Enhancement of cortical extracellular 5-HT by 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptor blockade restores the antidepressant-like effect of citalopram in non-responder mice

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Abstract

We recently found that the response of DBA/2 mice to SSRIs in the forced swim test (FST) was impaired and they also had a smaller basal and citalopram-stimulated increase in brain extracellular serotonin (5-HT) than ‘responder’ strains. We employed intracerebral microdialysis, FST and selective antagonists of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors to investigate whether enhancing the increase in extracellular 5-HT reinstated the anti-immobility effect of citalopram in the FST. WAY 100635 (0.3 mg/kg s.c.) or SB 242084 (1 mg/kg s.c.), respectively a selective 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptor antagonist, raised the effect of citalopram (5 mg/kg) on extracellular 5-HT in the medial prefrontal cortex of DBA/2N mice (citalopram alone 5.2 ± 0.3 fmol/20 µl, WAY 100635 + citalopram 9.9 ± 2.1 fmol/20 µl, SB 242084 + citalopram 7.6 ± 1.0 fmol/20 µl) to the level reached in ‘responder’ mice given citalopram alone. The 5-HT receptor antagonists had no effect on the citalopram-induced increase in extracellular 5-HT in the dorsal hippocampus. The combination of citalopram with WAY 100635 or SB 242084 significantly reduced immobility time in DBA/2N mice that otherwise did not respond to either drug singly. Brain levels of citalopram in mice given citalopram alone or with 5-HT antagonists did not significantly differ. The results confirm that impaired 5-HT transmission accounts for the lack of effect of citalopram in the FST and suggest that enhancing the effect of SSRIs on extracellular 5-HT, through selective blockade of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors, could be a useful strategy to restore the response in treatment-resistant depression.

Received 9 September 2008; Reviewed 11 October 2008; Revised 11 November 2008; Accepted 17 November 2008; First published online 6 January 2009

Key words: FST, intracerebral microdialysis, SSRIs, 5-HT<sub>1A</sub> receptors, 5-HT<sub>2C</sub> receptors, TPH-2.

Introduction

The serotonergic system plays an important role in many physiological functions in the brain (Jacobs & Azmitia, 1992) and a hypofunction of serotonin (5-HT) is believed to be involved in the pathogenesis of depression (Jans et al. 2007). The selective serotonin reuptake inhibitors (SSRIs), the most frequently used drugs for treatment of major depression, increase the synaptic availability of 5-HT (Fuller, 1994; Invernizzi et al. 1992, 1995, 1996) and this is considered the first step in the mechanism leading to the therapeutic activity of these drugs. Pharmacological inhibition of brain 5-HT synthesis and spontaneous mutations of tryptophan hydroxylase-2 (TPH-2), the enzyme responsible for the biosynthesis of brain 5-HT (Walther et al. 2003), are associated with reduced efficacy of SSRIs in depressed patients (Delgado et al. 1990; Shopsin et al. 1976; Zhang et al. 2005).

DBA/2N, DBA/2J and BALB/c inbred mice carry a mutated allele of TPH-2 (C1473G) and have lower brain 5-HT synthesis than mice homozygous for the 1473C allele such as C57BL/6N and C57BL/6J strains (Zhang et al. 2004). Studying strain differences in the response to SSRIs in the forced swim test (FST), a screening procedure for antidepressant compounds...
(Porsolt et al. 1977), we showed that citalopram and paroxetine reduced immobility time in C57BL/6 mice (‘responders’), but had no such effect in DBA/2 and BALB/c mice (Cervo et al. 2005; Gazzetti et al. 2008). The 5-HT precursor tryptophan restored the antidepressant effect of SSRIs in ‘non-responder’ mice (Cervo et al. 2005; Gazzetti et al. 2008). Interestingly, ‘non-responder’ strains had a lower basal and citalopram-induced increase in extracellular 5-HT in the medial prefrontal cortex (mPFC) and dorsal hippocampus (DH) than ‘responders’ (Calcagno et al. 2007). This suggested that insufficient 5-HT at its sites of action may explain why citalopram does not reduce immobility time in DBA/2 and BALB/c mice (Cervo et al. 2005).

