GABA_B Receptor Antagonist-Mediated Antidepressant-Like Behavior Is Serotonin-Dependent

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ABSTRACT

There is an emerging body of data purporting a role of γ-aminobutyric acid (GABA) in the pathophysiology of mood disorders. However, the role of metabotropic GABA_B receptors in depression is not well defined. The modified forced swim test has recently emerged as an excellent tool to assess behaviorally the role of monoamines in antidepressant action. To assess the role of GABA_B receptors in antidepressant-related behavior, we examined a number of selective GABA_B receptor ligands (novel positive modulators and antagonists) on behavior in the modified forced swim test. We demonstrate that the selective GABA_B receptor antagonists CGP56433A [3-{1-[S-(3-cyclohexylmethyl)hydroxy phosphinyl]-2-(S) hydroxy propyl]amino}ethyl]benzoic acid; 1–10 mg/kg] and [3-{1-[S-(3-dichlorophenyl)ethyl]amino}-2-(S)-hydroxy-propyl]phenylmethyl-phosphinic acid hydrochloride; 3–10 mg/kg] had a similar profile to the selective serotonin reuptake inhibitor fluoxetine; they decreased immobility and increased swimming behavior. The tricyclic antidepressant desipramine decreased immobility but increased climbing behavior. In contrast, the novel GABA_B receptor-positive modulator GS39783 (10–40 mg/kg) did not display antidepressant-like activity in the modified forced swim test. To further assess the possible interaction between GABA_B receptor antagonism and serotonin, rats were pretreated with the tryptophan hydroxylase inhibitor para-chlorophenylalanine. 5-Hydroxytryptamine depletion (>90%) abolished the antidepressant-like behavior of CGP56433A (10 mg/kg) by attenuating the increase in swimming. Together, these data demonstrate that GABA_B receptor antagonists via an interaction with the serotonergic system display antidepressant-like properties and therefore represent a novel approach for the treatment of depression.

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, where it acts on two receptor classes; ionotropic GABA_A and GABA_C receptors and metabotropic GABA_B receptors. The latter class form heterodimers comprised of GABA_B(1), of which there are two known splice variants, GABA_B(1a) and GABA_B(1b), and GABA_B(2) subunits, both of which are required for functionally active receptors (Calver et al., 2002). Emerging preclinical and clinical data implicate GABAergic dysfunction in the pathophysiology of mood disorders (Krystal et al., 2002; Brambilla et al., 2003). However, evidence for a specific role for GABA_B receptors in depression is limited and controversial, with rival hypotheses being purported that both positive or negative modulation of this receptor may be a useful antidepressant therapy (Lloyd et al., 1987; Nakagawa et al., 1999). Of late, more emphasis has been placed on GABA_B receptor antagonism as a potential therapeutic strategy for depression (Bowery et al., 2002). In support of this contention, we have recently demonstrated that GABA_B(1) subunit knockout mice display antidepressant-like activity in the mouse forced swim test (Mombereau et al., 2004). The forced swim test is currently the most widely used animal model for assessing depression related behavior in rodents (Porsolt et al., 1977; Borsini, 1995; Cryan et al., 2002a). When rats or mice are placed in an inescapable cylinder of water they develop an immobile posture, which is believed to reflect either 1) a failure to persist in escape-directed behavior after persistent stress or 2) the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli (Lucki, 1997; Cryan et al., 2002a; Cryan and Mombereau, 2004). The time that the animals spend in an immobile posture is decreased by acute or chronic treatment with numerous antidepressant compounds (Porsolt et al., 1977; Borsini, 1995; Cryan et al., 2002a). However, the forced swim test in its original form is largely unreliable in response to selective 5-HT reuptake inhibitors (SSRIs) (Page et al., 1999; Cryan et al., 2002a;
Harkin et al., 2003). Lucki and coworkers have made minor modifications to the forced swim test that have increased its sensitivity to SSRIs in addition to other antidepressants (for review, see Lucki, 1997). In the modified version of the test, the depth of the water is increased to 30 cm and a time-sampling technique is used, whereby the predominant behavior in each 5-s interval is recorded. These modifications enable differentiation of specific behaviors in the swim test; namely, climbing, swimming, and immobility (Detke et al., 1995; Lucki, 1997; Cryan et al., 2002a). It has been shown that swimming behavior is sensitive to serotonergic agonists and reuptake inhibitors, whereas climbing is modulated by catecholamine compounds (Detke et al., 1995; Lucki, 1997; Cryan and Lucki, 2000; Cryan et al., 2002a,b). Additionally, the increase in swimming behavior observed after fluoxetine has been shown to be attenuated by pretreatment with the tryptophan hydroxylase inhibitor para-chlorophenylalanine (pCPA; Page et al., 1999). Lesions of the ventral noradrenergic bundle have also been shown to attenuate the effects of noradrenaline reuptake inhibitor-induced climbing in the modified forced swim test (Cryan et al., 2002b). Therefore, the modified forced swim test has been proposed as a behavioral model to assess in vivo the role of monoamines in antidepressant action (Lucki, 1997; Page et al., 1999; Cryan et al., 2002a; Harkin et al., 2003).

