Augmentation of the neurochemical and behavioural effects of SSRIs
Rea, Kieran

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Chapter 8

Augmentation of antidepressant effects of SSRIs by GABA_B antagonists: mechanistic studies
Abstract

The slow onset of therapeutic effect of SSRIs has been linked to the gradual desensitization of 5-HT autoreceptors during treatment. Recently, it has been reported that 5-HT$_{2C}$ receptor antagonists augment the biochemical and behavioral effects of SSRIs. This augmentation has been attributed to an attenuation of GABA$_B$ induced feedback due to inhibition of GABA release by 5-HT$_{2C}$ antagonism. These experiments also showed that local infusion of GABA$_B$ antagonists in hippocampus augmented the effects of SSRIs in a similar fashion as 5-HT$_{2C}$ antagonists. In the current study we present data on the augmentation of biochemical and behavioral effects of SSRIs by GABA$_B$ antagonists. Using microdialysis coupled to HPLC, it was observed that systemic administration of phaclofen and the GABA$_B$ antagonists, CGP 463812 and SCH 50911, augmented the biochemical effects of citalopram on central 5-HT levels in raphe, and ventral hippocampus in a bell-shaped manner. While there was an augmentation, a bell-shaped profile of the combined administration of phaclofen and citalopram was not observed in the prefrontal cortex. Similar effects were observed in behavioural experiments involving the co-administration of GABA$_B$ compounds with citalopram. The present data clearly suggest that GABA$_B$ antagonists augment the efficacy of SSRIs. However, given indications for bell-shaped dose dependency, this approach might be difficult to use in the clinic.
Augmentation of antidepressant effects of SSRIs by GABA<sub>B</sub> antagonists: mechanistic studies

Introduction

Serotonin-containing neurons are localized in the midbrain and brainstem raphe nuclei and project to most regions of the brain, where they exert a wide array of physiological functions. A number of conditions such as depression, anxiety and various psychiatric illnesses are attributed, at least in part, to abnormalities of the serotonergic system. Current popular antidepressant treatment involves the inhibition of reuptake of monoamines such as noradrenaline and serotonin, or the manipulation of specific target receptors which modulate monoamine levels. The administration of selective serotonin reuptake inhibitors (SSRIs) dramatically increases the level of extracellular serotonin in the brain. It is also known that with the administration of SSRIs there is a certain amount of feedback to 5HT autoreceptors, and other 5HT receptors located on various other neurons which also impact on the release and firing of 5HT neurons. The firing and release of neurotransmitters from these serotonergic neurons is under a fine control of a number of different neurotransmitter systems such as serotonin itself (Barnes & Sharp, 1999), noradrenaline (Hjorth et al, 1995; Tao & Hjorth, 1992; de Boer et al, 1996), dopamine (Ferre et al, 1993, 1994), histamine (Schwarz et al, 1991; Barbera et al, 2002), glutamate (Ghersi et al, 2003; Pittaluga et al, 1987), and many others, including GABA (Tao & Auerbach, 2000).

The inhibitory neurotransmitter GABA plays a critical role in the regulation of firing of 5-HT neurons (Gallagher and Aghajanian, 1976; Abellan et al, 2000a; Tao & Auerbach, 1996; Bowery et al, 1987; Chu et al, 1990; Mennini et al, 1986). Indeed both GABA<sub>A</sub> (Gao et al, 1993; Rodriguez-Pallares et al, 2001) and GABA<sub>B</sub> receptors (Abellan et al, 2000b; Varga et al, 2002; Wirtshafter & Sheppard, 2001) have been shown to be located in the raphe on serotonergic neurons. Interestingly, GABA<sub>B</sub> receptors (Varga et al, 2002) as well as 5HT<sub>2C</sub> and 5HT<sub>1A</sub> receptors (Serrats et al, 2005) have also been localized in the raphe, on GABA interneurons which synapse with serotonergic neurons. Previous experiments have reported an increase in 5HT levels due to SSRI administration, which can be further augmented by 5HT<sub>2C</sub> and 5HT<sub>1A</sub> receptor antagonists (Cremers et al, 2004; Cremers et al, 2000; Hjorth et al, 1997; Hjorth et al, 1996; Gartside et al, 1997a, b; Dawson et al, 2002; Bosker et al,
Also, the activation of GABA<sub>B</sub> receptors has been shown to hyperpolarize 5-HT neurons (Innis & Aghajanian, 1987; Colmers & Williams, 1988; Innis <em>et al</em>, 1988). It seems plausible that there may be a negative feedback loop in the raphe involving 5HT receptors located on GABAergic neurons and GABA receptors on serotonergic neurons.