Thus, the aim of the present study was to assess whether intervention aimed at enhancing the effect of citalopram on extracellular 5-HT restored its ability to reduce immobility time in the mouse FST.

The ability of SSRIs to raise extracellular 5-HT, and possibly their clinical effect, is limited by the simultaneous activation of an autoinhibitory feedback controlling the activity of 5-HT neurons and the release of the neurotransmitter mainly through the activation of 5-HT1A auto- and post-synaptic receptors (Artigas et al. 1996, 2001; Invernizzi et al. 1992, 1997). Blockade of 5-HT1A receptors, preventing activation of the inhibitory feedback, enhances the increase of extracellular 5-HT caused by SSRIs in several brain regions (Artigas et al. 1996; Gartside et al. 1995; Hjorth, 1993; Hjorth & Auerbach, 1994; Invernizzi et al. 1992, 1996, 1997). Accordingly, the non-selective 5-HT1A/1B and β-adrenoceptor antagonist pindolol accelerated the effect of SSRIs in depressed patients, although some studies did not confirm this finding (Artigas et al. 2001).

5-HT2C receptors are also involved in the feedback control of 5-HT neurons (Sharp et al. 2007). 5-HT2C receptor antagonists potentiate the SSRIs’ effect on immobility time in the tail suspension test and enhance citalopram’s effect on extracellular 5-HT (Bootham et al. 2006b; Cremers et al. 2004). Desensitization of rat brain 5-HT2C receptors after long-term treatment with antidepressant drugs (Kennett et al. 1994) suggests that adaptive changes of these receptors might contribute to the development of the antidepressant action. Post-mortem studies showed abnormal mRNA editing of the 5-HT2C receptor, favouring the expression of less constitutively active receptor isoforms in the brain of suicide victims (Englander et al. 2005; Gurevich et al. 2002) while chronic administration of antidepressants to mice induced 5-HT2C receptor editing opposite to that found in the brain of depressed patients (Gurevich et al. 2002). Thus, the 5-HT2C receptor offers a promising target for enhancing the efficacy of SSRIs.

The present study examined whether WAY 100635 and SB 242084, respectively a selective 5-HT1A and 5-HT2C receptor antagonist, enhanced the effect of citalopram on mPFC and DH extracellular 5-HT in ‘non-responder’ DBA/2N mice and rescued its effect in the FST. These brain regions were chosen because they receive 5-HT fibres arising respectively from the dorsal raphe (DR) and median raphe (MR) nuclei (Azmitia & Segal, 1978; Kosofsky & Molliver, 1987). In addition, the effect of SSRIs on extracellular 5-HT in the mPFC of the rat seems to be larger than in lateral cortical regions (Beyer & Cremers, 2008) and there is evidence that the 5-HT1A-receptor-mediated inhibitory feedback of 5-HT neurons of the DR involves medial, but not lateral prefrontal cortex projections (Celada et al. 2001). Levels of citalopram in mice receiving 5-HT antagonists were measured to check for differences in drug brain concentrations across strains and treatments.

Materials and methods

Animals

Male C57BL/6N and DBA/2N mice (Charles River, Italy), aged 6–8 wk, were group-housed, with food and water freely available, under standard laboratory conditions (constant room temperature 21 ± 1 °C; relative humidity 60 ± 5%; 12-h light/dark cycle, lights on 07:00 hours).