The release of monoamines has been shown to be modulated via GABA<sub>B</sub> receptors (Bowery et al., 1980; Mannoury la Cour et al., 2004; Zhou et al., 2004). In particular, emerging evidence suggests a strong interaction between GABA<sub>B</sub> receptors and the serotonergic system. GABA<sub>B</sub> receptors are densely localized on and intricately interact with serotonergic neurons in the dorsal raphe nucleus (DRN) (Tao et al., 1996; Abellan et al., 2000a,b; Burman et al., 2003; Serrats et al., 2003). GABA<sub>B</sub> receptor activation is known to inhibit 5-HT cell firing in the raphe nucleus (Mannoury la Cour et al., 2004). In these studies, we hypothesize that GABA<sub>B</sub> receptor ligands by interacting with serotonergic neurotransmission may alter behavior in the modified forced swim test. Thus, we compared the behavioral profile of the two equipotent GABA<sub>B</sub> receptor antagonists CGP56433A and CGP55845A (Fraostl et al., 1995a,b; Pozza et al., 1999; Getova and Bowery, 2001; Macey et al., 2001) and the novel GABA<sub>B</sub> receptor-positive modulator GS39783 (Urwyler et al., 2003; Cryan et al., 2004) with that of known serotonergic and noradrenergic antidepressants. Furthermore, we examined the contribution of the serotonergic system to the behavioral effects of GABA<sub>B</sub> receptor antagonist CGP56433A.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River, Les Oncins, France) weighing between 250 and 350 g at the time of testing were used in these studies. The animals were housed in groups of four and maintained on a 12-h light/dark cycle (lights on 6:00 AM) in a temperature-controlled colony (22–24°C). The animals had free access to food and water. Animals were handled daily for at least 4 days before initiation of behavioral testing. The forced swim tests were performed in the afternoon (1:00–5:00 PM). All experimental procedures were subject to institutional review and conducted in accordance with the Veterinary Authority of Basel-Stadt, Switzerland.

Forced Swim Test. The modified rat forced swim test was conducted as described previously (Detke et al., 1995; Cryan and Lucki, 2000). Briefly, rats were individually placed into a Plexiglas cylinder (21 × 46 cm) filled with 25°C water to a depth of 30 cm. The rats were removed after 15 min, dried, and returned to their home cage. Water was changed between each test. Twenty-four hours after this first exposure, the rats were replaced in the swim cylinder (under the same conditions) for 5 min, and the session was recorded using a video camera placed above the cylinder for subsequent analysis. Drugs were administered s.c. three times (1, 5, and 23.5 h) before the test session. The rater of the test session was blind to the treatment group being scored. A time sampling technique was used where the predominant behavior, climbing, swimming, or immobility, in each 5-s period of the 300-s test was recorded (providing an overall total of 60 scores). Climbing behavior consisted of upward-directed movement of the forepaws, usually along the side of the swim cylinder. Swimming behavior consisted of horizontal movement throughout the swim chamber, which also included crossing into another quadrant. Immobility was defined as the animal floating in water without struggling and only making movements necessary to maintain its head above the water (see Cryan et al., 2002a for pictorial representations of observed behaviors).

Effect of Fluoxetine and Desipramine on Behavior in the Rat Forced Swim Test. Rats were assigned to vehicle (distilled water) or fluoxetine (20 mg/kg), whereas in a separate study, rats were assigned to saline (0.9% NaCl) or desipramine (20 mg/kg dissolved in water).