Emerging preclinical and clinical data implicate GABAergic dysfunction in the pathophysiology of mood disorders such as anxiety and depression (Cryan & Kaupmann, 2005). In a number of preclinical trials, GABA<sub>B</sub> antagonists were shown to have antidepressant effects in the forced swim test (Slattery <em>et al</em>, 2005; Mombereau <em>et al</em>, 2004a, b; Nakagawa <em>et al</em>, 1996; Borsini <em>et al</em>, 1986), learned helplessness model (Nakagawa <em>et al</em>, 1996) and Morris water maze (Nakagawa & Takashima, 1997). Similarly, the use of GABA<sub>B</sub> knockout mice in preclinical studies have reinforced the involvement of these receptors in the etiology of anxiety and depression (Mombereau <em>et al</em>, 2004; Kaupmann <em>et al</em>, 2003).

Following reports that there were 5-HT<sub>2C</sub> and 5-HT<sub>1A</sub> receptors localized on GABAergic neurons in the raphe (Serrats <em>et al</em>, 2005; Serrats <em>et al</em>, 2003), and that GABA<sub>B</sub> antagonists augment the SSRI-induced increase in extracellular 5-HT (Cremers <em>et al</em>, 2006 in print), it was decided to examine the effects of SSRIs in the presence of GABA<sub>B</sub> antagonists. Using microdialysis coupled to HPLC, 5-HT and GABA levels from raphe nuclei, prefrontal cortex and hippocampus were monitored, while animals were challenged with citalopram during systemic and local administration of a number of GABA<sub>B</sub> antagonists. The effects of the combination were also examined in the gerbil tail suspension test, to evaluate the effects in an animal model of depression.
Materials and Methods

Animals

*Neurochemical Studies*
Male albino rats of a Wistar-derived strain (300-350 g, Harlan, Zeist, The Netherlands) were used for the experiments. Animals were maintained on a standard 12-hour light/dark cycle at 24°C and allowed free access to food and water at all times. After surgery, rats were housed individually in plastic cages (35 x 35 x 40 cm), and had free access to food and water. Animals were kept on a 12 h light schedule (light on 7:00 a.m.).

*Behavioural Studies*
Experimentally naïve male Mongolian gerbils (*Meriones unguiculatus*, 60-80 g, Charles River, Germany) were used for all behavioural studies. Gerbils (n = at least 8/group) were housed four per cage, with food and water available *ad libitum*, in temperature and humidity controlled holding rooms. The animals were allowed 4-7 days to acclimatise to housing conditions prior to testing. All testing were conducted during the light phase of the light/dark cycle (lights on: 0600-18:00 h). Experiments were carried out in accordance with the ethical rules of the Danish Committee on Care and Use of Laboratory Animals.

*Materials*
Phaclofen, 2 hydroxysaclofen, CGP 46381, SCH 50911 and Citalopram hydrobromide were kindly donated by Lundbeck A.S., Denmark. For subcutaneous injections, drugs were dissolved in saline solution, while the drugs were dissolved in Ringer solution for infusion regimes.
Neurochemical Procedures

Surgery
Microdialysis of extracellular serotonin levels was performed using I-shaped microdialysis probes with a polyacrylonitrile/ sodium methyl sulfonate copolymer dialysis fibre (Brainlink, Groningen, the Netherlands). The dialysis probe was stereotactically implanted under the following conditions; isoflurane 2%: N\textsubscript{2}O 300 mL/ min: O\textsubscript{2} 300 mL/ min. Microdialysis probes were implanted at AP: -0.53, ML: +0.48, VD: -0.80 for hippocampus (4 mm dialysing membrane), AP: +0.33, ML: +0.08, VD: -0.60 for medial prefrontal cortex (3 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 et al, 1985). The microdialysis probes were permanently fixed to the skull using stainless steel screws and methylacrylic cement. Animals were allowed to recover 18-24 hours 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\textsubscript{2}, and 1.0 mM MgCl\textsubscript{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 minutes in HPLC vials containing 32.5 µL of 0.02 M acetic acid for serotonin and 7.5 µL for GABA analysis. The collected samples were stored in a freezer at -80 °C.