In vivo microdialysis and 5-HT assay

Surgery for microdialysis probe implantation was carried out under anaesthesia with 3 ml/kg Equithesin (composition in mM: pentobarbital 39, chloral hydrate 256, MgSO4 86, ethanol 10% v/v, propylene glycol 39.6% v/v, water 50.4% v/v), intraperitoneally (i.p.). Single vertical dialysis probes were prepared with cuprophan membrane (216 µm outer diameter, 3000 Da cut-off, Sorin Biomedica, Italy), essentially as described by Robinson & Whishaw (1988) for rats, except that the length of the exposed membrane
was 2 mm for the mPFC and 1 mm for the DH and the shaft of the mouse probe was shorter than in rats (1.2 mm vs. 1.5 mm). Mice were placed on a stereotaxic frame (model 900, David Kopf, USA) equipped with a mouse adapter for the incisor bar and a dialysis probe was implanted in the mPFC or the DH. The stereotaxic coordinates were AP +2.1 mm, L ±0.3 mm and V −2.5 mm for the mPFC and AP +2.3 mm, L ±1.5 mm and V −2.0 mm for the DH from bregma and dura surface, with the incisor bar positioned at 0.0 mm (Franklin & Paxinos, 1997). Surgery was performed between 10:00 and 12:00 hours.

About 20 h after surgery, the probes were perfused with artificial cerebrospinal fluid [aCSF, composition in mM: NaCl 145, CaCl₂ 1.26, KCl 3, MgCl₂ 1, Na₂HPO₄ 1.4 (pH 7.4), with 0.6 M NaH₂PO₄ at 1 µl/min with a CMA/100 pump (CMA/Microdialysis, Sweden). Probe perfusion began between 08:00 and 09:00 hours. After 1 h stabilization, samples of dialysate were collected every 20 min for 2 h and stored at 4 °C. Drugs were administered between 10:00 and 12:00 hours and 5-HT release monitored for 2 h after citalopram injection.

The 5-HT concentration in microdialysis samples (20 µl) was assayed by HPLC with electrochemical detection as described elsewhere (Invernizzi et al. 1992). Briefly, 5-HT was separated by a reverse-phase column (Supelcosil LC18-DB 3 µm, 150 × 4.6 mm; Supelchem, Italy) and a mobile phase consisting of 9 mM citric acid, 48 mM sodium acetate, 0.1 mM Na₃EDTA, 100 µM triethylamine and 40 µM acetanilide, pumped at 1 ml/min. The system consisted of a Coulochem II electrochemical detector equipped with a 5011 analytical cell (ESA Inc., USA) at the following potentials: E₁ 50 mV, E₂ 180 mV. 5-HT was read as the second electrode output signal. Detection limit was 1.1 fmol 5-HT on-column (signal-to-noise ratio 2).

**Forced swim test**

The FST procedure was essentially similar to that described elsewhere (Cervo et al. 2005). Briefly, mice were dropped individually into a clear Plexiglas cylinder (height 25 cm, diameter 10 cm) containing 15 cm of water, maintained at 25 ±1 °C, and their behaviour was videotaped for 6 min. The total period of immobility, during the last 4 min of the test, was timed by two observers unaware of the treatment the mice had received. A mouse was judged to be immobile when it floated in an upright position, making only small movements to keep its head above water (Porsolt et al. 1977).

**Open-field activity**

Separate groups of mice were used to assess whether treatments reducing immobility in the FST affected open-field activity. Mice receiving the same treatment of those in the FST were placed individually in an open field made of grey plastic (40 × 40 cm) with the floor divided into 25 equal squares. Open-field activity was videotaped for 6 min and quantified later by counting the number of squares crossed with all paws in the last 4-min, corresponding to the behavioural observation time in the FST.

**Citalopram measurement**

At the end of the FST, mice were killed by decapitation, the brain was rapidly removed and stored at −20 °C until analysis. Citalopram was determined by HPLC with UV detection (235 nm), according to Grignaschi et al. (1998). The lower limit for quantification (LOQ) was 0.08 µg/g using ~200 mg of brain tissue.

**Drug treatments**

Citalopram hydrobromide (Tocris Cookson, UK) and the 5-HT₁A receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100635; Pharmacia and Upjohn, Italy) were dissolved in saline (0.9% NaCl, 10 ml/kg) and injected respectively i.p. and subcutaneously (s.c.) at the doses indicated. The citalopram dose used in the present study maximally increased extracellular 5-HT in the mPFC and DH of DBA/2N and C57BL/6 mice (Calcagno et al. 2007) and maximally reduced immobility time in C57BL/6 mice (Cervo et al. 2005).