Effect of CGP56433A on Behavior in the Rat Forced Swim Test. This study examined the effect of three doses of CGP56433A on behavior in the forced swim test. Rats were assigned to one of four groups: vehicle (distilled water), CGP56433A (1 mg/kg), CGP56433A (3 mg/kg), or CGP56433A (10 mg/kg).

Effect of CGP55845A on Behavior in the Rat Forced Swim Test. This study examined the effect of three doses of CGP55845A on behavior in the forced swim test. Rats were assigned to one of three groups: vehicle (distilled water), CGP55845A (3 mg/kg), or CGP55845A (10 mg/kg).

Effect of GS39783 on Behavior in the Rat Forced Swim Test. This study examined the effect of three doses of GS39783 on behavior in the forced swim test. Rats were assigned to one of four groups: vehicle (distilled water + 1% Tween 80), GS39783 (10 mg/kg), GS39873 (20 mg/kg), or GS39783 (40 mg/kg).

Effect of CGP56433A and Fluoxetine on Locomotor Activity in the Rat. Locomotor activity was analyzed in a novel environment. The activity monitor consisted of a black-and-white video camera, mounted in the top-center of an enclosure (60 × 40 × 50 cm), whereby a cage (55 × 33 × 19 cm) was positioned in the enclosure. Each second a single video frame was acquired with a highly accurate, programmable, monochrome frame grabber board (type DT3155; Data Translation, Marlboro, MA). Using in-house developed software, digitized pixels of two successive frames were compared and the total number of pixels with altered intensity was counted (independently for pixels with increased and decreased intensity). This allowed the detection of the animal’s position within the cage (the center of the pixels with decreased intensity, because animals were dark compared with background). Total distance traveled (distance in centimeters between centers of activity when movement was >10% body size) was analyzed during a 30-min trial. The animals received three s.c. administrations 23.5, 5, and 1 h before the test. Rats were assigned to one of five groups: vehicle (distilled water), CGP56433A (1 mg/kg), CGP56433A (3 mg/kg), CGP56433A (10 mg/kg), or fluoxetine (20 mg/kg).

Effect of Pretreatment with pCPA Alone and on CGP56433A Behavior in the Rat Forced Swim Test. pCPA (150 mg/kg i.p.) or vehicle (0.9% NaCl i.p.) were administered once daily for three consecutive days. This treatment regimen has previously been shown to produce a 90% depletion of brain 5-HT concentrations in the rat (Cryan et al., 2000; Connor et al., 2001; Harkin et al., 2003). Seventy-two hours after test injection the animals were exposed to the 15-min preimmersion. Rats were assigned to one of four groups: vehicle, pCPA, vehicle + CGP56433A (10 mg/kg), or pCPA + CGP56433A (10 mg/kg).
Verification of 5-HT Depletion after pCPA Pretreatment. Immediately after the forced swim test test session, the rats were sacrificed by decapitation and the frontal cortex dissected on an ice-cold plate. 5-HT concentrations were measured by high-performance liquid chromatography coupled with electrochemical detection. Brain tissue was sonicated in 1 ml of the mobile phase for the high-performance liquid chromatography separation described below (without sodiumoctylsulfate), containing 100 ng of α-methyl-DOPA per extract as internal standard. Homogenates were centrifuged at 18,800 × g in a Sigma 4K10 refrigerated centrifuge for 15 min. Twenty microliters of the supernatant was automatically injected into a liquid chromatographic system (Hewlett Packard AminoQuant), which consists of an isocratic pump, a refrigerated microsampler with a 20-μl sample loop set at +8°C, an on-line ERC-3511 degasser (ERMA CR Inc., Tokyo, Japan), and an amperometric detector (cell potential set at +0.70 V). Chromatographic separation of 5-HT was achieved on a Zorbax 300SB-C18 analytical column, 4.6 mm i.d. × 15-cm length, with a 5-μm particle size (Agilent Technologies, Palo Alto, CA). The mobile phase contained 0.1 M chloroacetic acid, 0.3 mM sodiumoctylsulfate, 0.2 mM EDTA, and 2% (v/v) acetonitrile, and was adjusted to pH 2.6 with NaOH. The flow cell and the analytical column were maintained at the temperatures of 30 and 42°C, respectively. The flow rate was 1 ml/min. The chromatograms were integrated and stored using a computerized data acquisition system by using a ChemStation chromatographic software (Agilent Technologies) running onto a PC system; compound identification and peak quantification were achieved by comparison with known standards. Results were expressed as nanogram of 5-HT per gram of fresh weight of brain tissue.

