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RESEARCH ARTICLE

5-HT_{2A} receptor inactivation potentiates the acute antidepressant-like activity of escitalopram: involvement of the noradrenergic system

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Abstract Evidence suggests that the serotonin 2A receptor (5-HT_{2A}R) modulates the therapeutic activity of selective serotonin reuptake inhibitors (SSRIs). Indeed, among the genetic factors known to influence the individual response to antidepressants, the *HTR2A* gene has been associated with SSRIs response in depressed patients. However, in these pharmacogenetic studies, the consequences of *HTR2A* gene polymorphisms on 5-HT_{2A}R expression or function are lacking and the precise role of this receptor is still matter of debate. This study examined the effect of 5-HT_{2A}R agonism or antagonism with DOI and MDL100907, respectively, on the serotonergic system and the antidepressant-like activity of the SSRI escitalopram in mouse. The 5-HT_{2A}R agonist DOI decreased the firing rate of 5-HT neurons in the dorsal raphe (DR) nucleus of 5-$HT_{2A}R^{+/+}$ anesthetized mice. This inhibitory response persisted in 5-$HT_{2C}R^{-/-}$ but was completely blunted in 5-$HT_{2A}R^{-/-}$ mutants. Moreover, the suppressant effect of DOI on DR 5-HT neuronal activity in 5-$HT_{2A}R^{+/+}$ mice was attenuated by the loss of noradrenergic neurons induced by the neurotoxin DSP4. Conversely, in 5-$HT_{2A}R^{+/+}$ mice, the pharmacological inactivation of the 5-HT_{2A}R by the selective antagonist MDL100907 reversed escitalopram-induced decrease in DR 5-HT neuronal activity. Remarkably, in microdialysis experiments, a single injection of escitalopram increased cortical extracellular 5-HT, but not NE, levels in awake 5-$HT_{2A}R^{+/+}$ mice. Although the addition of MDL100907 did not potentiate 5-HT neurotransmission, it allowed escitalopram to increase cortical NE outflow and consequently to elicit an antidepressant-like effect in the forced swimming test. These results suggest that the blockade of the 5-HT_{2A}R may strengthen the antidepressant-like effect of escitalopram by facilitating the enhancement of the brain NE transmission. They provide support for the use of atypical antipsychotics with SSRIs as a relevant antidepressant augmentation strategy.

Keywords 5-HT · 5-HT_{2A} receptor · Antidepressants · Antipsychotics · Norepinephrine · SSRIs

Abbreviations

5-HT	Serotonin
5-HT_{2A}R	Serotonin 2A receptor
AAP	Atypical antipsychotic
DR	Dorsal raphe
FST	Forced swimming test
LC	Locus coeruleus
MDD	Major depressive disorder
NE	Norepinephrine
NET	Norepinephrine transporter
SERT	Serotonin transporter
SSRI	Selective serotonin reuptake inhibitor

Introduction

Substantial scientific evidence has linked the serotonin 2A receptor (5-HT_{2A}R) to the therapeutic response of antidepressant drugs (see Quesseveur et al. 2012 for review). For example, variations in the gene encoding for the 5-HT_{2A}R

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have been associated with the treatment outcome of selective serotonin reuptake inhibitors (SSRIs) in major depressive disorders (MDD) (Viikki et al. 2011; Lucae et al. 2010; Kishi et al. 2010; Kato et al. 2009; Peters et al. 2009; Wilkie et al. 2009; McMahon et al. 2006; Choi et al. 2005). A recent pharmacogenetic study also pointed out that specific single nucleotide polymorphisms related with 5-HT_{2A}R signaling pathways might influence the therapeutic activity of SSRIs in Chinese patients with MDD (Li et al. 2012). Unfortunately, in most cases, the consequences of these polymorphisms on 5-HT_{2A}R expression and/or function are lacking. Therefore, it is at this point uncertain whether a lower or a higher neurotransmission at the 5-HT_{2A}R is responsible for the apparent changes in antidepressant response. The observation that low doses of atypical antipsychotics (AAPs) that block the 5-HT_{2A}R display antidepressant activity and are effective adjuncts in depressed patients responding inadequately to SSRIs (Blier and Blondeau 2011; Blier and Szabo 2005) supports the hypothesis that inactivation of this receptor would facilitate the antidepressant response. Accordingly, it might be hypothesized that the progressive therapeutic activity of chronic treatment with SSRIs would be accompanied by a down-regulation of 5-HT_{2A}R-mediated neurotransmission (Meyer et al. 2001). However, the findings in support of this assumption are still matter of debate (Zanardi et al. 2001; Massou et al. 1997).

Preclinical studies are of great relevance to further elucidate the molecular mechanisms involved in 5-HT_{2A}R-mediated neurotransmission under basal conditions and in response to SSRIs. In vivo recordings in the dorsal raphe (DR) show that systemic administration of the preferential 5-HT_{2A}R agonist DOI attenuates the firing rate of 5-HT neurons (Boothman and Sharp 2005; Boothman et al. 2003; Bortolozzi et al. 2003; Martin-Ruiz et al. 2001; Garratt et al. 1991; Wright et al. 1990) and reduces basal extracellular 5-HT levels at nerve terminals (Martin-Ruiz et al. 2001). Behavioral studies demonstrate that 5-HT_{2A}R antagonists elicit antidepressant-like activities (Zaniewska et al. 2010; Pandey et al. 2010; Patel et al. 2004; Sibille et al. 1997) and potentiate the antidepressant-like responses of fluoxetine in rodents (Marek et al. 2005). However, in contradiction with these findings, 5-HT_{2A}R antagonists such as MDL100907 or the AAPs risperidone, olanzapine, clozapine and quetiapine fail to enhance SSRIs-induced increase in extracellular 5-HT levels (Huang et al. 2006; Marek et al. 2005; Denys et al. 2004; Zhang et al. 2000). Indeed, given the in vivo inhibitory effect of 5-HT_{2A}R on the serotonergic system, one would expect that 5-HT_{2A}R antagonists could prevent acute SSRIs-induced inhibition of DR 5-HT firing and consequently potentiate 5-HT release at nerve terminals. The absence of correlation between behavioral and neurochemical findings suggests the involvement of another neurotransmitter system to explain that an AAP augments the antidepressant activity of an SSRI. Among the multiple mechanisms of antidepressants, the role of the brain noradrenergic system arising from the locus coeruleus (LC) has been well studied (Dell'Osso et al. 2011; Brunello et al. 2002). Serotonergic and noradrenergic neurons have reciprocal anatomical and functional interactions (Guiard et al. 2008). Thus, the increase in extracellular 5-HT levels induced by escitalopram dampens the firing activity of LC NE neurons (Dremencov et al. 2007a) probably via stimulation of 5-HT_{2A}R on GABAergic interneurons, which results in increased GABA release and inhibition of the LC activity (Dremencov et al. 2007a; Szabo and Blier 2001; Szabo et al. 1999).