The 5-HT₆C receptor antagonist, 6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB 242084; GlaxoSmithKline, UK), was dissolved in DMSO: 4 µl tartaric acid:water (50:1:49) and injected s.c. Control mice received an injection of the vehicle (10 ml/kg).

WAY 100635 and SB 242084 were injected 20 min before citalopram. All drugs and vehicles were injected during the phase of stable 5-HT output defined as three consecutive baseline samples not differing by more than 15%. In behavioural experiments, mice were given the 5-HT receptor antagonists 20 min before citalopram and were submitted to the FST or open-field test 30 min after the last injection. Microdialysis and behavioural studies were done on separate groups of mice.

Doses of the 5-HT receptor antagonists were selected on the basis of blockade of the effects of selective
agonists (Boothman et al. 2006a; O’Neill & Conway, 2001).

**Histology**

At the end of the microdialysis experiment, mice were killed by decapitation. Brains were fixed in 4% paraformaldehyde and correct probe placements were checked by visual inspection of the probe tracks on Nissl-stained coronal sections (20 μm) from the mPFC and the DH of each mouse. Only mice with correct probe placement were considered in the results (100/107).

**Data analysis and statistics**

Extracellular levels of 5-HT, not corrected for *in vitro* recovery of the probe, were expressed as fmol/20 μl. Basal values of 5-HT in different experiments and in different strains of mouse were compared by one-way analysis of variance (ANOVA) or Student’s *t* test. All time-course data were analysed by ANOVA for repeated measures with treatments as between-subjects factor and time as within-subjects factor. Post-hoc comparisons between pre- and post-injection values and comparisons between treatments were done with Tukey–Kramer’s test.

Statistical analyses were done with the StatView 5.0 software (SAS Institute Inc., USA).

**Results**

**Basal level of extracellular 5-HT**

Mean (± S.E.M.) basal extracellular 5-HT in the mPFC and DH of DBA/2N mice was 2.5 ± 0.1 (n = 44) and 2.5 ± 0.1 (n = 45) fmol/20 μl, respectively (not corrected for probe recovery). No significant differences were found across different experiments (mPFC [F(7, 36) = 2.3, *p* > 0.05]; DH [F(7, 37) = 1.5, *p* > 0.05]).

**Blockade of 5-HT1A receptors enhanced the citalopram-induced increase in extracellular 5-HT in the mPFC**

As shown in Fig. 1a, 5 mg/kg citalopram increased extracellular 5-HT in the mPFC of DBA/2N mice, reaching 5.2 ± 0.3 fmol/20 μl at 60 min (210% of basal value). WAY 100635 (0.3 mg/kg) potentiated the overall effect of citalopram on extracellular 5-HT peaking at 9.9 ± 2.1 fmol/20 μl. ANOVA indicated a significant interaction between WAY 100635, citalopram and time [F(7, 119) = 3.3, *p* = 0.003] and a significant effect of citalopram [F(1, 17) = 42.0, *p* < 0.0001], WAY 100635 [F(1, 17) = 17.0, *p* = 0.0007] and their interaction [F(1, 17) = 12.0, *p* = 0.003]. WAY 100635 by itself had no effect on extracellular 5-HT.