Statistical Analysis. The effects of fluoxetine and desipramine were analyzed using an unpaired t test. The effects of other drugs were analyzed using one-way analysis of variance (ANOVA). Two-way ANOVA was used to analyze the interaction of CGP56433A treatment. Any overall statistical differences were analyzed using one-way analysis of variance (ANOVA). Two-way ANOVA was used to analyze the interaction of CGP56433A treatment. Any overall statistical differences were further analyzed using Fisher's post hoc tests. Data are expressed as group mean ± S.E.M.

Results

The Effect of Fluoxetine and Desipramine on Behavior in the Rat Forced Swim Test. As shown in Fig. 1A, fluoxetine (20 mg/kg) significantly decreased immobility (P < 0.001) and increased swimming time (P < 0.01), but did not alter climbing. Desipramine (20 mg/kg) significantly decreased immobility time (P < 0.01) via an increase in climbing behavior (P < 0.01) and did not effect swimming time (Fig. 1B). Similar effects of fluoxetine and desipramine in the modified forced swim test have been demonstrated previously, which is representative of other antidepressant drugs that enhance serotonergic or catecholamine neurotransmission, respectively (Lucki, 1997; Page et al., 1999; Cryan and Lucki, 2000).

The Effect of CGP56433A and CGP55845A on Behavior in the Rat Forced Swim Test. One-way ANOVA showed effects of CGP56433A on immobility time (F(3,60) = 4.4, P = 0.007), swimming duration (F(3,60) = 6.8, P = 0.001), and climbing time (F(3,60) = 3.8, P = 0.015). Post hoc tests revealed that CGP56433A (10 mg/kg) decreased immobility time (P = 0.002) while increasing swimming time (P = 0.003; Fig. 2A). No significant effects of CGP56433A (3 mg/kg) were observed, but CGP56433A (1 mg/kg) significantly increased climbing duration (P = 0.008). Furthermore, there was a trend toward a decrease in immobility time; however, this did not reach significance (P = 0.06; Fig. 2A).

One-way ANOVA showed effects of CGP55845A on swimming behavior (F(2,45) = 3.6, P < 0.05). Furthermore, there was a trend toward a decrease in immobility time; however, this did not reach significance (F(2,45) = 2.8, P = 0.07). No significant effect of treatment was observed on climbing score. Post hoc analysis revealed that both doses of CGP55845A (3 and 10 mg/kg) increased swimming time (P < 0.05; Fig. 2B).

The Effect of GS39783 on Behavior in the Rat Forced Swim Test. GS39783 at all doses tested (10, 20, or 40 mg/kg) failed to alter behavior in the forced swim test on any parameter analyzed (Fig. 3).

Effect of CGP56433A and Fluoxetine on Locomotor Activity in the Rat. There was a significant effect of treatment on total distance traveled (F(4,35) = 15.5, P < 0.001). Post hoc analysis revealed that fluoxetine and CGP56433A (3 and 10 mg/kg) significantly decreased distance traveled (P < 0.01), but there was no effect of CGP56433A (1 mg/kg) (Fig. 4).

Effect of Pretreatment with pCPA Alone and on CGP56433A Behavior in the Rat Forced Swim Test. Two-way ANOVA of immobility time revealed effects of pCPA [F(1,33) = 29.3, P < 0.001] and CGP56433A [F(1,33) = 5.4, P = 0.03] but no pCPA × CGP56433A interaction [F(1,33) = 1.2, P = 0.3]. Post hoc analyses showed that CGP56433A reduced immobility time in vehicle-pretreated rats (P < 0.05), an effect that was blocked by pCPA (Fig. 5). pCPA pretreatment significantly increased immobility time in the forced swim test (P < 0.05; Fig. 5). ANOVA of swimming time showed effects of CGP56433A [F(1,33) = 19.33, P < 0.001], pCPA × CGP56433A interaction [F(1,33) = 5.0, P = 0.03], but no effect of pCPA alone [F(1,33) = 1.8, P = 0.19]. Post hoc analyses demonstrated that CGP 56433A significantly increased swimming time (P < 0.001); an effect that was significantly attenuated by pCPA pretreatment (Fig. 5). Two-way ANOVA revealed effects of pCPA