Based on the above observations, we hypothesized that increased central NE transmission may contribute to 5-HT_{2A}R antagonist-mediated augmentation of the antidepressant-like activity of SSRIs. Here, we address this possibility by an integrated pharmacological, genetic and NE neurons lesion approach in mice including responses measured at the level of neuronal firing activity, extracellular neurotransmitter concentrations and behavior.

Materials and methods

Animals

Adult male 5-HT_{2A}R (Weisstaub et al. 2006) and 5-HT_{2C}R knock-out mice (Tecott et al. 1995) and their wild-type littermates were breed in our animal care facility. All mice were 5 weeks old and weighed 20–25 g at the beginning of the experiment. They were maintained on a 12-h light/dark cycle schedule (lights on at 6:00 A.M.) and housed in groups of five. Food and water were provided ad libitum. Behavioral testing occurred during the light phase between 8:00 A.M. and 10:00 A.M. All testing was conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee (Council directive 87–848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; permission 92-256B to D.J.D.).

Drugs

Escitalopram oxalate (H. Lundbeck A/S, Denmark) dissolved in DMSO 5 % was administered at a dose of 4 mg/kg. DSP4 (25 and 50 mg/kg), desipramine (8 mg/kg), DOI (1–10 mg/kg) and WAY100635 (0.5 mg/kg) were all dissolved in NaCl 9 % and purchased from Sigma-Aldrich (L'Isle d'Abeau Chesnes, Saint-Quentin Fallavier, France). MDL100907 was dissolved in hydroxy propyl-beta-cyclodextrin 20 % and administered at a dose of 2 mg/kg.

Lesion

The lesion of noradrenergic neurons was performed in adult mice through an intraperitoneal (i.p.) administration of DSP4 (25 or 50 mg/kg) and based on the experience reported by Cassano et al. (2009), we decided to undertake the recordings of DR 5-HT neurons 7 days after DSP4 administration. Using this procedure, it has been reported that NE levels were decreased by 48 and 62 % in the LC, by 58 and 77 % in the hippocampus and by 33 and 65 % in the prefrontal cortex following DSP4 25 and 50 mg/kg, respectively. Importantly, in these brain regions, DSP4 25 and 50 mg/kg had no major impact on 5-HT and DA levels (Cassano et al. 2009).

In vivo electrophysiology of DR 5-HT neurons

Mice were anaesthetized with chloral hydrate (400 m/kg; i.p.) and mounted in a stereotaxic frame. Additional anesthesia (50–100 mg/kg; i.p.) was given as necessary to maintain a full anesthetic state, characterized by the absence of response to a nociceptive tail pinch. Body temperature was maintained at 37 °C throughout the experiments using a thermistor-controlled heating pad (Astro-Med, Elancourt, France). The extracellular recordings of the 5-HT neurons in the DR were performed using single-barreled glass micropipettes (Stoelting, Dublin, Ireland) pulled on a pipette puller (Narishige, Tokyo, Japan) preloaded with a 2 M NaCl solution. Their impedance typically ranged between 2.5 and 5 MΩ. The single-barreled glass micropipettes were positioned 0.2–0.5 mm posterior to the interaural line on the midline and lowered using a hydraulic micropositioner (Kopf Instruments) into the DR, usually attained at a depth between 2.5 and 3.5 mm from the brain surface (Hof et al. 2000). To increase the signal-to-noise ratio, we used a current amplifier (BAK Electronics, Mount Airy, MD, USA) connected to the active filter Humbug (Quest scientific, DIPSI, Châtillon, France). The presumed DR 5-HT neurons were then identified according to the criteria of Aghajanian and Vandermaelen (1982), that is, a slow (0.5–2.5 Hz) and regular firing rate and long-duration (2–5 ms) bi- or triphasic extracellular waveform. All neuronal activity was recorded in real time using Spike2 software (Cambridge Electronic Design, Cambridge, UK), which was also used to analyze neurons offline. For all dose–response curves, only one neuron was recorded and tested from each animal.

In vivo intracerebral microdialysis

Mice anaesthetized with chloral hydrate (400 m/kg; i.p.) were bilaterally implanted with probes (CMA7 model, Carnegie Medicin, Stockholm, Sweden) located in the frontal cortex (FCx). Stereotaxic coordinates in mm from bregma were AP + 1.6, $L \pm 1.3$ and $V = -1.6$ (Hof et al. 2000). Animals were allowed to recover from the surgery overnight. The next day, ≈20 h after surgery, the probes were continuously perfused with aCSF (composition in mmol/L: NaCl 147, KCl 3.5, $CaCl_2$ 1.26, $MgCl_2$ 1.2, NaH_2PO_4 1.0, pH 7.4 ± 0.2) at a flow rate of 1.5 µl/min using CMA/100 pump (Carnegie Medicin, Stockholm, Sweden), while animals were awake and freely moving in their cage. The 5-HT and NE microdialysis samples were collected from different animals. After a 1-h stabilization period of the aCSF perfusion, four 15-min fractions were collected to determine monoamine baseline values for the individual mice (mean ± SEM of 4 values corresponding to corresponding to $t_{0\ \mathrm{min}}$). Drugs effects were expressed as percentage of the baseline value for the individual mouse. Effects of systemic administration of MDL100907 or its vehicle were evaluated in fractions $t_{15\ \mathrm{min}}$ to $t_{30\ \mathrm{min}}$. Subsequent dialysate samples were then collected after systemic administration of escitalopram for a 0–120-min period, that is, t_{45} to t_{150}. The samples were analyzed for 5-HT and NE by a High Performance Liquid Chromatography (HPLC) system (Ultremex C18, 4.6 × 75 mm, particle size 3 µm; Phenomenex, France) coupled to an amperometric detector (Decade II, Antec Leyden, Alpha M.O.S, France). The mobile phase for 5-HT and NE contained 107 or 100 mM NaH2PO4, 140 or 151 µM disodium EDTA, 0.77 or 3 mM I-octanesulphonic acid, respectively, and 20 % (v/v) methanol (pH adjusted between 4.1 and 4.3 with phosphoric acid). Its flow rate through the HPLC column was set at 0.7 mL/min using a ISO-3100A pump (Thermo Fischer Scientific group, Dionex, France). The limit of sensitivity for 5-HT or NE was ≈0.5 fmol/sample (signal-to-noise ratio = 2).