Fig. 1. Effect of 0.3 mg/kg WAY 100635 on citalopram-induced increase in extracellular 5-HT in (a) mPFC and (b) DH of DBA/2N mice. The first arrow indicates the injection of saline (Sal) or WAY 100635 (WAY) and the second the injection of saline (Sal) or 5 mg/kg citalopram (Cit). The dashed line indicates the effect of 5 mg/kg citalopram in C57BL/6N mice (not included in the statistical analysis). Mean basal levels of 5-HT in fmol/20 μl (± S.E.M.) were: (a) Sal + Cit, 2.5 ± 0.2 (n = 5); WAY + Sal, 2.3 ± 0.2 (n = 5); WAY + Cit, 2.2 ± 0.1 (n = 6); Sal + Sal, 2.3 ± 0.2 (n = 5); Sal + Cit C57BL/6N mice 3.6 ± 0.2 (n = 6). (b) Sal + Cit, 2.3 ± 0.2 (n = 6); WAY + Sal, 2.7 ± 0.1 (n = 6); WAY + Cit, 2.9 ± 0.3 (n = 6); Sal + Sal, 2.2 ± 0.2 (n = 5); Sal + Cit C57BL/6N mice 3.8 ± 0.3 (n = 6). Solid symbols indicate *p* < 0.05 vs. basal values; *p* < 0.05 Sal + Cit vs. Sal + Sal and *p* < 0.05 WAY + Cit vs. Sal + Cit (Tukey–Kramer’s test).
The increase in cortical and hippocampal extracellular 5-HT in DBA/2N mice after WAY 100635 and citalopram was similar to that in C57BL/6N mice given citalopram alone.

Effect of citalopram alone and in combination with WAY 100635 and SB 242084 on immobility time in the FST

As shown in Fig. 3, 5 mg/kg citalopram and 0.3 mg/kg WAY 100635 by themselves did not affect immobility time in DBA/2N mice, but together they significantly reduced it (WAY 100635 [F(1, 28) = 9.1, p = 0.0054]; citalopram [F(1, 28) = 8.2, p = 0.0078] and...
than in those given vehicle open-field activity in mice given SB 242084 was lower (Table 1).

However, differences between SB 242084 and saline or SB 242084 + citalopram (Cit) were killed at the end of the behavioural test. Mean + S.E.M., eight mice per group. * p < 0.05 vs. Veh + Sal, Veh + Cit and SB + Sal (Tukey-Kramer’s test).

**Open-field activity**

As reported in Table 1a, citalopram and WAY 100635 alone or in combination did not affect the open-field activity of DBA/2N mice. The vehicle used to dissolve SB 242084 strongly suppressed locomotor activity (p < 0.05 vs. saline + saline; Student’s t test). Overall, open-field activity in mice given SB 242084 was lower than in those given vehicle [F(1, 28) = 6.6, p = 0.02]. However, post-hoc comparisons showed no significant differences between SB 242084 + saline and vehicle + saline or SB 242084 + citalopram and vehicle + citalopram (Table 1b). Citalopram had no effect by itself [F(1, 28) = 0.26, p = 0.6] or in combination with SB 242084 [F(1, 28) = 1.4, p = 0.2].

We also measured crossing of the central and peripheral squares and found no differences across treatments in both cases (data not shown).

**Brain levels of citalopram**

The mean brain concentrations of citalopram at the end of the behavioural test are shown in Table 2. Pretreatment with WAY 100635 or SB 242084 did not significantly affect brain levels of the drug.

**Discussion**

In this study WAY 100635 and SB 242084, blocking respectively 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors, enhanced the citalopram-induced increase in cortical extracellular 5-HT and restored the anti-immobility effect of citalopram in DBA/2N mice that did not respond to the SSRI alone. We used doses of WAY 100635 and SB 242084 similar to or lower than those antagonizing the effects of 5-HT$_{1A}$ and 5-HT$_{2C}$ agonists in behavioural, biochemical and electrophysiological studies (Di Matteo et al. 2000; Kennett et al. 1997; Moser & Sanger, 1999; O’Neill & Conway, 2001; Pozzi et al. 2002; Quéré & Sharp, 2006; Trillat et al. 1998). In addition, WAY 100635 and SB 242084 belong to different chemical classes and are among the most selective antagonists available for each respective receptor.

