) mechanism may also modulate 5-HT release independently of DR activation.

5-HT2A receptors play a crucial role in the filtering of inputs reaching the somata of pyramidal neurons because their activation increases neuronal excitability. The activation by DOI of prefrontal 5-HT1A receptors increases the excitability of pyramidal neurons. At the same time, its action on (possibly) midbrain 5-HT2A receptors (Liu et al., 2000) reduces 5-HT cell firing and 5-HT release, which should result in a diminished activation of inhibitory pyramidal 5-HT1A receptors. Hence, the net balance of both effects would be an increased firing activity of projection neurons in layer V, as preliminarily observed (Puig, Celada, and Artigas, unpublished observations). This effect may mediate the hallucinogenic action of DOI. Hence, the blockade of 5-HT2A receptors or the direct or functional 5-HT1A receptor agonism exerted by atypical antipsychotics (Meltzer, 1999; Ichikawa et al., 2001) might be viewed as an attempt to restore the physiological balance between excitatory and inhibitory inputs onto prefrontal pyramidal neurons. Given the opposing roles of 5-HT1A and 5-HT2A receptors on the output of layer V pyramidal neurons, compounds with 5-HT2A antagonism and 5-HT1A agonism may be particularly useful to treat hallucinations and, more generally, positive symptoms in schizophrenia. Likewise, their direct action at 5-HT1A receptors might also improve negative (deficit) symptoms.

Further work is required to clarify the relative role of local and distal mechanisms in the control of 5-HT release by prefrontal 5-HT2A receptors and the precise localization of 5-HT2A receptors responsible for this effect of DOI.

REFERENCES


Aghajanian GK, Wang RY (1977) Habenular and other midbrain raphe