Behavior

The forced swimming test (FST) was performed in a separate group of mice. We used a modified version of the FST procedure with an increased sensitivity to antidepressant drugs (Holick et al. 2008). Briefly, mice were placed into clear plastic buckets, 20 cm in diameter and 23 cm deep filled 2/3 with water at 23–25 °C. Automated behavioral scoring was done using the software X'PERT FST developed by Bioseb (Vitrolles, France) (Rainer et al. 2012). Each bucket was instrumented with a sensor recording the vibrations produced by the movements of the mouse, and the behavior was recorded by a video. The system synchronizes the position of the mouse from the data calculated from the video recording and the vibration data. This information allows the system to compute characteristic values (based on speed of the animal, as well as different frequencies and powers of the vibrations through Fast Fourier Transform calculation) describing the animal behavior every second. Dependent variables were swimming and climbing duration. The test was performed 30 min after escitalopram administration.

Statistical analysis

Statistical analyses were performed using the computer software StatView 5.0. (Abacus Concepts, Inc., Berkley, CA, USA). Statistical comparisons of the effects of DOI in 5-$HT_{2A}R^{-/-}$, 5-$HT_{2C}R^{-/-}$ mice and their wild-type littermates were made using a two-way analysis with strain and genotype as main factors followed by Fisher's protected least significance difference post hoc test when appropriate. A one-way analysis on treatment factor was performed to compare the effect of DSP4 25 and 50 mg/kg to vehicle in 5-$HT_{2A}R^{+/+}$ mice. A one-way analysis on treatment factor was also applied to compare the effect of vehicle, escitalopram or the combination MDL100907 and escitalopram in 5-$HT_{2A}R^{+/+}$ mice submitted to microdialysis or the FST. Paired Student's *t* test was applied to determine the effect of MDL100907 on escitalopram-induced decrease in DR 5-HT neuronal activity in 5-$HT_{2A}R^{+/+}$ mice. The level of statistical significance was set at $p < 0.05$.

Results

Effects of DOI on DR 5-HT neuronal activity in 5-$HT_{2A}R^{-/-}$ and 5-$HT_{2C}R^{-/-}$ and corresponding wild-type mice

The effect of the preferential 5-$HT_{2A}R$ agonist DOI on DR 5-HT neuronal activity was investigated using cumulative dosing design (i.e., 1, 2, 4, 8 and 10 mg/kg; s.c.). A two-way ANOVA (strain × genotype) revealed a significant main drug effect on strain [$F_{1,17} = 5.12$; $p = 0.037$], genotype [$F_{1,17} = 10.13$; $p = 0.005$] and stain x genotype interaction [$F_{1,17} = 5.39$; $p = 0.032$]. In both control 5-$HT_{2A}R^{+/+}$ and 5-$HT_{2C}R^{+/+}$ mice, the s.c. administration of DOI elicited a dose-dependent inhibition of DR 5-HT firing rate (Fig. 1a–c). Of the 5

