Neuropsychopharmacology (2007) 32, 1550–1557

www.neuropsychopharmacology.org

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

Thomas IFH Cremers*[1,2,3,4,5], Kieran Rea[2], Fokko J Bosker[2,5], Håkan V Wikström[4], Sandra Hogg[3], Arne Mørk[3] and Ben HC Westerink[1,2]

[1]Brains-on-Line, Antonius Deusinglaan 1, Groningen, The Netherlands; [2]Department of Biomonitoring and Sensoring, University Centre for Pharmacy, Antonius Deusinglaan 1, Groningen, The Netherlands; [3]H Lundbeck A/S, Ottiliavej 9, Copenhagen, Denmark; [4]Department of Medicinal Chemistry, Rijksuniversiteit Groningen, Antonius Deusinglaan 1, Groningen, The Netherlands; [5]Department of Psychiatry, University Medical Centre of Groningen, Hanzeplein 1, Groningen, The Netherlands

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.
Neuropsychopharmacology (2007) **32,** 1550–1557; doi:10.1038/sj.npp.1301287; published online 3 January 2007

Keywords: microdialysis; citalopram; 5-HT_{2C} receptor; SB242084; GABA; augmentation

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).

*Correspondence: Dr TIFH Cremers, Department of Biomonitoring and Sensoring, University Centre for Pharmacy, Antonius Deusinglaan 1, Groningen 9713 AV, The Netherlands, Tel: +31 621506340, Fax: +31 503636908, E-mail: t.i.f.h.cremers@rug.nl
Received 17 February 2006; revised 21 September 2006; accepted 25 September 2006

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 heteroceptors 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; Slattery *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

(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 ($35 \times 35 \times 40\,cm^3$), 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 dissolve 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: isofluorane 2%, N_2O 300 ml/min, and O_2 300 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 $CaCl_2$, and 1.0 mM $MgCl_2$, 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 Na_2-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 $150 \times 2.1\,mm^2$ 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 Na_2-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 off-line by precolumn derivatization with *o*-phtaldialdehyde/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 derivitization by Lindroth and Mopper (1979). One hundred milligrams *o*-phtaldialdehyde was dissolved in 2 ml methanol and added to 200 ml 0.5 mol/l $NaHCO_3$ (pH adjusted to 9.5 with NaOH), containing 20 µl 2-mercaptoethanol. The reagent was freshly prepared daily.

Thirty microliters microdialysate samples were derivatized with 50 µl *o*-phtaldialdehyde/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 µM 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 $\lambda = 350$ nm, emission $\lambda = 450$ nm).

Data Presentation and Statistics

Four consecutive microdialysis samples with less then 20% variation were taken as control and set at 100%. Statistical analysis was performed using Sigmastat 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 Dunnet'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 µM 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 µM 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 µM SB242084 in the raphe, and the hippocampus, in combination with systemic citalopram administration produced different responses in 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 ($F(1, 14) = 0.227$,

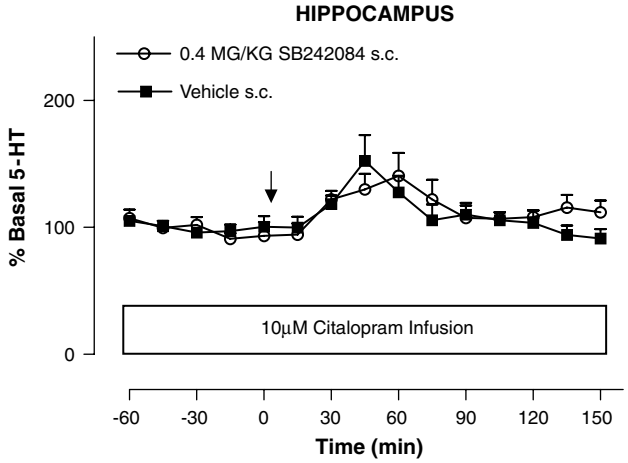

Figure 1 The effect of systemic co-administration of 0.4 mg/kg SB242084 with 10 µM citalopram infusion on 5-HT levels in ventral hippocampus. The horizontal bar represents the period of citalopram infusion. SB242084 and vehicle were administered systemically at $t = 0$. Data are expressed as percentage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

