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SB-649915-B, a Novel 5-HT$_{1A/B}$ Autoreceptor Antagonist and Serotonin Reuptake Inhibitor, is Anxiolytic and Displays Fast Onset Activity in the Rat High Light Social Interaction Test

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Preclinically, the combination of an SSRI and 5-HT autoreceptor antagonist has been shown to reduce the time to onset of anxiolytic activity compared to an SSRI alone. In accordance with this, clinical data suggest the coadministration of an SSRI and ($\pm$) pindolol can decrease the time to onset of anxiolytic/antidepressant activity. Thus, the dual-acting novel SSRI and 5-HT$_{1A/B}$ receptor antagonist, SB-649915-B, has been assessed in acute and chronic preclinical models of anxiolysis. SB-649915-B (0.1–1.0 mg/kg, i.p.) significantly reduced ultrasonic vocalization in male rat pups separated from their mothers (ED$_{50}$ of 0.17 mg/kg). In the marmoset human threat test SB-649915-B (3.0 and 10 mg/kg, s.c.) significantly reduced the number of postures with no effect on locomotion. In the rat high light social interaction (SI), SB-649915-B (1.0–7.5 mg/kg, t.i.d.) and paroxetine (3.0 mg/kg, once daily) were orally administered for 4, 7, and 21 days. Ex vivo inhibition of [³H]5-HT uptake was also measured following SI. SB-649915-B and paroxetine had no effect on SI after 4 days. In contrast to paroxetine, SB-649915-B (1.0 and 3.0 mg/kg, p.o., t.i.d.) significantly ($p < 0.05$) increased SI time with no effect on locomotion, indicative of an anxiolytic-like profile on day 7. Anxiolysis was maintained after chronic (21 days) administration by which time paroxetine also increased SI significantly. 5-HT uptake was inhibited by SB-649915-B at all time points to a similar magnitude as that seen with paroxetine. In conclusion, SB-649915-B is acutely anxiolytic and reduces the latency to onset of anxiolytic behavior compared to paroxetine in the SI model.

INTRODUCTION

Selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) are widely used, clinically efficacious antidepressant and anxiolytic agents. However, one of their major drawbacks, as with all antidepressants, is the time to onset of clinical effect, which can typically take 3–4 weeks. It has been hypothesized that this delayed efficacy of SSRIs may be alleviated by the concurrent blockade of 5-HT$_{1A}$ autoreceptors and serotonin reuptake inhibition. Artigas and colleagues (1994) reported that coadministration of paroxetine and the 5-HT$_{1A}$ receptor partial agonist and β-adrenergic receptor antagonist, ($\pm$) pindolol decreased the time to onset of antidepressant action compared to the SSRI alone from 2–3 weeks to 3–7 days. These findings have

been confirmed by numerous groups (Blier and Bergeron, 1995; Zanardi et al, 1997; Bordet et al, 1998; McAskill et al, 1998; Maes et al, 1999) and in a meta-analysis by Ballesteros and Callado (2004), but have been refuted by others (Berman et al, 1997, 1999). It is likely that the recent controversy surrounding the optimal dose of pindolol used in the augmentation of SSRI (Segrave and Nathan, 2005; Rabiner et al, 2001) may explain these inconsistencies.

The delay in clinical efficacy with SSRIs is, in part, thought to be due to their inability to acutely increase extracelluar 5-HT levels. Indeed, this is evident in rodents in which acute SSRI treatment decreases serotonergic cell firing via the activation of somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe (Blier et al, 1987; Gartside et al, 1995). It is not until the desensitization of these receptors has occurred that a sustained elevation in extracellular 5-HT levels is seen (Hensler et al, 1991; Le Poul et al, 1995; Mongeau et al, 1997). Furthermore, preclinical neuro-chemical studies have shown that blockade of the inhibitory 5-HT$_{1A}$ autoreceptors with ($\pm$) pindolol induces an acute, rapid elevation of extracellular forebrain 5-HT that is not seen with SSRIs alone (Artigas, 1993; Hjorth, 1996;

Dawson and Nguyen, 2000). Data with the highly selective 5-HT$_{1A}$ receptor antagonist WAY100635 (Forster et al, 1995; Fletcher et al, 1996) combined with SSRIs provide further evidence to support the role of 5-HT$_{1A}$ receptors in this response (Gartside et al, 1995; Dawson and Nguyen, 1998).

