Augmentation of SSRI Effects on Serotonin by 5-HT_{2C} Antagonists: Mechanistic Studies

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The treatment of depression may be improved by using an augmentation approach involving selective serotonin reuptake inhibitors (SSRIs) in combination with compounds that focus on antagonism of inhibitory serotonin receptors. Using microdialysis coupled to HPLC, it has recently been shown that the systemic co-administration of 5-HT_{2C} antagonists with SSRIs augmented the acute effect of SSRIs on extracellular 5-HT. In this paper, we have investigated the mechanism through which this augmentation occurs. The increase in extracellular 5-HT was not observed when both compounds were locally infused. However, varying the route of administration for both compounds differentially revealed that an augmentation took place when the 5-HT_{2C} antagonist was locally infused into ventral hippocampus and the SSRI given systemically, but not when systemic 5-HT_{2C} antagonist was co-administered with the local infusion of citalopram. This suggests that the release of extracellular serotonin in ventral hippocampus may be controlled by (an)other brain area(s). As 5-HT_{2C} receptors are not considered to be autoreceptors, this would implicate that other neurotransmitter systems are involved in this process. To investigate which neurotransmitter systems were involved in the interaction, systemic citalopram was challenged with several glutamatergic, GABA-ergic, noradrenergic, and dopaminergic compounds to determine their effects on serotonin release in ventral hippocampus. It was determined that the involvement of glutamate, norepinephrine, and dopamine in the augmentation did not seem likely, whereas evidence implicated a role for the GABA-ergic system in the augmentation.

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INTRODUCTION

The clinical treatment of depression is characterized by a high degree of nonresponse. Additionally, the onset of action of antidepressants typically takes 2–4 weeks. In order to enhance efficacy and shorten onset, augmentation strategies have been developed. Most augmentation strategies are derived from the hypothesis that chronic selective serotonin reuptake inhibitor (SSRI) treatment results in a desensitization of presynaptic serotonergic (5-HT) autoreceptors. This blunted autoreceptor control leads to increased effects of SSRIs on central serotonin levels in time, which is postulated to parallel the antidepressant effect (Hjorth *et al*, 2000; Blier, 2001; Blier *et al*, 1987).

Augmentation strategies focus on establishing enhanced SSRI responses on central serotonin levels. It should be mentioned, however, that many of these experiments reflect the response of 5-HT to acute SSRI administration, and not chronic administration. Over the last few decades, several such augmentation strategies have been developed. These add-on strategies range from antagonism of serotonergic autoreceptors like 5-HT_{1A} and 5-HT_{1B} (Hjorth *et al*, 2000; Blier *et al*, 1998; Artigas *et al*, 1994, 1996; Bosker *et al*, 2001; Celada *et al*, 2001; Cremers *et al*, 2000a,b), but also comprise heteroreceptors such as adrenoceptors (Bengtsson *et al*, 1998; Szabo and Blier, 2001; Besson *et al*, 2000; Hopwood and Stamford, 2001; Bortolozzi and Artigas, 2003; Pudovkina *et al*, 2003; Rouquier *et al*, 1994; Amargos-Bosch *et al*, 2003; Weikop *et al*, 2004; Gobert *et al*, 1997; Gobert and Millan, 1999; De Boer *et al*, 1996), 5-HT_{2A} receptors (Szabo and Blier, 2002), GABA_B receptors (Abellan *et al*, 2000; Slatery *et al*, 2005; Mombereau *et al*, 2004; Nakagawa *et al*, 1996), and substance P receptors (Guiard *et al*, 2004), to mention but a few.