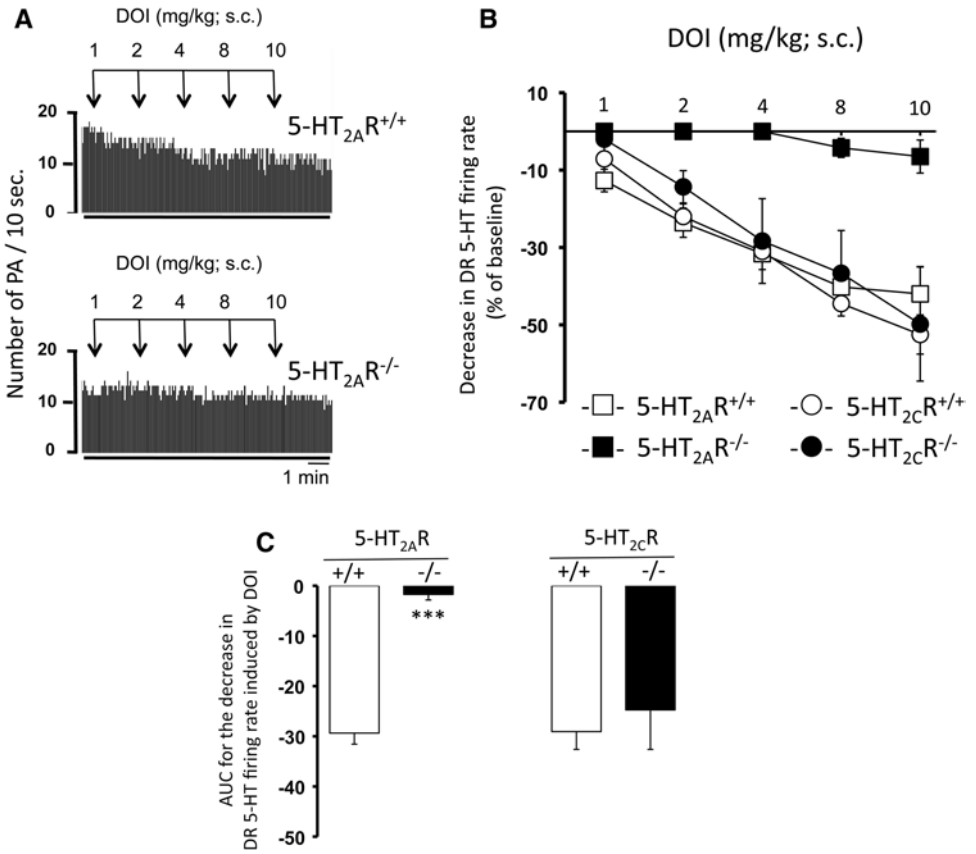

Fig. 1 Effects of the 5-$HT_{2A}R$ agonist DOI on the firing rate of DR 5-HT neurons in 5-$HT_{2A}R^{-/-}$ and 5-$HT_{2C}R^{-/-}$ mice and their wild-type littermates. DOI was administered using a cumulative dosing regimen, that is, all mice received 1, 2, 4, 8 and 10 mg/kg (s.c.) with a 3-min interval between each injection. **a** Examples of integrated firing histograms showing the effects of cumulative doses of DOI on the spontaneous activity of DR 5-HT neurons in 5-$HT_{2A}R^{+/+}$ (*upper panel*) and 5-$HT_{2A}R^{-/-}$ mice (*lower panel*). **b** Data presented as means ± SEM of percent decrease in basal firing rate in 5-$HT_{2A}R^{-/-}$ mice (*filled square*), 5-$HT_{2C}R^{-/-}$ mice (*filled circle*) and their wild-type 5-$HT_{2A}R^{+/+}$ (*open square*) and 5-$HT_{2C}R^{+/+}$ (*open circle*) littermates. **c** Data presented as means ± SEM of the integrated area under the curve (AUC) of the percent decrease in basal firing rate induced by DOI in 5-$HT_{2A}R^{-/-}$ and 5-$HT_{2C}R^{-/-}$ mice and their wild-type littermates. ($n = 5$–6 mice per group). ***$p < 0.001$: significantly different from 5-$HT_{2A}R^{+/+}$ mice

presumed serotonergic neurons recorded in 5-$HT_{2A}R^{+/+}$ or 5-$HT_{2C}R^{+/+}$ mice, all of them were partially inhibited by DOI. The maximal decrease in basal firing rate was observed at the highest dose tested (−42 ± 7 and −52 ± 5 %, respectively). In 5-$HT_{2A}^{-/-}$ mice, the inhibitory effect of DOI on DR 5-HT firing rate was completely blunted compared to their wild-type littermates ($p < 0.001$; Fig. 1a–c). Of the 5 neurons recorded in 5-$HT_{2A}R^{-/-}$ mice, only 2 displayed a marginal decrease in basal firing rate at the highest dose of DOI tested. In marked contrast, the dose-dependent inhibitory effect of DOI persisted in 5-$HT_{2C}R^{-/-}$ mice and was not different from that observed in their wild-type littermates ($p = 0.65$; Fig. 1b, c). Of the 6 neurons recorded in 5-$HT_{2C}R^{+/+}$ or 5-$HT_{2C}R^{-/-}$ mice, 5 were inhibited by DOI and the maximal decrease in basal firing rate was observed at the highest dose tested (−52 ± 5 % and −49 ± 14 %, respectively).

Effects of DOI on DR 5-HT neuronal activity in 5-$HT_{2A}R^{+/+}$ mice pre-treated with the noradrenergic neurotoxin DSP4

Using the same dosing regimen as described above, pretreatment with the noradrenergic neurotoxin, DSP4 to 5-$HT_{2A}R^{+/+}$ mice, significantly attenuated the effect of cumulative s.c. dosing of DOI on DR 5-HT neuron firing rate. A one-way ANOVA revealed a significant main effect of treatment factor [$F_{2,12} = 7.05$; $p = 0.009$]. In 5-$HT_{2A}^{+/+}$ mice, DSP4 25 and 50 mg/kg significantly attenuated the inhibitory effect of DOI on DR 5-HT firing rate compared to sham mice ($p = 0.01$ and $p = 0.004$, respectively, Fig. 2a–c). Of the 5 neurons recorded in each group of DSP4 pre-treated mice, all of them were inhibited by DOI; albeit to a lower extend than in sham mice. No significant differences between DSP4 25 and DSP4 50 mg/kg were observed on the electrophysiological effects of DOI ($p = 0.66$; Fig. 2a–c).

Effects of MDL100907 on escitalopram's effects on DR 5-HT neuronal activity, cortical extracellular 5-HT and NE levels and behavioral responses in the FST in 5-$HT_{2A}R^{+/+}$mice

In order to unveil the specific contribution of the 5-$HT_{2A}R$ in the inhibitory effect of escitalopram on DR 5-HT neuronal activity, mice were pre-treated with the 5-$HT_{1A}R$ antagonist WAY100635. In these conditions, MDL100907 significantly counteracted escitalopram-induced decrease in DR 5-HT neurons ($t_{1,8} = 0.49$; Fig. 3).

In microdialysis studies, escitalopram significantly increased basal cortical extracellular 5-HT levels compared to control group (AUC values: 308 ± 45 vs. 98 ± 5, respectively). Pretreatment with the 5-$HT_{2A}R$ antagonist MDL100907 had no significant effect on escitalopram response (AUC value: 338 ± 40; one-way ANOVA,