![Fig. 1. Effects of fluoxetine and desipramine in the modified forced swim test. A, fluoxetine decreased immobility time in the forced swim test (t test; P < 0.001) and increased swimming behavior (t test; P < 0.01; n = 9–10). B, desipramine decreased immobility time in the forced swim test (t test; P < 0.01) and increased climbing behavior (t test; P < 0.01; n = 10). Data represent mean ± S.E.M. **, P < 0.01 and ***P < 0.001 represent statistical difference compared with vehicle.](image-url)
Fig. 2. Effects of the GABA<sub>B</sub> receptor antagonists CGP56433A and CGP55845A in the modified forced swim test. A, CGP56433A at 10 mg/kg significantly decreased immobility time in the forced swim test via an increase in swimming scores, whereas at 1 mg/kg significantly increased climbing with a trend toward a decrease in immobility (P = 0.06; n = 16). B, CGP55845A significantly alters swimming time (ANOVA; P < 0.05; n = 16). Data represent mean ± S.E.M. One-way ANOVA with Fisher’s post hoc test was performed.

Verification of 5-HT Depletion after pCPA Pretreatment. Two-way ANOVA showed effects of pCPA pretreatment on 5-HT [F(1,30) = 199.1; P < 0.0001] levels in the prefrontal cortex. Post hoc analyses revealed that pCPA pretreatment significantly decreased climbing time in the representative treatment groups (P < 0.05; Fig. 5).

Discussion

The present studies demonstrate that GABA<sub>B</sub> receptors play a role in antidepressant-like behavior in the modified forced swim test. GABA<sub>B</sub> receptor antagonists decreased immobility time via an increase in swimming. This profile is similar to that produced by serotonergic antidepressants in the test, suggesting that GABA<sub>B</sub> receptor antagonists may recruit serotonergic mechanisms to elicit their behavioral effects. To verify this hypothesis, we demonstrated that pretreatment with the tryptophan hydroxylase inhibitor pCPA, which depleted brain 5-HT levels, abolished the antidepressant-like behavioral effects of CGP56433A.

The emerging evidence purporting a dysfunction of the
GABAergic system in mood disorders (Brambilla et al., 2003; Krystal et al., 2002) suggested that targeting the GABA<sub>B</sub> receptor may represent a novel strategy for antidepressant drug development. Previously, we have demonstrated that GABA<sub>B(1)</sub> subunit knockout mice show antidepressant-like activity in the mouse forced swim test compared with wild-type mice (Mombereau et al., 2004). These effects were also recapitulated in pharmacological studies using CGP56433A in the mouse forced swim test (Mombereau et al., 2004). In the present studies, we demonstrate that both GABA<sub>B</sub> receptor antagonists tested, CGP56433A and CGP55845A, selectively increase swimming time in the rat modified forced swim test (Fig. 2). This pattern of behavior was qualitatively similar to that seen after administration of the SSRI fluoxetine (Fig. 1A). In contrast, the norepinephrine reuptake inhibitor desipramine decreased immobility with a concomitant increase in climbing (Fig. 1B). CGP55845A does not alter climbing behavior at any dose tested (Fig. 2B). However, at the lowest dose tested CGP56433A (1 mg/kg) decreased immobility time with a concurrent increase in climbing behavior (Fig. 2A), which is akin to selective catecholaminergic antidepressants (Lucki, 1997; Cryan et al., 2002a,b). This suggests that at lower doses CGP56433A may have some effect on catecholamine neurotransmission. The fact that CGP55845A does not induce similar effects and that they are not present at higher doses of CGP56433A make it difficult to interpret these findings presently.