Our group recently reported on the acute augmentation of 5-HT levels by combining SSRIs with 5-HT_{2C} antagonists

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(Cremers *et al*, 2004). The 5-HT_{2C} receptor antagonist SB242084 was chosen as it has a 100-fold affinity over other 5-HT receptors (Kennett *et al*, 1997; Bromidge *et al*, 1997). As 5-HT_{2C} receptors were not described to be prominently involved in controlling serotonin release, this observation was surprising. Furthermore, as no effects of 5-HT_{2C} antagonists are observed during basal conditions, this receptor is only mediating an effect on serotonin neurotransmission in the presence of an SSRI. It is also of interest that along with the recent finding that 5-HT_{2C} receptors are located on GABA-ergic cells in the raphe (Serrats *et al*, 2005), a number of investigators had proposed theories involving the manipulation of dopamine (DA) (Alex *et al*, 2005; Eberle-Wang *et al*, 1997; Stanford and Lacey, 1996; Giorgetti and Tecott, 2004; Di Giovanni *et al*, 2001; Pozzi *et al*, 2002), and noradrenaline (Gobert *et al*, 2000; Millan *et al*, 2005) by 5-HT_{2C} receptors localized on GABA-ergic neurons in the VTA, SN, and LC and some terminal areas.

5-HT_{2C} receptors are described to be located postsynaptically to the serotonergic neuron, and as such, the mechanism through which the augmentation of SSRI responses occurs involves alternative transmitter systems. Using microdialysis coupled to HPLC, we evaluated which neurotransmitter systems and receptors are involved in the augmentation strategy.

MATERIALS AND METHODS

Animals

Male albino rats of a Wistar-derived strain (285–320 g; Harlan, Zeist, The Netherlands) were used for the experiments. After surgery, rats were housed individually in plastic cages (35 × 35 × 40 cm³), and had free access to food and water. Animals were kept on a 12 h light schedule (light on 0700 hours). The experiments were concordant with the declarations of Helsinki and were approved by the animal care committee of the faculty of mathematics and natural science of the University of Groningen, The Netherlands.

Drugs

Citalopram hydrobromide, phaclofen, and SB242084 dihydrochloride were obtained from H Lundbeck A/S (Copenhagen, Denmark). Prazosin hydrochloride and DNQX were purchased from Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands. Citalopram was dissolved in saline, whereas SB242084 dihydrochloride and prazosin were first prepared in 20% v/v solutol solution and further diluted in saline or Ringer solution depending on whether they were required for systemic injections or local infusions. DNQX and phaclofen were dissolved in Ringer solution for infusions.

Surgery

Microdialysis of extracellular serotonin levels was performed using I-shaped microdialysis probes with a polyacrylonitrile/sodium methyl sulfonate copolymer dialysis fiber (Brainlink, Groningen, The Netherlands). The dialysis probe was stereotactically implanted under the following conditions: isoflurane 2%, N₂O 300 ml/min, and O₂ 300 ml/min. Microdialysis probes were implanted at AP: −0.53,

ML: +0.48, VD: −0.80 for hippocampus (4 mm dialysing membrane) and intra aural: +0.12, ML: +0.14, VD: −0.90 (4 mm dialysing membrane) at a 10° angle for raphe nuclei, according to coordinates from bregma (Paxinos and Watson, 1986). The microdialysis probes were permanently fixed to the skull using stainless steel screws and methylacrylic cement. Animals were allowed to recover 18–24 h before microdialysis experiments commenced.

Microdialysis Experiments

Rats were allowed to recover for at least 24 h. Probes were perfused with artificial cerebrospinal fluid containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, and 1.0 mM MgCl₂, at a flow rate of 1.5 µl/min by TSE Univentor 802 syringe pump (Technical and Scientific Equipment (TSE), Bad Homburg, Germany). Microdialysis samples were collected every 15 min in HPLC vials containing 32.5 µl 0.02 M acetic acid for monamine analysis, and 7.5 µl for GABA analysis. For drug infusion regimes, the tubing was momentarily disconnected and filled with required drug, and then reattached ensuring no air was present in the tube. The collected samples were stored in a freezer at −80°C.

Monoamine Analysis

Serotonin, norepinephrine (NE), and DA concentrations were determined using HPLC coupled with electrochemical detection. Microdialysis fractions were injected via a Gilson 223 XL autoinjector (Gilson, Villiers Le Bel, France) onto respective columns for 5-HT or NE/DA analysis.