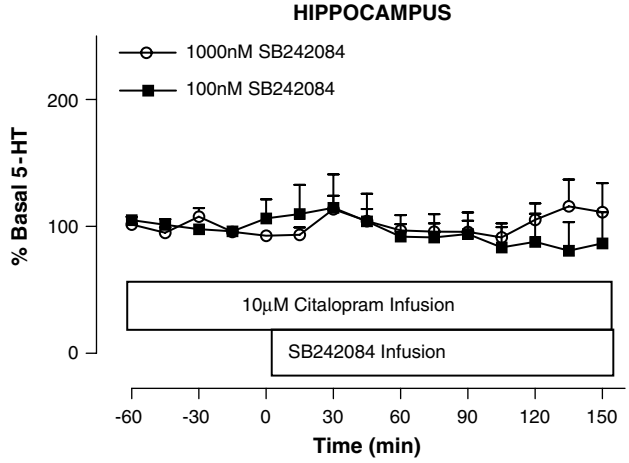

Figure 2 The effect of co-infusion of 100 and 1000 nM SB242084 with 10 µM citalopram infusion on 5-HT levels in ventral hippocampus. The horizontal bars represent the period of infusion of citalopram and SB242084, respectively. Data are expressed as percentage of basal levels ± SEM ($n = 5$).

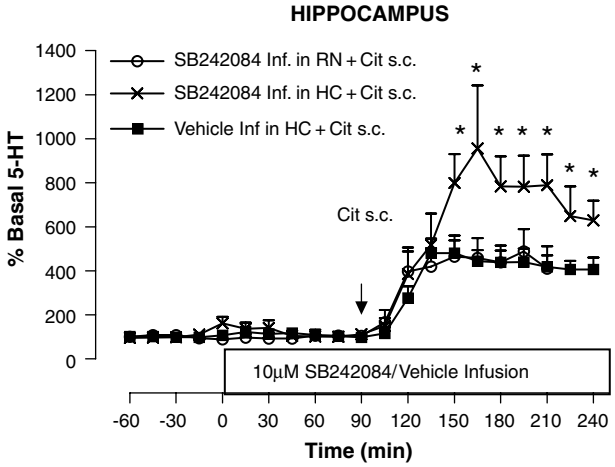

Figure 3 Time course of the effect of co-infusion of 1 μM SB242084 in RN and HC, respectively, with 3.0 mg/kg citalopram s.c. on 5-HT levels in ventral hippocampus. The horizontal bar represents the period of infusion of SB242084. Citalopram was administered systemically at $t = 90$. Data are expressed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

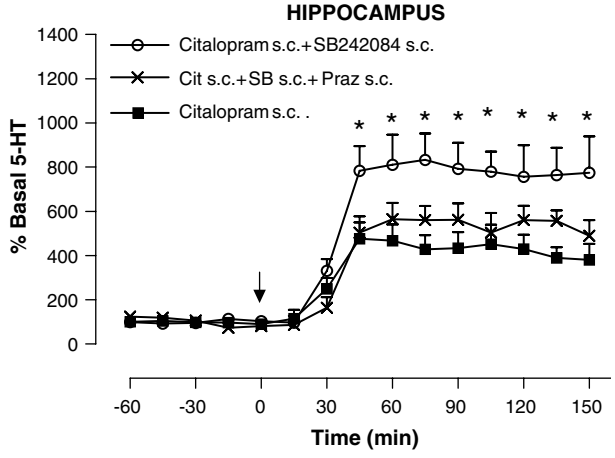

Figure 4 Reversal of the augmenting effects of the combination of citalopram (3.0 mg/kg s.c.) with SB242084 (0.4 mg/kg s.c.) on 5-HT levels in ventral hippocampus by prazosin (0.4 mg/kg s.c.). Citalopram, prazosin, and SB242084 were administered systemically at $t = 0$. Data are expressed as percentage of basal levels ± 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,