**Table 1.** Open-field activity of DBA/2N mice given citalopram alone and in combination with WAY 100635 or SB 242084

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Open-field activity (squares crossed/4 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 0.3 mg/kg WAY 100635</td>
<td></td>
</tr>
<tr>
<td>Saline + saline</td>
<td>210.4 ± 22.5</td>
</tr>
<tr>
<td>Saline + citalopram</td>
<td>187.4 ± 20.0</td>
</tr>
<tr>
<td>WAY 100635 + saline</td>
<td>165.5 ± 11.0</td>
</tr>
<tr>
<td>WAY 100635 + citalopram</td>
<td>181.1 ± 19.2</td>
</tr>
<tr>
<td>(b) 1 mg/kg SB 242084</td>
<td></td>
</tr>
<tr>
<td>Vehicle + saline</td>
<td>63.5 ± 14.7*</td>
</tr>
<tr>
<td>Vehicle + citalopram</td>
<td>92.9 ± 27.7</td>
</tr>
<tr>
<td>SB 242084 + saline</td>
<td>39.8 ± 5.6*</td>
</tr>
<tr>
<td>SB 242084 + citalopram</td>
<td>28.0 ± 13.3</td>
</tr>
</tbody>
</table>

Mice were given (a) 0.3 mg/kg WAY 100635, (b) 1 mg/kg SB 242084, or respective vehicles 20 min before receiving 5 mg/kg citalopram and open-field activity was evaluated 30 min later. Mean + S.E.M., eight animals per group. * p < 0.05 vs. saline + saline (Student’s t test).

**Table 2.** Effect of WAY 100635 and SB 242084 on brain levels of citalopram in DBA/2N mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citalopram (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + citalopram</td>
<td>1.17 ± 0.28</td>
</tr>
<tr>
<td>WAY 100635 + citalopram</td>
<td>1.10 ± 0.29</td>
</tr>
<tr>
<td>Vehicle + citalopram</td>
<td>1.03 ± 0.17</td>
</tr>
<tr>
<td>SB 242084 + citalopram</td>
<td>1.05 ± 0.19</td>
</tr>
</tbody>
</table>

Mice (the ones used in the FST) were given 0.3 mg/kg WAY 100635 or 1 mg/kg SB 242084 20 min before 5 mg/kg citalopram that was given 30 min before the 6 min FST. Mice were killed at the end of the behavioural test. Mean ± S.E.M., eight mice per group.
5-HT antagonists reverse resistance to citalopram

DH is in line with previous reports that rat 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors

The enhancement of citalopram’s effect on extracellular 5-HT by 5-HT$_{1A}$ and 5-HT$_{2C}$ receptor antagonists agrees with previous findings that pharmacological blockade and/or genetic deletion of these receptors suppressed the inhibitory feedback regulating the activity of 5-HT neurons and enhanced the effects of SSRIs on extracellular 5-HT in several regions of the rat and mouse brain (Bortolozzi et al. 2004; Cremers et al. 2004; Invernizzi et al. 1992, 1997; Píneyro & Blier, 1999). The fact that doses of WAY 100635 and SB 242084 enhancing the citalopram-induced increase in extracellular 5-HT in the mPFC but not in the DH reinstated citalopram’s effect in the FST supports 5-HT’s fundamental role in the anti-immobility effect of SSRIs and suggests a preferential involvement of DR mPFC 5-HT neurons in this effect.