For 5-HT determination, 20 µl of the microdialysis sample was injected onto a 100 × 2.0 mm C18 Hypersil 3 µm column (Bester, Amstelveen, The Netherlands) and separated with a mobile phase consisting of 4.1 g/l sodium acetate, 500 mg/l Na₂-EDTA, 50 mg/l heptane sulfonic acid, 4.5% methanol v/v, and 30 µl/l of triethylamine, pH 4.75 at a flow rate of 0.4 ml/min by Shimadzu LC-10 AD pumps (Shimadzu, 's Hertogenbosch, The Netherlands). 5-HT was detected amperometrically at a glassy carbon electrode at 500 mV vs Ag/AgCl (Antec Leyden, Leiden, The Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µl sample (signal-to-noise ratio 3).

For the determination of NE and DA concentrations, 20 µl microdialysate fractions were injected onto a 150 × 2.1 mm² C18 Hypersil Keystone 3 µm BDS column (Bester, Amstelveen, The Netherlands) and separated with a mobile phase consisting of 4.1 g/l sodium acetate, 150 mg/l Na₂-EDTA, 150 mg/l octane sulfonic acid, and 2.5% methanol v/v, pH 4.1 at a flow rate of 0.35 ml/min by Shimadzu LC-10 AD pumps (Shimadzu, 's Hertogenbosch, The Netherlands). NE and DA were detected amperometrically at a glassy carbon electrode at 500 mV vs Ag/AgCl (Antec Leyden, Leiden, The Netherlands). The detection limit was 0.5 fmol NE per 20 µl sample (signal-to-noise ratio 3).

GABA Analysis

GABA concentrations in the dialysates were determined offline by precolumn derivatization with *o*-phthalaldehyde/mercaptoethanol reagent, and separation by reverse-phase HPLC on a Supercosil LC-18-DB column (Rea *et al*, 2005).

Samples were derivatized as follows, based on the derivatization by Lindroth and Mopper (1979). One hundred milligrams *o*-phthalaldehyde was dissolved in 2 ml methanol and added to 200 ml 0.5 mol/l NaHCO₃ (pH adjusted to 9.5 with NaOH), containing 20 μ l 2-mercaptoethanol. The reagent was freshly prepared daily.

Thirty microliters microdialysate samples were derivatized with 50 μ l *o*-phthalaldehyde/mercaptoethanol reagent, mixed, and allowed to react for 2 min. Fifty microliters of the reaction mixture was then injected by a Gilson 231 XL sampling injector (Gilson, Villiers Le Bel, France) onto the HPLC apparatus. The mobile phase consisted of 30% methanol, 70 mM di-sodium hydrogen phosphate, 400 μ M EDTA, and 0.15% tetrahydrofluoran, and orthophosphoric acid was added dropwise until a pH of 5.25 was obtained. Fluorescent detection was performed off-column using a JASCO FP-1520 detector (excitation λ = 350 nm, emission λ = 450 nm).

Data Presentation and Statistics

Four consecutive microdialysis samples with less than 20% variation were taken as control and set at 100%. Statistical analysis was performed using Sigmapstat for Windows (Jandel Corporation). Treatments were compared using two-way ANOVA for repeated measurements, followed by Student's Newman Keuls *post hoc* analysis. Effect were compared *vs* baseline using one-way ANOVA for repeated measurements on ranks, followed by Dunnett's test. Level of significance was set at $p < 0.05$.

RESULTS

Baseline levels in dialysates from hippocampus samples were determined as 5.26 ± 0.49 fmol/sample for 5-HT ($n = 63$), 6.54 ± 0.52 fmol/sample for NE ($n = 18$), and 604.99 ± 41.21 fmol/sample for GABA, respectively ($n = 17$). In raphe samples, baseline levels were determined as 33.93 ± 4.58 fmol/sample for 5-HT, 11.39 ± 1.04 fmol/sample for DA ($n = 16$), and 15.20 ± 1.94 fmol/sample for NE ($n = 17$), respectively. Basal dialysates were not corrected for *in vitro* recovery.