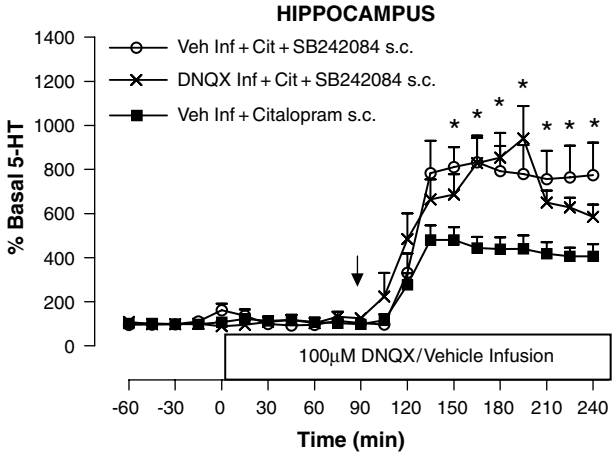

Figure 5 The investigation of the effect of 3.0 mg/kg citalopram s.c. and 0.4 mg/kg SB242084 s.c. combination, with 100 μM DNQX preinfusion on 5-HT levels in ventral hippocampus. Citalopram and SB242084 were administered systemically at $t = 90$. The horizontal bar represents the period of infusion of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

5-HT levels were monitored while the AMPA/kainate antagonist, DNQX (100 μM), 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 μM 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 bicucculine (50 μM) 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

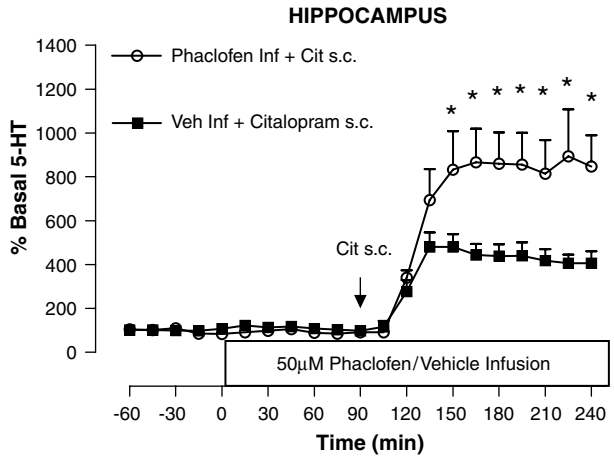

Figure 6 Time course of the effect of 3.0 mg/kg citalopram s.c. and 50 μM phaclofen on 5-HT levels in ventral hippocampus. Citalopram was administered systemically at $t = 90$. The horizontal bar represents the period of infusion of phaclofen or vehicle. Data are expressed as percentage of basal levels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

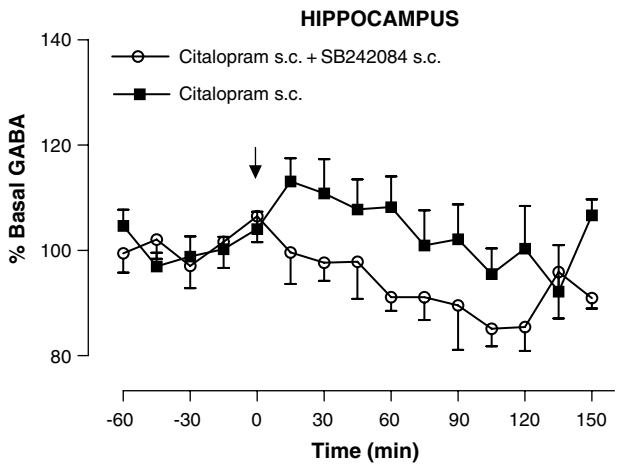

Figure 8 Investigation of the effect of 3.0 mg/kg citalopram s.c. and 0.4 mg/kg SB242084 s.c. co-administration on GABA levels in ventral hippocampus. Citalopram and SB242084 were administered systemically at $t = 0$. Data are expressed as percentage of basal levels ± SEM ($n = 6$).