Our current data confirm that GABA<sub>B</sub> receptor antagonists have antidepressant potential. Furthermore, the selective antidepressant-like effects of GABA<sub>B</sub> receptor antagonists in the forced swim test are qualitatively similar to that observed after enhanced serotonergic neurotransmission. Compounds that increase locomotor activity can often provide false positive results in the forced swim test; however, in the present studies the GABA<sub>B</sub> receptor antagonist CGP56433A actually dose dependently decreased locomotor activity in a novel environment (Fig. 4). Therefore, antidepressant-like effects cannot be attributed to alterations in locomotion. Fluoxetine also decreased total distance traveled as shown previously (Sills et al., 2000; Stanford et al., 2002).

Previous evidence for a role for GABA<sub>B</sub> receptors in antidepressant-like effects has been demonstrated by Nakagawa et al. (1996b). These authors demonstrated, using an injection protocol similar to that in the present studies (15-min preswim and injections 23.5 and 0.5 h before 5-min test), that the GABA<sub>B</sub> receptor agonist baclofen attenuated the decrease in immobility caused by antidepressants in the traditional forced swim test. Furthermore, using the learned helplessness model, it has been shown that the GABA<sub>B</sub> receptor antagonist CGP36742 had an antidepressant-like response (Nakagawa et al., 1999), whereas baclofen increased susceptibility to helplessness and attenuated the effects of antidepressants in this test (Nakagawa et al., 1996a,b). Of note, GABA<sub>B</sub> receptor antagonists (including CGP56433A) increase brain-derived neurotrophic factor expression in the hippocampus and cortex (Heese et al., 2000), which may contribute to their antidepressant-like effects (Conti et al., 2002; Shirayama et al., 2002). Together, our current data support the contention that antagonism of GABA<sub>B</sub> receptors may be a suitable target for the development of antidepressant agents.

It has been shown previously that pretreatment with pCPA attenuates the increased swimming behavior evoked by fluoxetine (Page et al., 1999) without affecting baseline behavior. In the current studies, we demonstrate that the decrease in immobility elicited by CGP56433A (10 mg/kg) is abolished after pCPA pretreatment, corresponding to an attenuation of the increase in swimming time (Fig. 5). This indicates that GABA<sub>B</sub> receptor antagonists display antidepressant-like activity via an interaction with the serotonergic system. pCPA administration depleted 5-HT levels by 90% compared with vehicle (Table 1), corresponding with previous studies (Page et al., 1999; Connor et al., 2001; Harkin et al., 2003). As in previous studies (Page et al., 1999; Harkin et al., 2003), pretreatment with pCPA did not alter basal swimming levels. However, we also observed an increase in immobility with a corresponding decrease in climbing scores in pCPA-pretreated animals, which was not observed previously. Presently, the mechanisms underlying these changes are unclear.

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**Fig. 4.** Effects of the GABA<sub>B</sub> receptor antagonist CGP56433A and fluoxetine on locomotor activity. CGP56433A dose dependently decreased total distance traveled and fluoxetine also decreased total distance traveled in 30 min. Data represent mean ± S.E.M. (n = 8). One-way ANOVA was performed with Dunnett’s post hoc test, where *, P < 0.05 and **, P < 0.01 represent statistical difference compared with vehicle.