In order to examine the augmentation of the SSRI-induced 5-HT release by the 5-HT_{2C} antagonist, we performed a number of combination studies at the level of the hippocampus and raphe nuclei. Experiments involving the local infusion of 10 μ M citalopram in hippocampus, with concomitant administration of the 5-HT_{2C} antagonist (0.4 mg/kg SB242084 s.c.) caused no change in basal 5-HT levels as compared to citalopram infusion with systemic vehicle administration ($F(1, 10) = 0.355$, $p = 0.564$) (Figure 1). Similarly, no effect was observed with the co-infusion of 100 nM ($F(1, 8) = 0.977$, $p = 0.352$) or 1000 nM ($F(1, 8) = 0.620$, $p = 0.454$) SB242084 with 10 μ M citalopram infusion, respectively, as compared to citalopram infusion alone (Figure 2). The absolute value of citalopram infusion in hippocampus was determined as 52.09 ± 8.39 fmol/sample for 5-HT.

The local administration of 1 μ M SB242084 in the raphe, and the hippocampus, in combination with systemic citalopram administration produced different responses in

Figure 1 The effect of systemic co-administration of 0.4 mg/kg SB242084 with 10 μ M citalopram infusion on 5-HT levels in ventral hippocampus. The horizontal bar represents the period of citalopram infusion. SB242084 and vehicle were administered systemically at $t = 0$. Data are expressed as percentage of basal levels $\pm$ SEM ($n = 6$).

Figure 2 The effect of co-infusion of 100 and 1000 nM SB242084 with 10 μ M citalopram infusion on 5-HT levels in ventral hippocampus. The horizontal bars represent the period of infusion of citalopram and SB242084, respectively. Data are expressed as percentage of basal levels $\pm$ SEM ($n = 5$).

hippocampal 5-HT release (Figure 3). It was determined that there was no augmentation of the citalopram-induced response in hippocampus when SB242084 was locally infused in the raphe ($F(1, 13) = 0.012$, $p = 0.915$), but there was a significant increase in 5-HT levels when the SB242084 was locally infused in the hippocampus ($F(1, 13) = 12.087$, $p = 0.004$). Systemic citalopram administration alone significantly increased extracellular 5-HT as compared to pre-drug administration levels ($F(1, 13) = 11.17$, $p = 0.015$).

The co-administration of systemic SB242084 with systemic citalopram (Figure 4) increased 5-HT levels in hippocampus to almost 800% of basal levels ($F(1, 8) = 9.19$, $p = 0.013$). No effects were observed on extracellular NE levels in hippocampus ($F(1, 14) = 0.227$,

Figure 3 Time course of the effect of co-infusion of 1 μ M SB242084 in RN and HC, respectively, with 3.0 mg/kg citalopram s.c. on 5-HT levels in ventral hippocampus. The horizontal bar represents the period of infusion of SB242084. Citalopram was administered systemically at $t=90$. Data are expressed as percentage of basal levels $\pm$ SEM ($n=5-9$). *Represents significance ($p<0.05$) vs citalopram.

Figure 4 Reversal of the augmenting effects of the combination of citalopram (3.0 mg/kg s.c.) with SB242084 (0.4 mg/kg s.c.) on 5-HT levels in ventral hippocampus by prazosin (0.4 mg/kg s.c.). Citalopram, prazosin, and SB242084 were administered systemically at $t=0$. Data are expressed as percentage of basal levels $\pm$ SEM ($n=5-9$). *Represents significance ($p<0.05$) vs citalopram.

$p=0.641$) or raphe nuclei ($F(1, 16)=0.989$, $p=0.335$) with the co-administration of citalopram (3.0 mg/kg s.c.) and SB242084 (0.4 mg/kg s.c.), vs citalopram administration alone (data not shown). However, the systemic administration of the α -1 antagonist, prazosin (0.4 mg/kg), prevented the 5-HT_{2C}-mediated augmentation in 5-HT levels in hippocampus ($F(1, 14)=0.227$, $p=0.641$).

No effect on extracellular DA release was observed in the raphe nuclei with systemic citalopram administration (3.0 mg/kg) alone ($F(1, 12)=0.334$, $p=0.574$) or in combination with systemic SB242084 (0.4 mg/kg) ($F(1, 6)=0.641$, $p=0.339$) (data not shown).