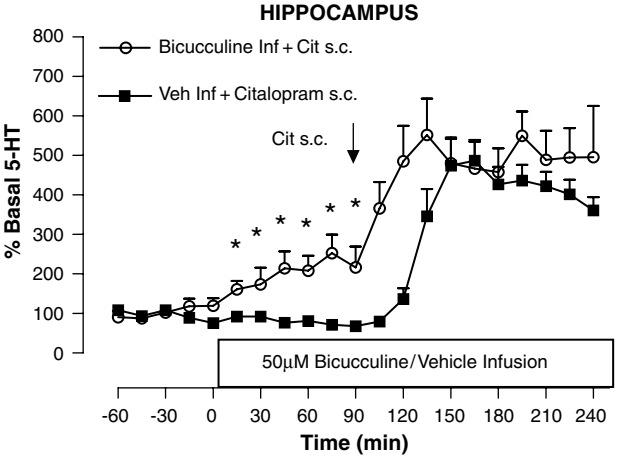

Figure 7 Time course of the effect of 3.0 mg/kg citalopram s.c. and 50 μM bicuculline on 5-HT levels in ventral hippocampus. Citalopram was administered systemically at $t = 90$. The horizontal bar represents the period of infusion of bicuculline or vehicle. Data are expressed as percentage of basal levels ± 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 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 heteroceptor 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 D$_1$-specific compounds had no effect on the turnover of 5-HT (Lappalainen *et al*, 1991), and that the DA D$_2$ receptor agonist increased local 5-HT release in RN (Ferre S and Artigas F, 1993), although this effect may be mediated at D$_2$ 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-HT$_2$ 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.

REFERENCES

Abellan MT, Jolas T, Aghajanian GK, Artigas F (2000). Dual control of dorsal raphe serotonergic neurons by GABA$_B$ receptors. Electrophysiological and microdialysis studies. *Synapse* **36**: 21–34.

Abi-Saab WM, Bubser M, Roth RH, Deutch AY (1999). 5-HT$_2$ receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacology* **20**: 92–96.

Alex KD, Yavanian GJ, McFarlane HG, Pluto CP, Pehek EA (2005). Modulation of dopamine release by striatal 5-HT$_{2C}$ receptors. *Synapse* **55**: 242–251.

Amargos-Bosch M, Adell A, Bortolozzi A, Artigas F (2003). Stimulation of alpha1-adrenoceptors in the rat medial prefrontal cortex increases the local *in vivo* 5-hydroxytryptamine release: reversal by antipsychotic drugs. *J Neurochem* **87**: 831–842.

Artigas F, Perez V, Alvarez E (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch Gen Psychiatry* **51**: 248–251.

Artigas F, Romero L, de Montigny C, Blier P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT$_{1A}$ antagonists. *Trends Neurosci* **19**: 378–383.

Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* **38**: 1083–1152.

Bengtsson HJ, Kullberg A, Millan MJ, Hjorth S (1998). The role of 5-HT$_{1A}$ autoreceptors and alpha 1 adrenoceptors in the modulation of 5-HT release-III clozapine and the novel putative antipsychotic S16924. *Neuropharmacology* **37**: 349–356.

Besson A, Haddjeri N, Blier P, de Montigny C (2000). Effects of the co-administration of mirtazapine and paroxetine on serotonergic neurotransmission in the rat brain. *Eur Neuropsychopharmacol* **10**: 177–188.

Blier P (2001). Pharmacology of rapid-onset antidepressant treatment strategies. *J Clin Psychiatry* **62**: 12–17.