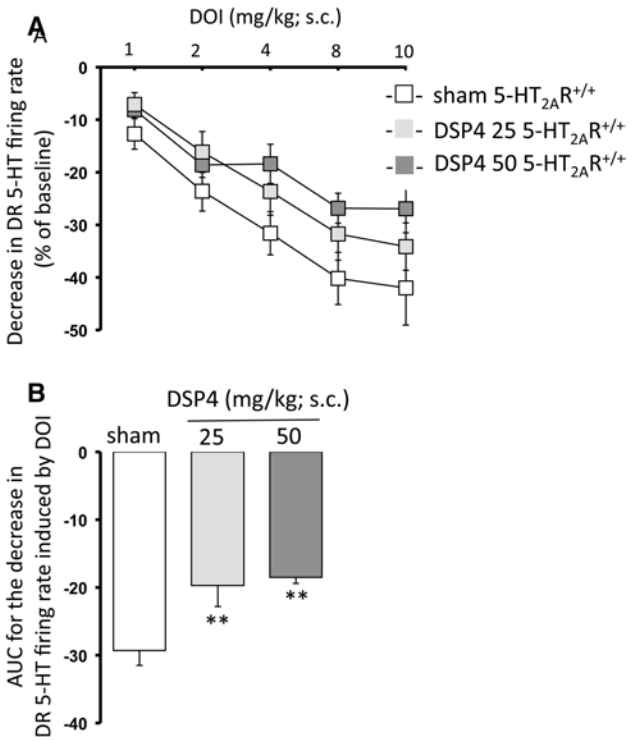

Fig. 2 Effects of the 5-$HT_{2A}R$ agonist DOI on the firing rate of DR 5-HT neurons in 5-$HT_{2A}R^{+/+}$ mice pre-treated with the neurotoxin DSP4. DOI was administered using a cumulative dosing regimen, that is, all mice received 1, 2, 4, 8 and 10 mg/kg (s.c.) with a 3-min interval between each injection. **a** Data presented as means ± SEM of percent decrease in basal firing rate in 5-$HT_{2A}R^{+/+}$ mice pretreated with vehicle (sham: *open square*) or DSP4 at the dose 25 (*light gray square*) or 50 mg/kg (*dark gray square*) by the intraperitoneal route (i.p.). **b** Data presented as means ± SEM of the integrated area under the curve (AUC) of the percent decrease in basal firing rate in 5-$HT_{2A}^{+/+}$ sham and DSP4 pre-treated mice. ($n = 5$ mice per group). $^{**}p < 0.01$: significantly different from sham 5-$HT_{2A}R^{+/+}$ mice

$F_{2,26} = 25.94$; $p < 0.001$; Fig. 4a–b). In contrast, escitalopram did not modify basal cortical extracellular NE levels compared to control group (AUC values: 83 ± 11 vs. 101 ± 10, respectively). However, a significant increase in extracellular NE levels was detected in the group of mice treated with MDL100907 and escitalopram compared to escitalopram alone (AUC values: 126 ± 10 vs. 83 ± 11; $p < 0.05$; one-way ANOVA, $F_{2,23} = 3.6$; $p = 0.041$; Fig. 4c–d).

The swimming and climbing times were measured during the last 4 min of the 6-min test of 5-$HT_{2A}R^{+/+}$ mice. Whereas escitalopram alone failed to increase swimming time in the FST, the combination with MDL100907 significantly increased this parameter compared to mice administered the vehicle (one-way ANOVA, $F_{2,38} = 3.27$; $p = 0.049$; Fig. 5a). Climbing behavior was not significantly changed by drug treatment (one-way ANOVA, $F_{2,38} = 0.757$; $p = 0.47$; Fig. 5b) It is noteworthy that the NE reuptake inhibitor desipramine did not modify the swimming time

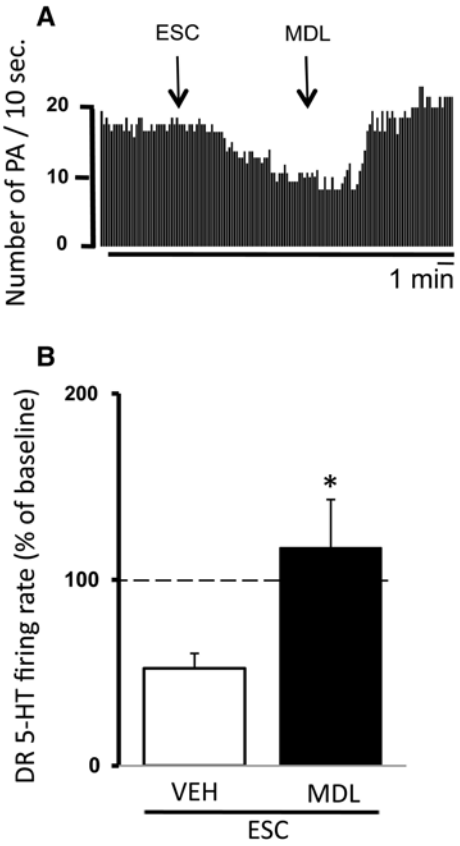

Fig. 3 Effects of the 5-HT_{2A}R antagonist MDL100907 on escitalopram-induced decrease in DR 5-HT neuronal activity in 5-$HT_{2A}R^{+/+}$ mice. MDL100907 (MDL: 2 mg/kg; s.c. was administered when the maximal inhibitory effect of the escitalopram (ESC: 4 mg/kg; s.c.) on DR 5-HT neuronal activity has been reached. These electrophysiological experiments were performed in the presence of the 5-HT_{1A}R antagonist WAY100635 (0.5 mg/kg) administered subcutaneously 15 min before escitalopram injection. **a** Example of integrated firing histograms showing the effects a single injection of MDL100907 on escitalopram-induced decrease in DR 5-HT neurons in 5-$HT_{2A}R^{+/+}$. **b** Data are presented as means ± SEM of percent DR 5-HT firing rate. ($n = 5$ mice per group). *$p < 0.05$: significantly different from vehicle pre-treated 5-$HT_{2A}R^{+/+}$ mice

compared to mice administered the vehicle ($t_{1,28} = 0.27$) but significantly increased the climbing time ($t_{1,28} = 0.042$) (data not shown).

Discussion

In summary, the present study confirms the literature reporting that the stimulation of the 5-HT_{2A}R reduces the neuronal activity of DR 5-HT neurons and extends this observation to the fact that such a decrease involves the noradrenergic system. This study also demonstrates that the 5-HT_{2A}R antagonist MDL100907 enhances the acute antidepressant-like effect of escitalopram as measured in the FST and suggest that increased cortical NE outflow contributes to this effect. Together these data provide a mechanistic rationale for the use of AAPs as adjunct to SSRIs to strengthen the therapeutic activity of this class of antidepressant drugs.