The role of 5-HT is further supported by several studies. First, DBA/2N mice have a lower brain synthesis rate and extracellular levels of 5-HT (Cervo et al. 2005; Zhang et al. 2004) and less effect for citalopram on extracellular 5-HT in the mPFC and DH than mice responding to citalopram in the FST (Calcagnò et al. 2007; Cervo et al. 2005; Jacobsen et al. 2008; Zhang et al. 2004). Second, 5-HT synthesis inhibition with pCPA abolished the anti-immobility effect of citalopram in ‘responder’ strains (Cervo et al. 2005) and the anti-immobility effect of tryptophan + paroxetine combination in DBA/2N mice (Guzzetti et al. 2008). Third, boosting 5-HT synthesis with the 5-HT precursor tryptophan restored the antidepressant-like effect of SSRIs in DBA/2N, DBA2J and BALB/c mice, which synthesize less 5-HT than C57BL/6J and C57BL/6N mice (Cervo et al. 2005; Guzzetti et al. 2008). This latter finding was confirmed by a recent study showing that the insensitivity of NMRI mice to SSRIs in the tail suspension test was associated with a reduction of tissue and extracellular 5-HT in the mPFC and was reversed by the 5-HT precursor 5-hydroxytryptophan (Jacobsen et al. 2008). Thus, it is quite likely that the reduction of immobility time obtained by combining citalopram and WAY 100635 or SB 242084 is due to the fact that both these antagonists raise citalopram’s effect on extracellular 5-HT in the mPFC to the level reached in ‘responder’ mice. The fact that WAY 100635 had no effect on the citalopram-induced increase in extracellular 5-HT in the DH is in line with previous reports that rat 5-HT$_{1A}$ receptors exert strong inhibitory control over the activity of 5-HT neurons arising from the DR, such as the mPFC. In contrast, the DH of the rat, an area innervated by 5-HT neurons arising from the MR (Azmitia & Segal, 1978; Kosofsky & Molliver, 1987) containing far fewer 5-HT$_{1A}$ receptors and 5-HT uptake sites than the DR (Adell et al. 2002; Hrdina et al. 1990; Lechin et al. 2006; Weissmann-Nanopoulos et al. 1985), was less affected by the 5-HT$_{1A}$ Receptor or not at all (Beck et al. 2004; Blier et al. 1990; Invernizzi et al. 1991; Lorens & Guldberg, 1974; Sinton & Fallon, 1988). Thus, 5-HT$_{1A}$ receptor antagonists preferentially enhanced the effects of SSRIs on extracellular 5-HT in rat brain regions innervated by the DR such as the mPFC (Hervas et al. 2000; Invernizzi et al. 1997; Romero & Artigas, 1997).

The fact that SB 242084 enhanced the citalopram-induced increase in extracellular 5-HT in the mPFC but not DH suggests that 5-HT$_{2C}$ receptors preferentially regulate 5-HT neurons arising from the DR. The fact that SB 242084 enhanced the effect of SSRIs on extracellular 5-HT in the ventral hippocampus of the rat (Cremers et al. 2007), a region innervated also by the DR (Azmitia & Segal, 1978), support this interpretation and suggests that the increase of extracellular 5-HT in the ventral hippocampus might contribute to the effect of SSRIs in the FST.

To our knowledge, the anatomical distribution and cellular localization of 5-HT$_{2C}$ receptors and the susceptibility of DR and MR 5-HT neurons to 5-HT$_{2C}$ receptor regulation in the mouse brain have not been studied. Therefore, the clear-cut difference in the effects of SB 242084 on the citalopram-induced increase in 5-HT in the mPFC and DH remain to be investigated.

Although the enhancement of extracellular 5-HT probably plays a major role in the ability of WAY 100635 and SB 242084 to reinstate the anti-immobility effect of citalopram, different mechanisms are likely to be involved in the action of these drugs. WAY 100635 blocks 5-HT$_{1A}$ receptors that are expressed by 5-HT neurons of the raphe (Miquel et al. 1992) and directly inhibits the activity of these cells by inducing membrane hyperpolarization through an action on potassium and calcium channels (Aghajanian & Lakoski, 1984; Penington & Fox, 1994). Post-synaptic 5-HT$_{1A}$ receptors located in the mPFC are also involved in the auto-regulation of 5-HT neurons through a long feedback loop (Ceci et al. 1994; Celada et al. 2001; Hajos et al. 1999) and may contribute to the action of WAY 100635.

5-HT$_{2C}$ receptors are essentially localized on non-serotonergic neurons. Immunocytochemical studies showed that 5-HT$_{2C}$ receptors are expressed in glutamic acid decarboxylase-positive, GABAergic
neurons in the raphe and other brain regions (Pazos et al. 1985; Serrats et al. 2005). GABAergic neurons synapse upon 5-HT cells in the raphe (Wang et al. 1992) and control their activity (Gallager & Aghajanian, 1976). The fact that 5-HT applied to rat brain slices containing the DR causes a GABA- and 5-HT2C-mediated inhibition of 5-HT neurons (Liu et al. 2000) suggests that SB 242084 might enhance citalopram’s effects by acting on 5-HT2C receptors on GABAergic neurons intrinsic to the raphe that in turn inhibit 5-HT cells. We did not address this issue in the present study, but preliminary findings in our laboratory show that SB 242084 infused into the DR enhanced citalopram’s effect on extracellular 5-HT in the mPFC of DBA/2N mice (see Supplementary Fig. S1, available online). Thus, 5-HT2C receptors of the DR are probably mainly involved in the mechanism by which SB 242084 restored citalopram’s anti-immobility effect in DBA/2N mice. However, extra-raphe 5-HT2C receptors may also contribute (Cremers et al. 2007; Sharp et al. 2007).