To examine the potential involvement of the glutamate system in the 5-HT_{2C} receptor-mediated augmentation,

Figure 5 The investigation of the effect of 3.0 mg/kg citalopram s.c. and 0.4 mg/kg SB242084 s.c. combination, with 100 μ M DNQX preinfusion on 5-HT levels in ventral hippocampus. Citalopram and SB242084 were administered systemically at $t=90$. The horizontal bar represents the period of infusion of DNQX, or vehicle. Data are expressed as percentage of basal levels $\pm$ SEM ($n=5-9$). *Represents significance ($p<0.05$) vs citalopram.

5-HT levels were monitored while the AMPA/kainate antagonist, DNQX (100 μ M), was infused during the administration of SB242084 (0.4 mg/kg s.c.) with citalopram (3.0 mg/kg s.c.) (Figure 5). The infusion of DNQX did not modify the increase in 5-HT observed with the combination of SB242084 and citalopram ($F(1, 10)=0.0292$, $p=0.868$).

The local infusion of the GABA_B antagonist phaclofen was also shown to augment the effect of the SSRI citalopram on 5-HT in hippocampus (Figure 6). A 50 μ M phaclofen infusion augmented the citalopram-induced increase in 5-HT in a manner similar to the augmentation seen with the 5-HT_{2C} antagonist SB242084 ($F(1, 12)=12.813$, $p=0.004$). Infusion of the GABA_A antagonist bicuculline (50 μ M) significantly increased basal 5-HT levels ($F(1, 9)=11.97$, $p=0.007$) in hippocampus (Figure 7), although the combination of systemic citalopram (3.0 mg/kg) produced no further effect on 5-HT levels ($F(1, 13)=0.158$, $p=0.698$). The combination of SB242084 (0.4 mg/kg s.c.) with citalopram (3.0 mg/kg s.c.) resulted in a slight, yet significant decrease in extracellular GABA in hippocampus ($F(1, 18)=5.053$, $p=0.037$), when compared to citalopram alone (Figure 8).

DISCUSSION

Classical augmentation of SSRI effects by 5-HT_{1A} and 5-HT_{1B} antagonists is related to serotonin autoreceptor blockade, which, in turn facilitate serotonin release. Interestingly, the augmentation of 5-HT with the combination of citalopram and 5-HT_{2C} receptor antagonists was of similar magnitude as previously reported for compounds that block 5-HT_{1A} and 5-HT_{1B} autoreceptors (Cremers *et al*, 2000a, b, 2001, 2004). However, the current mechanism of augmentation is more complex, as 5-HT_{2C} receptors are not located on serotonergic neurons (see below). Multiple neurotransmitter systems are likely to be involved in the

Figure 6 Time course of the effect of 3.0 mg/kg citalopram s.c. and 50 μ M phaclofen on 5-HT levels in ventral hippocampus. Citalopram was administered systemically at $t=90$. The horizontal bar represents the period of infusion of phaclofen or vehicle. Data are expressed as percentage of basal levels $\pm$ SEM ($n=5-9$). *Represents significance ($p<0.05$) vs citalopram.

Figure 7 Time course of the effect of 3.0 mg/kg citalopram s.c. and 50 μ M bicuculline on 5-HT levels in ventral hippocampus. Citalopram was administered systemically at $t=90$. The horizontal bar represents the period of infusion of bicuculline or vehicle. Data are expressed as percentage of basal levels $\pm$ SEM ($n=5-9$). *Represents significance ($p<0.05$) vs citalopram.

effects of 5-HT_{2C} antagonists, eventually leading to the augmentation of SSRI effects.

Distribution of 5-HT_{2C} receptors in the brain is abundant. The highest densities are found in the chorioid plexus, cerebral cortex, hippocampus, striatum, and substantia nigra (Barnes and Sharp, 1999; Hoyer *et al*, 2002). Evidence that 5-HT_{2C} receptors might be involved in the regulation of serotonin release is scarce. In fact, a number of researchers have reported slight attenuations in basal NE and DA levels with no effect on basal 5-HT (Millan *et al*, 1998, 2005; Gobert *et al*, 2000).