Blier P, de Montigny C, Chaput Y (1987). Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J Clin Psychopharmacol* **7**: 24S–35S.

Blier P, Pineyro G, el Mansari M, Bergeron R, de Montigny C (1998). Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann N Y Acad Sci* **861**: 204–216.

Bonanno G, Maura G, Raiteri M (1986). Pharmacological characterization of release-regulating serotonin autoreceptors in rat cerebellum. *Eur J Pharmacol* **126**: 317–321.

Bortolozzi A, Artigas F (2003). Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain. Role of alpha-adrenoceptors. *Neuropsychopharmacology* **28**: 421–434.

Bosker FJ, Cremers TI, Jongsma ME, Westerink BH, Wikstrom HV, den Boer JA (2001). Acute and chronic effects of citalopram on postsynaptic 5-hydroxytryptamine(1A) receptor-mediated feedback: a microdialysis study in the amygdala. *J Neurochem* **76**: 1645–1653.

Bromidge SM, Duckworth M, Forbes IT, Ham P, King FD, Thewlis KM *et al* (1997). 6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB-242084): the first selective and brain penetrant 5-HT2C receptor antagonist. *J Med Chem* **40**: 3494–3496.

Celada P, Puig MV, Casanovas JM, Guillazo G, Artigas F (2001). Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, GABA(A), and glutamate receptors. *J Neurosci* **21**: 9917–9929.

Cremers TI, de Boer P, Liao Y, Bosker FJ, den Boer JA, Westerink BH *et al* (2000a). Augmentation with a 5-HT$_{1A}$, but not a 5-HT$_{1B}$ receptor antagonist critically depends on the dose of citalopram. *Eur J Pharmacol* **397**: 63–74.

Cremers TI, Giorgetti M, Bosker FJ, Hogg S, Arnt J, Mork A *et al* (2004). Inactivation of 5-HT$_{2C}$ receptors potentiates consequences of serotonin reuptake blockade. *Neuropsychopharmacology* **29**: 1782–1789.

Cremers TI, Spoelstra EN, de Boer P, Bosker FJ, Mork A, den Boer JA *et al* (2000b). Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram. *Eur J Pharmacol* **397**: 351–357.

Cremers TI, Wiersma LJ, Bosker FJ, den Boer JA, Westerink BH, Wikstrom HV (2001). Is the beneficial antidepressant effect of co administration of pindolol really due to somatodendritic autoreceptor antagonism? *Biol Psychiatry* **50**: 13–21.

de Boer TH, Nefkens F, van Helvoirt A, van Delft AM (1996). Differences in modulation of noradrenergic and serotonergic transmission by the alpha-2 adrenoceptor antagonists, mirtazapine, mianserin and idazoxan. *J Pharmacol Exp Ther* **277**: 852–860.

de Deurwaerdere P, Navailles S, Berg KA, Clarke WP, Spampinato U (2004). Constitutive activity of the serotonin$_{2C}$ receptor inhibits *in vivo* dopamine release in the rat striatum and nucleus accumbens. *J Neurosci* **24**: 3235–3241.

Di Giovanni G, Di Matteo V, La Grutta V, Esposito E (2001). m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience* **103**: 111–116.

Di Matteo V, Di Mascio M, Di Giovanni G, Esposito E (1999). SB 242084, a selective serotonin$_{2C}$ receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology* **38**: 1195–1205.

Di Matteo V, Di Mascio M, Di Giovanni G, Esposito E (2000). Acute administration of amitriptyline and mianserin increases dopamine release in the rat nucleus accumbens: possible involvement of serotonin$_{2C}$ receptors. *Psychopharmacology* **150**: 45–51.

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF (1997). Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. *J Comp Neurol* **384**: 233–247.

Ferre S, Artigas F (1993). Dopamine D2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. *J Neurochem* **61**: 772–775.

Ferre S, Cortes R, Artigas F (1994). Dopaminergic regulation of the serotonergic raphe-striatal pathway: microdialysis studies in freely moving rats. *J Neurosci* **14**: 4839–4846.