There is compelling clinical evidence for antidepressant efficacy of AAPs in particular in depressed patients responding inadequately to SSRIs (Thase et al. 2007; Shelton et al. 2005; Hirose and Ashby 2002; Ostroff and Nelson 1999; Ghaemi and Katzow 1999). In the last few years, aripiprazole, olanzapine and quetiapine have obtained FDA approvals for treatment of resistant depression in combination with SSRIs (DeBattista and Hawkins 2009) and the use of AAPs in this pathology has become one of the leading augmentation strategies in depression (Chen et al. 2011). Despite the clinical evidence, the mechanism of action of AAPs is still poorly understood. AAPs have a high affinity for the 5-HT_{2A}R in common, and a lot of the preclinical studies have focused on the role of this receptor subtype in the modulation of brain monoaminergic transmission.

The present study confirms that systemic administration of the preferential 5-HT_{2A}R agonist DOI to mice decreases the firing rate of DR 5-HT neurons as reported previously notably in rats (Boothman and Sharp 2005; Boothman et al. 2003; Bortolozzi et al. 2003; Wright et al. 1990; Martin-Ruiz et al. 2001; Garratt et al. 1991). Due to the limited selectivity of 5-HT_2R ligands for specific 5-HT_2R subtypes (Quesseveur et al. 2012), we also assessed whether the inhibitory effect of DOI on DR 5-HT neuronal activity specifically involves the 5-HT_{2A}R. The finding that DOI is practically devoid of electrophysiological effect on DR 5-HT firing rate in 5-$HT_{2A}R^{-/-}$ mice whereas its inhibitory activity persists in 5-$HT_{2C}R^{-/-}$ mice confirms the involvement of the 5-HT_{2A}R in DOI's response. There are different possible ways to explain how the activation of the 5-HT_{2A}R decreases the discharge of DR 5-HT neurons. Thus, the presence of 5-HT_{2A}R mRNA in the DR (Xie et al. 2002), together with the observation that the local application of DOI in this brain region induces a dose-dependent increase in the frequency of inhibitory postsynaptic currents (IPSCs) (Liu et al. 2000), supports the existence of a local inhibitory mechanism. In line with this, systemic administration of DOI increases c-fos immunoreactivity in GABAergic interneurons of the DR (Boothman and Sharp 2005) raising the possibility that DOI reduces the firing of 5-HT neurons by increasing GABA release in the DR. Alternatively, it has been reported that sub-acute (2 days) administration of escitalopram or sustained administration of paroxetine, citalopram or fluoxetine in rat enhances 5-HT levels in the LC, which in turn produces a marked inhibition of NE neurons (Dremencov et al. 2007a, b; Seager et al. 2004; Szabo and Blier 2001; Szabo et al. 1999, 2000). It has been proposed that such an inhibitory action results from the activation of the 5-HT_{2A}R located on GABA interneurons in the LC