5-HT analysis
Serotonin concentrations were determined using HPLC coupled with electrochemical detection. 20µL of microdialysate fractions were injected via an autoinjector (Gilson 223 XL, The Netherlands) 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\textsubscript{2}-EDTA, 50 mg/L heptane sulphonic acid, 4.5 %
methanol v/v, and 30 µL/L of triethylamine, pH 4.75 at a flow rate of 0.4 mL/min (Shimadzu LC-10 AD). 5-HT was detected amperometrically at a glassy carbon electrode at 500 mV vs Ag/AgCl (Antec Leyden, Leiden, Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µL sample (signal-to-noise ratio 3).

**GABA analysis**

GABA concentrations in the dialysates were determined off-line by pre-column derivatization with o-phtaldialdehyde/mercaptoethanol reagent, and separation by reverse-phase HPLC on a Supercosil LC-18-DB column as previously described (Rea et al., 2005). Samples were derivatized as follows, based on the derivitisation by Lindroth and Mopper (1979). 100 mg o-phtaldialdehyde 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 µL 2-mercaptoethanol. The reagent was freshly prepared daily.

30 µL microdialysate samples were derivatized with 50 µL o-phtaldialdehyde/mercaptoethanol reagent, mixed, and allowed to react for two minutes. 50 µL 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, 70mM di-sodium hydrogen phosphate, 400µM EDTA and 0.15% tetrahydrofuran, 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).

**Behavioural Procedures**

**Tail Suspension Test**

The tail suspension procedure was a modified version of that validated for NMRI mice by Steru and colleagues (Porsolt et al., 1987; Steru et al., 1987). Animals were transported a short distance from the holding facility to the testing room and left undisturbed for at least 30 mins. Subjects were randomly allocated to treatment conditions and tested in counterbalanced order. Thirty minutes after injection, gerbils were suspended by the tail with the use of adhesive tape with one end of the tape
wrapped around the animal’s tail (approx 2 cm from the tip of the tail) and the other pushed through a hook which was connected to a PC. During a 6 min test session, immobility time was recorded via an automated system. A reduction in immobility is considered to reflect an antidepressant-like response.

**Expression of results and statistics**

The neurochemical data are expressed as area under the curve (AUC). The average concentration of five stable baseline samples was set at 100%. Statistic analysis was performed using one way ANOVA followed by Dunnet’s $t$-test for parametric data.

For the Tail Suspension Test, dose-response studies were analysed by one factor ANOVA followed by Dunnett’s $t$-tests, or two factor ANOVA followed by Student Newmann Keuls post hoc test.
Augmentation of antidepressant effects of SSRIs by GABA<sub>B</sub> antagonists: mechanistic studies

Results

Baseline levels in dialysates from hippocampus (n = 94), prefrontal cortex (n = 31) and raphe nuclei (n = 24) samples were determined as 7.71 ± 0.67 fmol/ sample, 5.72 ± 0.82 fmol/ sample and 37.84 ± 2.51 fmol/ sample for 5-HT; and 318.78 ± 26.77 and 337.08 ± 17.18 fmol/ sample GABA for hippocampus, and raphe nuclei respectively. Basal dialysates were not corrected for in vitro recovery. The average concentration of five stable baseline samples was set at 100%, and all data were corrected correspondingly. The area under the curve for all treatments was then determined and statistics performed on these results.