Further studies are needed to establish whether 5-HT1A and 5-HT2C receptor antagonists’ ability to enhance the effects of citalopram is maintained after chronic treatment and whether 5-HT-related side-effects may limit the application of this strategy.

Differences in the behavioural response between mice given citalopram alone or with 5-HT1A or 5-HT2C receptor antagonists cannot be attributed to pharmacokinetic factors. In fact, brain levels of citalopram at the end of the behavioural tests were essentially similar in mice given citalopram alone or with the 5-HT receptor antagonists. The fact that citalopram alone or combined with WAY 100635 or SB 242084 did not increase open-field activity makes it unlikely that gross changes in motor performance were involved in the effect of these treatments in the FST.

A clear reduction of open-field activity was found in mice receiving the SB 242084 vehicle. This effect was observed in two separate experiments (data shown in Table 1 concerns the first experiment) confirming the reliability of this finding.

The fact that reduced open-field activity was found in all groups receiving SB 242084 vehicle, while immobility time was only reduced in mice given SB 242084 + citalopram makes it unlikely that the anti-immobility effect of this drug combination reflects changes in locomotor activity.

The blockade of 5-HT1A and 5-HT2C receptors with the antagonists had no effect by itself on extracellular 5-HT and immobility time, confirming that these receptors do not exert tonic control on the activity of serotonergic neurons (Adell et al. 2002; Boothman et al. 2006b; Cremers et al. 2004; Gartside et al. 1995; Invernizzi et al. 1997) and behaviour in the FST (Cremers et al. 2004; O’Neill & Conway, 2001; Tatarczynska et al. 2004).

One of the strengths of the present study is that we used a mouse strain that does not respond to citalopram in the FST, to show that the response can be restored by enhancing citalopram’s effect on 5-HT transmission. Previous studies aimed at improving the antidepressant response were mostly done in rats or mice already responding to the antidepressant alone, and gave conflicting results. WAY 100635 did not further reduce immobility time in the FST in mice and rats responding to SSRIs alone (Guilloux et al. 2006; Moser & Sanger, 1999). Moreover, genetic deletion or pharmacological blockade of 5-HT2C receptors, that clearly enhanced the effect of SSRIs on extracellular 5-HT, only marginally enhanced their anti-immobility effect in the mouse tail suspension test (Cremers et al. 2004). In contrast, the present study clearly found that blockade of 5-HT1A or 5-HT2C receptors restores citalopram’s antidepressant-like effect in non-responder mice to the drug alone. These findings therefore suggest that strategies aimed at enhancing 5-HT transmission might be more effective in improving the antidepressant efficacy in subjects with hypofunctioning brain 5-HT transmission such as DBA/2N mice. The results also show that enhancing the effect of SSRIs on extracellular 5-HT confers sensitivity to citalopram in the FST to DBA/2N mice, which are otherwise insensitive to citalopram alone. Augmentation of the response with 5-HT1A or 5-HT2C receptor antagonists may be useful to restore the antidepressant response to SSRIs, particularly in treatment-resistant depression associated with hypofunctioning 5-HT neurotransmission.

Note

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org).

Acknowledgements

The financial support of Ing. P. De Sanctis is greatly appreciated. We are grateful to Mrs E. Mancini for a fellowship to support the work of E.C. and to J. D. Baggott for language editing. We thank A. Di Clemente for his help in behavioural studies.

Statement of Interest

None.
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