Giorgetti M, Tecott LH (2004). Contributions of 5-HT$_{2C}$ receptors to multiple actions of central serotonin systems. *Eur J Pharmacol* **29**: 1–9.

Gobert A, Millan MJ (1999). Serotonin (5-HT)$_{2A}$ receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. *Neuropharmacology* **38**: 315–317.

Gobert A, Rivet JM, Cistarelli L, Melon C, Millan MJ (1997). Alpha-2 adrenergic receptor blockade markedly potentiates duloxetine- and fluoxetine-induced increases in noradrenaline, dopamine, and serotonin levels in the frontal cortex of freely moving rats. *J Neurochem* **69**: 2616–2619.

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP *et al* (2000). Serotonin$_{2C}$ receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse* **36**: 205–221.

Guiard BP, Przybylski C, Guilloux JP, Seif I, Froger N, De Felipe C *et al* (2004). Blockade of substance P (neurokinin 1) receptors

enhances extracellular serotonin when combined with a selective serotonin reuptake inhibitor: an *in vivo* microdialysis study in mice. *J Neurochem* **89**: 54–63.

Hajos M, Hoffman WE, Weaver RJ (2003). Regulation of septo-hippocampal activity by 5-hydroxytryptamine$_{2C}$ receptors. *J Pharmacol Exp Ther* **306**: 605–615.

Hjorth S, Bengstsson HJ, Kullberg A, Carlzon D, Peilot H, Auerbach SB (2000). Serotonin autoreceptor function and antidepressant drug action. *J Psychopharmacol* **14**: 177–185.

Hopwood SE, Stamford JA (2001). Noradrenergic modulation of serotonin release in rat dorsal and median raphe nuclei via alpha-1 and alpha-2A adrenoceptors. *Neuropharmacology* **41**: 433–442.

Hoyer D, Hannon JP, Martin GR (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* **71**: 533–554.

Kennett GA, Wood MD, Bridge F, Trail B, Riley G, Holland V *et al* (1997). SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology* **36**: 609–620.

Koch S, Perry KW, Bymaster FP (2004). Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology* **46**: 232–242.

Lappalainen J, Hietala J, Koulu M, Sjoholm B, Syvalahti E (1991). Effects of acute administration of SCH 23390 on dopamine and serotonin turnover in major dopaminergic areas and mesencephalic raphe nuclei—comparison with ritanserin. *Prog Neuropsychopharmacol Biol Psychiatry* **15**: 861–872.

Lindroth P, Mopper K (1979). High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivitization with opthalaldehyde. *Anal Chem* **51**: 1667–1674.

Linner L, Wiker C, Arborelius L, Schalling M, Svensson TH (2004). Selective noradrenaline reuptake inhibition enhances serotonergic neuronal activity and transmitter release in the rat forebrain. *J Neural Transm* **111**: 127–139.

Liu R, Jolas T, Aghajanian G (2000). Serotonin 5-HT$_2$ receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res* **873**: 34–45.

Lucas G, Spampinato U (2000). Role of striatal serotonin$_{2A}$ and serotonin$_{2C}$ receptor subtypes in the control of *in vivo* dopamine outflow in the rat striatum. *J Neurochem* **74**: 693–701.

Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G *et al* (2001a). Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J Neurosci* **21**: 9856–9866.

Martin-Ruiz R, Ugedo L, Honrubia MA, Mengod G, Artigas F (2001b). Control of serotonergic neurons in rat brain by dopaminergic receptors outside the dorsal raphe nucleus. *J Neurochem* **77**: 762–775.

Maura G, Roccatagliata E, Raiteri M (1986). Serotonin autoreceptor in rat hippocampus: pharmacological characterization as a subtype of the 5-HT$_1$ receptor. *Naunyn Schmiedebergs Arch Pharmacol* **334**: 323–326.