The effect of citalopram during GABA<sub>B</sub> antagonist challenges on 5-HT in ventral hippocampus and raphe nuclei

The systemic administration of citalopram (3.0 mg/kg) increased basal output by 600% in ventral hippocampus. The systemic co-administration of 3.0 mg/kg citalopram with varying doses of the GABA<sub>B</sub> antagonist phaclofen and 2-hydroxy saclofen (2.0 mg/kg) (Fig. 1) augmented the increase in extracellular 5-HT ($F(5, 42) = 5.676$, $p < 0.001$). Post hoc analysis determined that the systemic application of 2.0 mg/kg phaclofen in combination with citalopram significantly augmented the extracellular increase in 5-HT in hippocampus ($p = 0.03$), while the other doses of phaclofen had no significant effect on the output of 5-HT. 2-hydroxysaclofen was also shown to augment the effect of citalopram on extracellular 5-HT ($p = 0.018$).
Figure 1 The effect of varying doses of phaclofen, and 2-hydroxy saclofen, on the citalopram-induced increase in extracellular 5-HT, in hippocampal dialysates. Results are expressed as mean ± SEM (n = 5-12). * represents a significant difference as compared to citalopram + vehicle controls ($p < 0.05$).

The effects of the acute systemic administration of citalopram on extracellular 5-HT in hippocampal dialysates was also augmented when co-administrated with CGP46381 ($F(3, 33) = 9.823, p < 0.001$) (Fig. 2). Post hoc analysis determined that the effect of citalopram was significantly augmented by the systemic co-administration of 0.5 mg/kg, and 2.0 mg/kg CGP 46381 ($p = 0.001$, and 0.012 respectively) while the augmentation was not apparent at higher concentrations (10.0 mg/kg) of CGP46381 ($p = 0.961$).
Figure 2 The effect of varying doses of CGP 46381, on the citalopram-induced increase in extracellular 5-HT, in hippocampal dialysates. Results are expressed as mean ± SEM (n = 5-12). * represents a significant difference as compared to citalopram + vehicle controls (p < 0.05).

Similarly, the effect of citalopram on 5-HT was augmented in a bell-shaped dose response by systemic SCH50911 administration (F(3, 33) = 2.835, p = 0.055) (Fig. 3). The lower dose (2.0 mg/kg), and the higher dose (30.0 mg/kg) had no significant effect (p = 0.939, and 0.894 respectively) on the citalopram-induced increase in 5-HT, while a dose of 10.0 mg/kg resulted in a significant augmentation of the response (p = 0.023).
Figure 3 The effect of varying doses of SCH 50911, on the citalopram-induced increase in extracellular 5-HT, in hippocampal dialysates. Results are expressed as mean ± SEM (n = 5-12). * represents a significant difference as compared to citalopram + vehicle controls (p < 0.05).

The effect of citalopram on 5-HT during phaclofen co-administration was also examined in raphe nuclei ($F(2, 15) = 3.173, p = 0.075$) (Fig. 4). It was determined that the systemic administration of 2.0 mg/kg phaclofen significantly augmented the citalopram effect ($p = 0.011$), while 10.0 mg/kg phaclofen displayed no significant difference from the effect seen with citalopram administration alone ($p = 0.676$). The administration of phaclofen, 2-hydroxy saclofen, CGP 46381, or SCH 50911 alone had no significant effect on basal 5-HT levels as compared to vehicle treated animals (data not shown).
Figure 4 The effect of systemic co-administration of 2.0 mg/kg, and 20.0 mg/kg phaclofen, on the citalopram-induced increase in extracellular 5-HT, in dialysates from the raphe nuclei. Results are expressed as mean ± SEM (n = 5-8). * represents a significant difference as compared to citalopram + vehicle controls (p < 0.05).

The local infusion of phaclofen (50µM) (p = 0.017) and CGP 46381 (1µM) (p = 0.002) also augmented the increase in hippocampal 5-HT as compared to the systemic administration of citalopram (F(2, 26) = 23.008, p < 0.001) (Fig. 5).
Figure 5 The effect of local infusion of 50µM phaclofen, and 1.0 µM CGP 46381 on the citalopram-induced increase in extracellular 5-HT, in hippocampal dialysates. Results are expressed as mean ± SEM (n = 5-12). * represents a significant difference as compared to citalopram controls ($p < 0.05$).