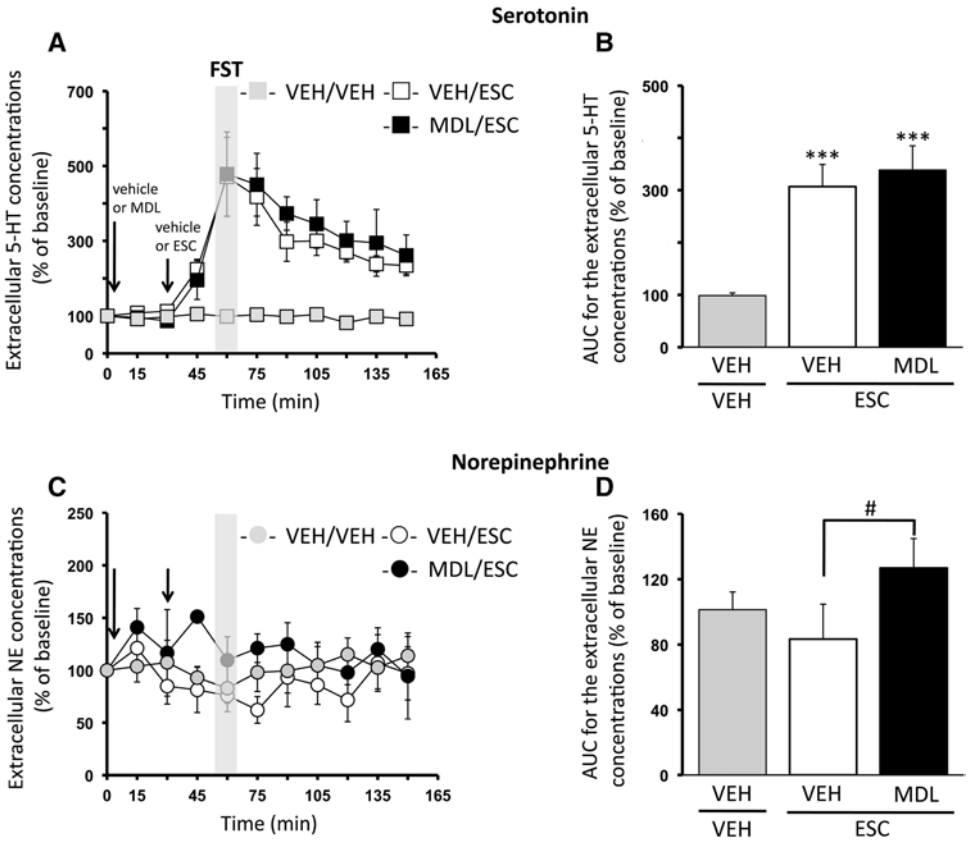

Fig. 4 Effects of acute administration of the SSRI escitalopram given either alone or in combination with the 5-HT_{2A}R antagonist MDL100907 on extracellular levels of 5-HT and NE in the frontal cortex in 5-$HT_{2A}R^{+/+}$ mice. **a** Time course. Data are means ± SEM of extracellular 5-HT levels ($[5\text{-HT}]_{ext}$) expressed as percent of baseline (B_0) following exposure to vehicle/vehicle (*light gray square*: VEH/VEH), vehicle/escitalopram (*open square*: VEH/ESC 4 mg/kg; s.c.) or MDL100907/escitalopram (*filled square*: MDL 2 mg/kg; s.c./ESC 4 mg/kg; s.c.). Baseline 5-HT in vehicle/vehicle, vehicle/escitalopram and MDL/escitalopram were 3.1 ± 0.5, 2.6 ± 0.4 and 3.6 ± 1.3 fmol/sample, respectively. **b** AUC values (means ± SEM) were calculated for the amount of 5-HT outflow collected during the 45–165 min post-escitalopram administration. **c** Time course. Data are means ± SEM of extracellular cortical levels of NE ($[NE]_{ext}$) expressed as percent of baseline (B_0) following exposure to vehicle/vehicle (*light gray circle*: VEH/VEH), vehicle/escitalopram (*open circle*: VEH/ESC 4 mg/kg; s.c.) or MDL100907/escitalopram (*filled circle*: MDL 2 mg/kg; s.c./ESC 4 mg/kg; s.c.). Baseline NE in vehicle/vehicle, vehicle/escitalopram and MDL100907/escitalopram were 1.9 ± 0.4, 2.8 ± 0.5 and 2.5 ± 0.4 fmol/sample, respectively. **d** AUC values (means ± SEM) were calculated for the amount of NE outflow collected during the 45–165 min post-escitalopram administration. As indicated by the *arrows*, NaCl or MDL10907 was administered at t0 and escitalopram at t30. The gray area illustrates the time relative to drug treatment when FST was undertaken in separate groups of mice ($n = 4$–7 mice per group). $^{*}p < 0.05$ and $^{***}p < 0.001$: significantly different from vehicle/vehicle-treated mice. $^{\#}p < 0.05$: significantly different from vehicle/escitalopram-treated mice

(Szabo and Blier 2001). In light of the prominent excitatory NE innervation of the DR (Mongeau et al. 1997; Vandermaelen and Aghajanian 1983; Baraban and Aghajanian 1980), the impairment of DR 5-HT neuronal activity induced by DOI could also be secondary to its inhibitory effect on LC NE neurons. In support of this latter hypothesis, we demonstrate here that the lesion of noradrenergic neurons with the neurotoxin DSP4 significantly attenuates DOI-induced decrease in DR 5-HT neuronal activity. It is noteworthy that the attenuations of DOI's effects are modest, and this is likely due to a partial depletion of the noradrenergic system induced by DSP4 (Cassano et al. 2009). However, our results strongly suggest that any increase in endogenous 5-HT availability due to SERT inhibition may participate in the acute inhibitory property of escitalopram on serotonergic firing rate through the activation of 5-HT_{2A}R located in the DR and/or the LC. In line with previously reported data in rats after intravenous administration of SSRIs (Mansari et al. 2007; El Mansari et al. 2005), the subcutaneous administration of escitalopram decreased the firing rate of DR 5-HT neurons in mice. Since it has long been recognized that the inhibitory effect of SSRI on 5-HT firing rate is mediated by the overactivation of somatodendritic 5-HT_{1A} autoreceptor in the DR (Gardier et al. 1996), we blocked this mechanism by using the 5-HT_{1A} receptor antagonist WAY100635. In these conditions, the 5-HT_{2A}R

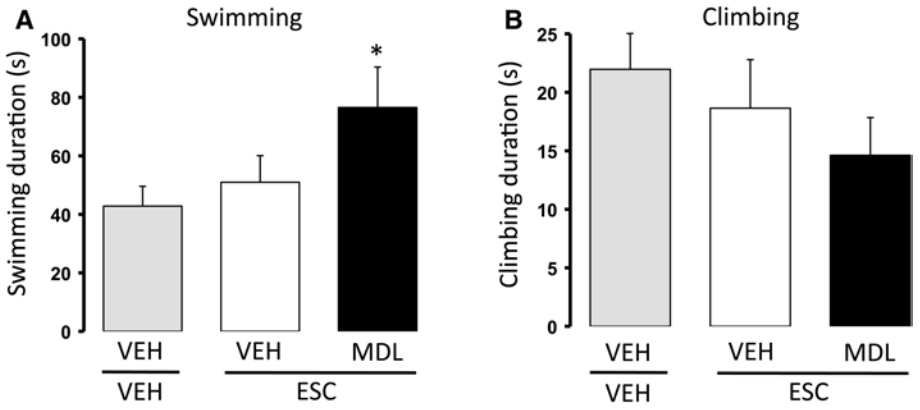

Fig. 5 Effects of acute administration of the SSRI escitalopram given either alone or in combination with the 5-HT_{2A}R antagonist of MDL100907 in the forced swimming test (FST) in 5-$HT_{2A}R^{+/+}$ mice. Antidepressant-like effect of vehicle/escitalopram (VEH/ESC: 4 mg/kg; s.c.) or MDL100907/escitalopram (MDL: 2 mg/kg; s.c./ESC: 4 mg/kg; s.c.) measured at duration of swimming (**a**) or climbing (**b**) behaviors. Data are means ± SEM of swimming and climbing duration in seconds. MDL100907 or its vehicle was administered 30 min before escitalopram. ($n = 12$–15 mice per group). *$p < 0.05$: significantly different from the vehicle/vehicle group of mice

antagonist MDL100907 reversed the residual inhibition of 5-HT firing induced by escitalopram. These results may appear surprising given the compelling evidence showing that WAY100635 completely prevents or counteracts acute SSRIs-induced decrease in 5-HT cell firing. They also stand in contrast with recent findings showing that MDL100907 did not block the electrophysiological effects of low dose of citalopram on DR 5-HT neuronal activity in rats (Sotty et al. 2009). Such a discrepancy may come from the use of a higher dose of escitalopram in the present study which might have recruited indirect pathways involving 5-HT_{2A}R in the modulation of the serotonergic system, whereas low doses could preferentially trigger 5-HT_{1A} autoreceptor located on 5-HT neurons in the DR. Despite this divergence, our results concur with previous findings showing that various AAPs counteract SSRI-induced inhibition of DR 5-HT neuronal activity (Dremencov et al. 2007a, b). They also emphasize the notion that the pharmacological inactivation of the 5-HT_{1A} autoreceptor is necessary but likely not sufficient to prevent the acute inhibitory effects of SSRIs on DR 5-HT neuronal activity.