Similarly, no effect has been found when potassium-evoked serotonin release from brain homogenate was studied *in vitro* in the presence of 5-HT_{2C} antagonist

Figure 8 Investigation of the effect of 3.0 mg/kg citalopram s.c. and 0.4 mg/kg SB242084 s.c. co-administration on GABA levels in ventral hippocampus. Citalopram and SB242084 were administered systemically at $t=0$. Data are expressed as percentage of basal levels $\pm$ SEM ($n=6$).

ketanserin (Bonanno *et al*, 1986; Maura *et al*, 1986). These data were confirmed by the absence of effects when both the SSRI and the 5-HT_{2C} antagonist were applied locally in the hippocampus in the present study. Interestingly, these experiments indicate that the augmentation originates from blockade of 5-HT_{2C} receptors in terminal areas, but does not comprise a mere local interaction, as the SSRI has to be administered systemically. Thus, the SSRI activates systems outside the vicinity of the microdialysis probe that are required for 5-HT_{2C} antagonists to become relevant.

The 5-HT_{2C} receptor is located as a heteroreceptor on GABA-ergic neurons (Serrats *et al*, 2005), and may also be located on glutamatergic, noradrenergic, or dopaminergic neurons (see below). Theoretically, all these systems might be involved in the mechanism through which 5-HT_{2C} antagonists augment the effects of SSRIs on serotonin levels in the brain.

Norepinephrine

Several studies have shown that NE release is increased upon systemic administration of 5-HT_{2C} antagonists (Millan *et al*, 2005, 1998; Gobert *et al*, 2000). Additional studies show that the manipulation of NE release affects serotonin release through adrenoceptors which are postulated to be present in the dorsal raphe nucleus (Hopwood and Stamford, 2001; Bortolozzi and Artigas, 2003; Pudovkina *et al*, 2003) and possibly terminal areas (Rouquier *et al*, 1994; Amargos-Bosch *et al*, 2003; Linner *et al*, 2004; Koch *et al*, 2004; Weikop *et al*, 2004; Gobert *et al*, 1997; De Boer *et al*, 1996). However, if NE would be involved in the present mechanism, it is to be expected that NE levels would be enhanced when the SSRI was administered in conjunction with the 5-HT_{2C} antagonist. As no effects on NE levels were observed in hippocampus or in raphe nuclei, the direct involvement of NE in the mechanism of augmentation is less likely. It is possible that the co-administration of these compounds is affecting the noradrenergic system at regions

other than those in this study, which may indirectly be mediating effects on 5-HT release.

Interestingly, after investigation of a series of receptor-specific antagonists, we determined that the α_1 -adrenoceptor antagonist prazosin completely abolished the augmentation by 5-HT_{2C} receptor antagonists, implying that a norepinephrinergic tone on the serotonergic neurons is necessary but not directly involved in the augmentation at the level of the hippocampus.

Dopamine

Interactions between the serotonergic system and the dopaminergic system have also been described (Martin-Ruiz *et al*, 2001b; Ferre *et al*, 1994). Antagonism of 5-HT_{2C} receptors in the prefrontal cortex has been shown to elevate DA and NE (Alex *et al*, 2005; Giorgetti and Tecott, 2004; Lucas and Spampinato, 2000; Gobert *et al*, 2000; Millan *et al*, 1998; De Deurwaerdere *et al*, 2004; Di Matteo *et al*, 1999, 2000). As dopaminergic innervation of the hippocampus is low, no augmentation of DA levels were detectable. It has been previously shown that DA D₁-specific compounds had no effect on the turnover of 5-HT (Lappalainen *et al*, 1991), and that the DA D₂ receptor agonist increased local 5-HT release in RN (Ferre S and Artigas F, 1993), although this effect may be mediated at D₂ receptors localized outside the raphe nuclei (Martin-Ruiz *et al*, 2001b). However, no effect was observed on extracellular DA levels in the raphe nuclei after a single administration, or the combination of the 5-HT_{2C} antagonist with citalopram, which renders an interaction with DA neurons less likely.