**Fig. 5.** Effect of pCPA pretreatment (150 mg/kg i.p.; 3 d) on CGP56433A (10 mg/kg) in the modified forced swim test. pCPA pretreatment significantly increased immobility and decreased climbing in both treatment groups compared with the relevant pretreatment group. pCPA pretreatment significantly decreased swimming time after CGP56433A administration but did not affect vehicle group. CGP56433A significantly decreased immobility time and increased swimming after vehicle pretreatment but did not alter any behavior measured after pCPA pretreatment. Data represent mean ± S.E.M. (n = 9–10). Two-way ANOVA followed by Fisher’s post hoc test was performed. *, P < 0.05 and ***, P < 0.001 compared with relevant pretreatment group. #, P < 0.05 and ##, P < 0.01 compared with same treatment group.
immunoreactive cell bodies in the raphe complex were coposita-tems. It has been recently demonstrated that above that which show anxiolytic-like effects. These data incorrect dose selection because the doses used were equal or of full agonists such as baclofen (Cryan et al., 2004). The lack because it lacks the sedative and muscle-relaxant properties of full agonists such as baclofen (Cryan et al., 2004). The lack of effects in the forced swim test is probably not due to incorrect dose selection because the doses used were equal or above that which show anxiolytic-like effects. These data further confirm a dissociation of a role for GABA receptors in the modulation of anxiety and antidepressant-like behavior (Mombereau et al., 2004). Given that serotonin can modulate anxiety and depression in opposite manners, with high serotonergic activity being associated with anxiety and low activity with depression (Graeff et al., 1996; Cryan and Leonard, 2000), it is plausible that differential interaction of GABA receptors on 5-HT neuronal firing at the level of the DRN may be in part responsible for the behavioral effects subsequent to differential pharmacological manipulations of GABA receptors. There are a number of studies providing evidence for a link between GABA receptors and serotonergic systems. It has been recently demonstrated that >95% of 5-HT immunoreactive cell bodies in the raphe complex were copositative for GABA receptors (Varga et al., 2002). Furthermore, Innis et al. (1988) demonstrated that GABA and 5-HT receptors are coupled to the same potassium (K\(^+\)) channel, GirK, in serotonergic neurons. It is also noteworthy that GABA released from dorsal raphe interneurons, and afferents from the amygdala, periaqueductal gray, and habenula inhibit 5-HT cell firing in the DRN, whereas GABA injected directly into the raphe decreased 5-HT cell firing by >50% (Abellan et al., 2000a). Furthermore, in a series of elegant studies, Mannoury la Cour et al. (2001, 2004) showed altered GABA-induced responses in 5-HT transporter (5-HTT) knockout mice. These animals exhibit a functional desensitization and down-regulation of 5-HT\(_T\_A\) autoreceptors in the DRN, but no change in postsynaptic 5-HT\(_T\_A\) receptors. Specifically, the GABA receptor agonist baclofen inhibited 5-HT cell firing in the DRN, which occurred with a 30-fold decrease in potency in 5-HTT knockout mice and was blocked by administration of the GABA\(_B\) receptor antagonists saclofen and CGP52432 (Mannoury la Cour et al., 2004). Additionally, guanosine 5′-O-(3-thio)triphosphate binding was signifi-

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### TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5-HT Concentration</th>
<th>Percentage of Control</th>
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<tbody>
<tr>
<td>Vehicle/vehicle</td>
<td>255 ± 9 (n=9)</td>
<td>N/A</td>
</tr>
<tr>
<td>pCPA/vehicle</td>
<td>27 ± 1 (n=7)</td>
<td>10 ± 0</td>
</tr>
<tr>
<td>Vehicle/CGP56433A</td>
<td>256 ± 28 (n=9)</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>pCPA/CGP56433A</td>
<td>27 ± 2 (n=9)</td>
<td>10 ± 1</td>
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</tbody>
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N/A, not applicable.

Interestingly, the novel GABA\(_B\) receptor-positive modulator GS39783 (Cryan et al., 2004) did not alter any behavior in the modified forced swim test at any dose tested (Fig. 3), which is in agreement to that demonstrated in the mouse forced swim test after chronic treatment (Mombereau et al., 2004). GS39783 is an excellent tool for behavioral analysis because it lacks the sedative and muscle-relaxant properties of full agonists such as baclofen (Cryan et al., 2004). The lack of effects in the forced swim test is probably not due to incorrect dose selection because the doses used were equal or above that which show anxiolytic-like effects. These data further confirm a dissociation of a role for GABA receptors in the modulation of anxiety and antidepressant-like behavior (Mombereau et al., 2004). Given that serotonin can modulate anxiety and depression in opposite manners, high serotonergic activity being associated with anxiety and low activity with depression (Graeff et al., 1996; Cryan and Leonard, 2000), it is plausible that differential interaction of GABA receptors on 5-HT neuronal firing at the level of the DRN may be in part responsible for the behavioral effects subsequent to differential pharmacological manipulations of GABA receptors.

In summary, the present studies demonstrate that GABA\(_B\) receptor antagonists but not positive modulators display antidepressant-like activity in the modified forced swim test. Furthermore, this behavior is mediated via an interaction between the GABA\(_B\) and serotonergic systems as the selective increase in swimming behavior after administration is no longer present in serotonin-depleted animals. These studies suggest that GABA\(_B\) receptor antagonists may represent a novel approach to the treatment of depression.

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