Neither the combination of 2.0 mg/kg or 10.0 mg/kg phaclofen with citalopram resulted in a significant change in GABA levels (Fig. 6) as compared to citalopram alone in hippocampus ($F(2, 19) = 0.907, p = 0.422$) or raphe ($F(2, 12) = 0.856, p = 0.454$).
Figure 6 The effect of systemic co-administration of 2.0 mg/kg, and 10.0 mg/kg phaclofen, with 3.0 mg/kg citalopram on extracellular GABA in dialysates from the hippocampus, and raphe nuclei. Results are expressed as mean ± SEM (n = 4-7).

The effect of citalopram during phaclofen challenges on 5-HT in prefrontal cortex

The systemic administration of phaclofen at 2.0 mg/kg (p = 0.010) and 20.0 mg/kg (p = 0.001) significantly augmented the citalopram-induced increase in 5-HT (F(2, 29) = 8.661, p = 0.001). Contrary to results obtained in the ventral hippocampus, the administration of phaclofen with citalopram in the prefrontal cortex did not display the same bell-shaped dose dependency (Figure 7). Again, the administration of phaclofen alone did not significantly alter basal 5-HT levels as compared to vehicle administration (data not shown).
Figure 7 The effect of systemic co-administration of 2.0 mg/kg, and 20.0 mg/kg phaclofen, on the citalopram-induced increase in extracellular 5-HT, in dialysates from the prefrontal cortex. Results are expressed as mean ± SEM (n = 5-8). * represents a significant difference as compared to citalopram + vehicle controls ($p < 0.05$).

The effect of the co-administration of phaclofen with citalopram in the Gerbil Tail Suspension Test

A dose response curve was first constructed (Fig. 8) to determine which concentration of citalopram should be used in the study to allow for the detection of an antidepressant effect. While the oral doses of 1.0, 2.0, 4.0 and 16.0 mg/kg citalopram were significantly different from vehicle dose ($F(5, 47) = 10.004, p < 0.01$), it was decided to use the 0.5 mg/kg citalopram dose. Despite the fact that it did not produce a significant decrease in antidepressant response ($p = 0.445$), there was
an inclination towards a decrease in immobility, and hence towards an antidepressant-like response. This dose allowed for an easier detection of a potentiation in the antidepressant effect of citalopram.

**Figure 8** The effect of various orally administered doses of citalopram on immobility in the gerbil tail suspension test. Results are expressed as mean ± SEM (n = 8). * represents a significant difference as compared to vehicle controls (p < 0.05).
Using this citalopram dose (0.5 mg/kg p.o.), a study was performed investigating the effect of oral phaclofen administration on immobility (2.5, 5.0 and 10.0 mg/kg). There was an overall effect of citalopram treated groups as compared to vehicle treated groups ($F(1, 63) = 17.084, p < 0.001$), while the administration of phaclofen had no significant effect at any of the oral doses tested (2.5, 5.0 and 10.0 mg/kg) as compared to vehicle ($F(3, 63) = 0.807, p = 0.496$). There was no significant decrease in immobility with the combination of the two treatments as compared to their respective controls ($F(3, 63) = 1.742, p = 0.169$), except when 2.5 mg/kg phaclofen was co-administered with 0.5 mg/kg citalopram ($p = 0.005$) (Fig. 9).

**Fig. 9**

![Bar chart showing the effect of oral phaclofen administration alone and in combination with 0.5 mg/kg citalopram on immobility in the gerbil tail suspension test. Results are expressed as mean ± SEM (n = 8). * represents a significant difference as compared to control ($p < 0.05$).](image)

**Figure 9** The effect oral phaclofen administration alone, and in combination with 0.5 mg/kg citalopram, on immobility in the gerbil tail suspension test. Results are expressed as mean ± SEM (n = 8). * represents a significant difference as compared to control ($p < 0.05$).
Discussion