Glutamate

A study by Martin-Ruiz *et al* (2001a) has shown that modulation of glutamatergic neurotransmission by 5-HT₂ receptors might also be involved in control of serotonin release. Evidence suggests that the 5-HT_{2A/2C} receptor agonist, 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane, increases glutamate levels in terminal areas (Scruggs *et al*, 2003), and it has been reported that enhanced glutamatergic release facilitates serotonin release through activation of kainate receptors which are located on the serotonergic neuron (Martin-Ruiz *et al*, 2001a). The administration of SSRIs would increase extracellular 5-HT, and thus activate both 5-HT_{2A} and 5-HT_{2C} receptors on glutamatergic neurons. The application of SB242084 would block 5-HT_{2C} receptor-mediated regulation of glutamate release, leaving the response to be mediated by 5-HT_{2A} receptors, which have been shown to be located on glutamatergic neurons and increase glutamate release after stimulation (Martin-Ruiz *et al*, 2001a). However, if this were the underlying mechanism, it should have been possible to prevent the augmentation of serotonin release by glutamate with the local administration of AMPA/kainate antagonist DNQX. As no attenuation of augmentation was observed, glutamatergic involvement is also not likely. In addition, evaluation of the effects of citalopram with and without SB242084 on glutamate levels did not show any effects (data not included). Of course, the limited relevance of glutamate levels as measured with microdialysis should be taken into account. Various authors have expressed serious doubts

whether glutamate sampled by microdialysis is of synaptic origin (Timmerman and Westerink, 1997). One key finding to support this is that very few researchers have reported a decrease in glutamate levels with the administration of tetrodotoxin, whereas there is a dramatic decrease in levels of neurotransmitters such as serotonin, noradrenaline, or DA.

GABA

Several studies have shown that 5-HT_{2C} receptors facilitate GABA release (Liu *et al*, 2000; Hajos *et al*, 2003; Abi-Saab *et al*, 1999), and recently 5-HT_{2C} receptors have been shown to be localized on GABA cells in the raphe nucleus (Serrats *et al*, 2005). Arguably, a diminished GABA-ergic tone owing to 5-HT_{2C} receptor antagonists in this area may contribute to the increased effect of SSRIs. Several studies have shown that inhibition of 5-HT release by GABA is mediated by GABA_A and GABA_B receptors (Pei *et al*, 1989; Tao and Auerbach, 2000, 2003). This would indicate that there is a reciprocal relationship between the activity of GABA-ergic and serotonergic neurons. However, to date, there are no studies localizing the 5-HT_{2C} receptors to GABA-ergic neurons in terminal regions.

The present study clearly shows a prominent interaction between the serotonergic and the GABA-ergic system. In the presence of an SSRI, the extent of 5-HT release is governed by a GABA_B receptor-mediated feedback control. This effect was not apparent under pre-SSRI administration conditions. In contrast, a GABA_A receptor-induced feedback was present under basal conditions, but not in the presence of the SSRI. Although the involvement of the GABA_B receptor in the augmentation of the serotonin response by citalopram is apparent, a pronounced increase of GABA levels in hippocampus upon administration of the SSRI was not observed. However, a slight, yet significant decrease in GABA levels was reported with the combination of citalopram and SB242084 as compared to citalopram alone. The mechanism of the 5-HT_{2C}-mediated augmentation of the SSRI response on 5-HT levels may be explained by this decrease in GABA release, which may in turn affect the GABA tone on terminal serotonin neurons. As the GABA receptors have an inhibitory effect on 5-HT release, the co-administration of SB242084 may serve to reverse these effects, thus allowing a further increase in extracellular 5-HT.

Conclusion

In the present study, we have evaluated several mechanisms through which 5-HT_{2C} antagonists might augment the effects of SSRIs on serotonin release. Whereas the involvement of dopaminergic and glutamatergic systems does not seem likely, NE and GABA-ergic systems are crucial for the observed effects. A basal stimulatory input from the NE system is required for the augmentation, although it is not thought to underlie the mechanism under evaluation. Enhanced GABA-ergic feedback through activation of terminal GABA_B receptors was found in the presence of systemic SSRI treatment. The absence of pronounced effects of the SSRI on GABA levels in hippocampus, remains to be explained, but might be related to the differential origin of

GABA levels as quantified by microdialysis. However, the decrease in GABA levels with the combination of SB242084 and citalopram, as compared to citalopram alone, as well as the fact that phaclofen augmented the citalopram-induced increase in 5-HT, indicates that the GABA system is indeed involved in the mechanism of the augmentation.

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