Millan MJ, Brocco M, Gobert A, Dekeyne A (2005). Anxiolytic properties of agomelatine, an antidepressant with melatoninergic and serotonergic properties: role of 5-HT$_{2C}$ receptor blockade. *Psychopharmacology (Berlin)* **177**: 448–458.

Millan MJ, Dekeyne A, Gobert A (1998). Serotonin 5-HT$_{2C}$ receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex *in vivo*. *Neuropharmacology* **37**: 953–955.

Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF (2004). Genetic and pharmacological evidence of a role for GABA$_B$ receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology* **29**: 1050–1062.

Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M, Takashima T (1996). Involvement of GABA$_B$ receptor systems in experimental depression: baclofen but not bicuculline exacerbates helplessness in rats. *Brain Res* **741**: 240–245.

Paxinos G., Watson C (1986). *The Rat Brain in Stereotactic Coordinates*. Academic Press: London.

Pei Q, Zetterstrom T, Fillenz M (1989). Both systemic and local administration of benzodiazepine agonists inhibit the *in vivo* release of 5-HT from ventral hippocampus. *Neuropharmacology* **28**: 1061–1066.

Pozzi L, Acconacia S, Ceglia I, Invernizzi RW, Samann R (2002). Stimulation of 5-hydroxytryptamine 5-HT$_{2C}$ receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J Neurochem* **82**: 93–100.

Pudovkina OL, Cremers TI, Westerink BH (2003). Regulation of the release of serotonin in the dorsal raphe nucleus by alpha-1 and alpha-2 adrenoceptors. *Synapse* **50**: 77–82.

Rea K, Cremers TI, Westerink BHC (2005). HPLC conditions are critical for the detection of GABA by microdialysis. *J Neurochem* **94**: 672–679.

Rouquier L, Claustre Y, Benavides J (1994). Alpha-1 adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: a microdialysis study. *Eur J Pharmacol* **261**: 59–64.

Scruggs JL, Schmidt D, Deutch AY (2003). The hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) increases cortical extracellular glutamate levels in rats. *Neurosci Lett* **346**: 137–140.

Serrats J, Mengod G, Cortes R (2005). Expression of serotonin 5-HT$_{2C}$ receptors in GABAergic cells of the anterior raphe nuclei. *J Chem Neuroanat* **29**: 83–91.

Slattery DA, Desrayaud S, Cryan JF (2005). GABA$_B$ receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. *J Pharmacol Exp Ther* **312**: 290–296.

Stanford IM, Lacey MG (1996). Differential actions of serotonin, mediated by 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors, on GABA-mediated synaptic input to rat substantia nigra pars reticulata neurons *in vitro*. *J Neurosci* **16**: 7566–7573.

Szabo ST, Blier P (2001). Effects of the selective norepinephrine reuptake inhibitor reboxetine on norepinephrine and serotonin transmission in the rat hippocampus. *Neuropsychopharmacology* **25**: 845–857.

Szabo ST, Blier P (2002). Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT$_{2A}$ receptor antagonism on the firing activity of norepinephrine neurons. *J Pharmacol Exp Ther* **302**: 983–991.

Tao R, Auerbach SB (2000). Regulation of serotonin release by GABA and excitatory amino acids. *J Psychopharmacol* **14**: 100–113.

Tao R, Auerbach SB (2003). Influence of inhibitory and excitatory inputs on serotonin efflux differs in the dorsal and median raphe nuclei. *Brain Res* **961**: 109–120.

Timmerman W, Westerink BH (1997). Brain microdialysis of GABA and glutamate: what does it signify? *Synapse* **27**: 242–261.

Weikop P, Kehr J, Scheel-Kruger J (2004). The role of alpha-1 and alpha-2 adrenoreceptors on venlafaxine-induced elevation of extracellular serotonin, noradrenaline and dopamine levels in the rat prefrontal cortex and hippocampus. *J Psychopharmacol* **18**: 395–403.