Recently, it has been reported that 5-HT\textsubscript{2C} antagonists are capable of augmenting the biochemical and behavioral effects of SSRIs (Cremer \textit{et al}, 2004). There is also evidence supporting the localization of 5-HT\textsubscript{2C} and 5-HT\textsubscript{1A} receptors on GABAergic neurons (Serrats \textit{et al}, 2003; Eberle-Wang \textit{et al}, 1997), and that these serotonergic receptors activate local GABA inhibitory circuits (Liu \textit{et al}, 2000) which impact on GABA\textsubscript{B} receptors located on raphe serotonergic neurons (Serrats \textit{et al}, 2003; Abellan \textit{et al}, 2000; Varga \textit{et al}, 2002; Wirtshafter & Sheppard, 2001). Activation of GABA\textsubscript{B} receptors has also been shown to inhibit 5-HT levels in terminal areas (Tao & Auerbach, 2000). While investigating the mechanisms involved in the augmentation of the citalopram-induced increase in 5-HT by 5-HT\textsubscript{2C} antagonists, we observed an increase in hippocampal 5-HT when phaclofen was locally infused prior to systemic citalopram administration. However, to date no GABA\textsubscript{B} receptors have been localized to serotonergic neuron terminals. The increase in serotonin was similar in magnitude to the augmentation seen with co-administration of 5-HT\textsubscript{1A} (Hjorth \textit{et al}, 1997) and 5-HT\textsubscript{2C} (Cremer \textit{et al}, 2004) antagonists with citalopram. This augmentation was also demonstrated when the GABA\textsubscript{B} antagonist 2-hydroxy saclofen was systemically co-administered with citalopram. Using microdialysis coupled to HPLC, we examined the effect of citalopram on hippocampal 5-HT when systemically challenged with a series of doses of phaclofen. Interestingly we observed that the combination strategy did not display a simple dose response curve but a bell-shaped response. Similarly, this bell-shaped dose response curve was observed in hippocampus when citalopram was challenged with the more selective GABA\textsubscript{B} antagonists, CGP43681, and SCH50911. This effect was again seen in the raphe when phaclofen was co-administered with citalopram. However, when we examined GABA levels, there was no significant attenuation in extracellular GABA levels in raphe or hippocampal dialysate samples as compared to citalopram controls. In this study we show that the local infusion of phaclofen augments the citalopram-induced increase in 5-HT in hippocampus. In an earlier study (Rea \textit{et al}, 2005), we showed that the local infusions of GABA\textsubscript{B} agonists and antagonists decreased, and
increased GABA levels respectively. However, the infusion of these compounds had no effect on 5-HT levels at the concentrations infused (data not shown). It is possible that the local infusion of these GABA\textsubscript{B} compounds influences the GABA tonus of the serotonergic neuron, and facilitates a further augmentation of the effects of SSRIs without directly affecting the basal release of 5-HT.

Interestingly, the bell-shaped effect on 5-HT with the combination of an SSRI with a GABA\textsubscript{B} antagonist was not apparent in the prefrontal cortex. An augmentation in the citalopram-induced increase in 5-HT was still observed with the co-administration of 20.0 mg/kg phaclofen s.c. This may be due to a difference in GABA receptor density or function (Amantea \textit{et al}, 2004), or possibly due to the interaction of phaclofen on GABA\textsubscript{B} receptors on cortical neurons other than serotonergic or GABA\textsubscript{ergic} neurons (Santiago \textit{et al}, 1993).

We also show evidence for a bell-shaped dose response phenomenon in an animal model for depression. It was determined in the gerbil tail suspension test that the administration of a dose of 2.0 mg/kg phaclofen with citalopram was significantly different to the amount of time spent immobile as compared to citalopram alone. This effect was not seen when citalopram was co-administered with higher (5.0 or 10.0 mg/kg) doses. The tail suspension test is classically used for the discrimination of antidepressant compounds. While SCH50911 showed a tendency for a bell shaped response in combination with citalopram, the results were not significant (data not shown). The compound CGP43681 showed little or no response alone, or in combination with citalopram (data not shown). The doses of citalopram used in this study were lower than those of the microdialysis experiments, as the lower doses of citalopram sufficiently decreased immobility (Varty \textit{et al}, 2003), as compared to controls, and it would still be possible to observe a shift in the dose response curve, or a further augmentation of the immobility response. Although a bell-shaped dose response change in behaviour was observed with the combination studies, it is possible that a more pronounced effect would be observed at higher doses of citalopram in combination with the GABA\textsubscript{B} antagonists.