From these electrophysiological data, we anticipated that the pharmacological inactivation of the 5-HT_{2A}R might potentiate the effects of escitalopram on cortical extracellular 5-HT and/or NE levels. Surprisingly, MDL100907 did not potentiate escitalopram-induced increase in cortical 5-HT outflow. Although this lack of potentiating effect of MDL100907 seems to be a common feature of AAPs (Quesseveur et al. 2012), such a property appears paradoxical given the inhibitory effect of 5-HT_{2A}R activation on the serotonergic system itself. It might be possible that a sustained administration of MDL100907 is required to unveil a significant effect at nerve terminals. Alternatively, the expected neurochemical effects of MDL100907 on 5-HT release could be apparent only after sustained administration of escitalopram when the 5-HT_{1A} autoreceptor is partially or completely desensitized (Guiard et al. 2012). Remarkably, a single systemic injection of escitalopram failed to modify cortical extracellular NE levels. However, MDL100907 pretreatment allowed escitalopram to increase cortical NE. This finding strengthens the hypothesis that the activation of 5-HT_{2A}R in response to escitalopram administration exerts a negative control on the noradrenergic system. In agreement with this assumption, we have recently shown that the increase in brain 5-HT transmission resulting from the genetic inactivation of the 5-HT transporter (5-HTT) reduces the basal cortical extracellular NE levels (Nguyen et al. 2012). To the best of our knowledge, this is the first time that such an observation is reported with MDL100907 and this concurs with the synergic effect of AAPs and SSRIs to enhance NE release (Huang et al. 2006; Denys et al. 2004; Koch et al. 2004; Zhang et al. 2000). Although this enhancement of cortical NE release might result from the blockade of escitalopram-induced decrease in LC NE neuronal activity (Dremencov et al. 2007a, b), it is also possible that MDL100907 acts locally in the FCx to stimulate NE release. However, this latter possibility can be ruled out since evidence demonstrates that 5-HT_{2A}R agonists, but not antagonists, increase cortical extracellular NE levels (Franberg et al. 2012; Gobert and Millan 1999; Marek and Aghajanian 1999). Thus, the putative excitatory effect of MDL100907 on cortical NE release most likely involves the inactivation of inhibitory 5-HT_{2A}R in the LC.

Despite the ability of escitalopram to enhance 5-HT transmission as described herein, it did not elicit an antidepressant-like effect in the FST as previously reported at the same dose (Nguyen et al. 2012). It can be assumed that even if antidepressant-like activity has not been unveiled,

escitalopram might have produced effects on monoamines levels that do not allow reaching a threshold leading to the expected response. The observation that the administration of MDL100907 30 min before an inactive dose of escitalopram unveils an antidepressant-like effects suggests that a simultaneous increase in cortical extracellular levels of 5-HT and NE (~340 and 130 % of baseline, respectively) is necessary to produce antidepressant-like activity and that sufficient concentrations of these monoamines for producing antidepressant-like are reached with the addition of the 5-HT_{2A}R antagonist. The FST is particularly relevant in the context of this study since it allows the determination of two measures, that is, the swimming and the climbing reflecting the activation of brain serotonergic (Page et al. 1999; Detke et al. 1995) or noradrenergic neurons (Nguyen et al. 2012; Cryan et al. 2002), respectively. Despite these considerations, MDL100907 potentiates cortical extracellular NE levels induced by escitalopram while having no effect on the duration of climbing in response to the SSRI. This lack of correlation cannot be attributed to technical failure since in the present study we controlled that the NE reuptake inhibitor desipramine significantly increased the climbing duration (data not shown). It is thus possible that the pharmacological inactivation of the 5-HT_{2A}R triggered other neuronal circuits contributing to cancel this behavior. On the other hand, despite the lack of potentiation of escitalopram-induced increase in cortical 5-HT levels, MDL100907 significantly increases the swimming duration induced by escitalopram. Therefore, it can be assumed that the sole increase in NE transmission was responsible for the antidepressant-like activity observed by combining MDL100907 with escitalopram. This is in agreement with the earlier findings showing that different classes of drugs enhancing NE levels such as desipramine and nortryptiline or relatively selective NE reuptake inhibitors like reboxetine and atomoxetine produce antidepressant-like activity (Petit-Demouliere et al. 2005).

Conclusion

The present study strongly supports a role of the 5-HT_{2A}R in the regulation of the serotonergic system, particularly in response to SSRIs. From this set of data, it clearly appears that the 5-HT_{1A} autoreceptors and the 5-HT_{2A} heteroreceptors act in concert to exert a strong inhibitory feedback control on DR 5-HT neurons in response to SSRI thereby limiting their ability to enhance 5-HT neurotransmission. It also appears that the potentiating effects of 5-HT_{2A}R inactivation on SSRI antidepressant activity may rely at first on the enhancement of noradrenergic neurotransmission supporting the interest of developing drugs combining 5-HT reuptake blockade and 5-HT_{2A}R antagonism properties in a single compound or using augmentation strategies based on the combination of AAPs with SSRIs. Moreover, recent clinical and preclinical evidence also suggests a role for the 5-HT_{2C}R in the regulation of the serotonergic system in response to SSRIs and AAPs (Quesseveur et al. 2012). 5-HT_{2C}R constitutes therefore an additional target but the modalities of augmentation strategies involving all these serotonergic receptors have yet to be determined. In particular, the examination of the long-term effects of AAP administration on SSRIs' effects is required by electrophysiological, neurochemical and behavioral tests in animal models of depression. Finally, the role of the dopaminergic system in the beneficial effects of this therapeutic strategy should also draw our attention for future investigations.

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Conflict of interest None.

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