Similarly, in agreement with the current data, no significant antidepressant effects were observed in the tail suspension test when GABA\textsubscript{B} antagonists were
administered alone (Cryan et al, 2004; Cryan et al, 2005; Mombereau et al, 2004). It is of interest however, that administration of GABA<sub>B</sub> antagonists were found to have antidepressant-like qualities in a number of other animal models of depression including the forced swim test (Mombereau et al, 2004; Nakagawa et al, 1996; Slattery et al, 2005; Borsini et al, 1986) and learned helplessness models (Nakagawa et al, 1996; Nakagawa et al, 1997). It is also interesting that when co-administered with the GABA<sub>B</sub> agonist, baclofen, the effects of antidepressants are blocked in certain animal models of depression (Nakagawa et al, 1996; Nakagawa et al, 1997). This however may be due to the sedative nature of baclofen. While there appears to be a role for GABA<sub>B</sub> receptors in mediating an antidepressant response in many of these animal models, the mechanism behind how the GABA<sub>B</sub> receptor is mediating its effect is unclear.

It is possible that in the presence of an SSRI, the resulting surplus of serotonin activates 5-HT<sub>1A</sub> and/or 5-HT<sub>2C</sub> receptors localized on GABA<sub>A</sub>ergic cells. Indeed, this was shown to be the case with 5-HT<sub>2C</sub> antagonism significantly impacting on GABA release in the presence of citalopram (Cremers et al, 2006 in print). It seems plausible that the administration of citalopram may increase the release of GABA, which in turn could activate GABA<sub>B</sub> receptors located on serotonergic neurons. The resulting decrease in serotonergic firing would reduce the amount of serotonin being released from serotonergic terminals. The supposition is that by co-administering a 5-HT<sub>2C</sub> or 5-HT<sub>1A</sub> antagonist, or GABA<sub>B</sub> antagonist, we are preventing the effect of GABA on serotonergic firing either indirectly by preventing GABA release, or directly by GABA<sub>B</sub> receptor antagonism. This would lead to an increase in 5-HT release due to disinhibition, and antagonism of effects of GABA on serotonergic neurons, respectively. Despite the fact that these proposed augmentations of GABA effects were not observed in the present study, it is possible that these effects are being mediated at sites in other brain areas outside the vicinity of the microdialysis probe. It is likely that, when co-administered with citalopram, the homeostatic balance between the GABA and serotonergic neurons becomes compromised, and the effects of the GABA<sub>B</sub> antagonist become more pronounced. It should be noted however, that with GABA<sub>B</sub> antagonist administration, there will be antagonism at both presynaptic
GABA\textsubscript{B} receptors on GABAergic neurons and postsynaptic GABA\textsubscript{B} receptors on serotonergic neurons. It has been implied that some GABA\textsubscript{B} agonists and antagonists express a preference for either the pre- or post-synaptic receptor due to differences in the heterodimer composition of the receptors (Phelan, 1999; Yamada \textit{et al}, 1999; Pozza \textit{et al}, 1999). The GABA\textsubscript{B} receptor has recently been shown to be an allosterically modulated G-protein receptor (Urwyler \textit{et al}, 2001) and although the compounds used in this study have not been tested for allosteric modulating activity, a possible explanation for the bell-shaped dose response phenomenon is that in excess amounts, GABA\textsubscript{B} antagonists bind to other allosteric sites on the GABA\textsubscript{B} receptor resulting in conformational changes which prevents the binding of the antagonist. A recent theory is that the GABA\textsubscript{B} heterodimer undergoes a conformational change (Venus Fly Trap model) when bound by endogenous GABA which allows the allosteric modulation of response by affecting the stability of the GABA-GABA\textsubscript{B} receptor complex (Pin \textit{et al}, 2004). The current data suggest that the GABAergic and 5-HTergic systems are involved in regulating the levels of their respective systems, and perhaps by manipulating certain receptors we can provide novel therapeutic targets. However, given indications for bell-shaped dose dependency, the manipulation of GABA\textsubscript{B} receptors may compromise its use in the clinic.
Augmentation of antidepressant effects of SSRIs by GABA\(_B\) antagonists: mechanistic studies

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Augmentation of antidepressant effects of SSRIs by GABA<sub>B</sub> antagonists: mechanistic studies


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