In addition to the 5-HT$_{1A}$ autoreceptor, 5-HT$_{1B/D}$ autoreceptors also play a part in regulating 5-HT release from the nerve terminal (Hoyer and Middlemiss, 1989) and 5-HT synthesis (Hjorth et al, 1995). There is also evidence that 5-HT$_{1B}$ receptor desensitization is seen following chronic SSRI treatment indicating that these receptors may play a part in mediating the effects seen with SSRIs (Blier and Bouchard, 1994; Newman et al, 2004; Shalom et al, 2004). Preclinical microdialysis studies have demonstrated that the selective 5-HT$_{1B}$ receptor antagonist SB-224289 augments the 5-HT increase seen with paroxetine, and that WAY100635 further potentiates this effect (Roberts et al, 1999). Another 5-HT$_{1B/D}$ receptor antagonist, GR127935, also augments an SSRI effect in microdialysis (Rollema et al, 1996; Gobert et al, 1997) and potentiates the effects of an SSRI/5-HT$_{1A}$ receptor antagonist combination (Sharp et al, 1997; Dawson and Nguyen, 2000). Therefore, 5-HT$_{1A/B/D}$ receptor antagonism may play a beneficial role in the augmentation of the effects of SSRIs.

Anxiety tests such as the marmoset human threat test and rat pup ultrasonic vocalization test have been shown to identify clinically efficacious anxiolytic and antidepressant agents, for example, benzodiazepines and SSRIs (Gardner, 1985; Olivier et al, 1998; Costall et al, 1988b). However, although these studies may, at least in part, predict the clinical efficacy of this class of compounds, they do not model the temporal effects of SSRIs seen in the clinic. Lightowler et al (1994) demonstrated that in the high light social interaction (SI) test, a rodent test of anxiety-related behavior (File and Hyde, 1978, 1979; File, 1980), chronic, but not acute, administration of paroxetine significantly increased time spent in active SI (indicative of an anxiolytic profile). These data demonstrated that the high light SI test may be a useful model to evaluate the temporal nature of SSRI-induced anxiolysis. Furthermore, Duxon et al (2000) reported that coadministration of paroxetine and WAY100635 reduced the latency to anxiolysis from 21 days with paroxetine alone to 7 days with the combination. Hence, these findings suggest that the rat high light SI test may be used to model the rapid onset of action seen in the clinic with the combination of an SSRI and ($\pm$) pindolol.

We report here the behavioral assessment of SB-649915-B, a novel SSRI and 5-HT$_{1A/B}$ autoreceptor antagonist (Atkinson et al, 2005) that has high affinity for recombinant human and rat native tissue 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (Scott et al, 2006). [^{3}H]citalopram binding and [^{3}H]5-HT uptake studies have also shown that SB-649915-B has high affinity for human recombinant and rat native SERT (Scott et al, 2006). SB-649915-B also has a good selectivity profile against other 5-HT, dopamine, and adrenergic (α_{1B} and β_2) receptors (Atkinson et al, 2005; Scott et al, 2006). In vivo SB-649915-B shows antagonism at 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors as determined by the dose-dependent inhibition of 8-OH-DPAT-induced hyperlocomotion and SKF-99101-H-induced increase in seizure threshold (Hughes et al, 2007) respectively. Ex vivo [^{3}H]5-HT uptake studies have also determined the functional activity of SB-649915-B at

the serotonin transporter following oral dosing in rodents (Hughes et al, 2007). The studies described herein determine the effects of SB-649915-B after acute administration in the rat pup ultrasonic vocalization and marmoset human threat tests, and evaluate the temporal nature of these effects in the high light SI model.

METHODS

Animals

All in vivo studies conformed to GlaxoSmithKline ethical standards and were conducted in accordance with either the United Kingdom Animals (Scientific Procedures) Act, 1986 or Italian law (art. 7, Legislative Decree no. 116, 27 January 1992), which acknowledges the European Directive (86/609/EEC).

Male Sprague–Dawley rat pups (Charles River, Italy) used in the vocalization study were housed under standard laboratory conditions on a 12 h light/dark cycle with lights on at 0500 GMT and food and water available ad libitum.

Human threat test studies utilized laboratory-bred male (vasectomized) and female common marmosets over 2 years of age, weighing 300–500 g. The animals were caged in couples, in a housing room maintained at $25 \pm 1^\circ C$, 60% humidity, and a 12 h light/dark cycle (lights on at 0500 GMT, with 30 min-simulated dawn and twilight). Water was available ad libitum and food was administered twice daily (0700 and 1400 h).

In the SI studies, male Sprague–Dawley rats (Charles River, UK, 265–384 g on the day of testing) were group-housed until 5 days before testing, after which they were singly housed to increase their drive for SI. Animals were housed under standard laboratory conditions, had free access to food and water, and were maintained under a 12 h light/dark cycle with lights on at 0600 GMT.

Ultrasonic Vocalizations in Rat Pups

Rat pups were housed with their mothers and littermates under standard laboratory conditions. At the age of 9–12 days, pups were screened over a 1 min session for their ability to emit ultrasonic vocalization (42 kHz) after removal from their home cage. During the test period, animals were maintained at $22 \pm 1^\circ C$ and their vocalizations were recorded in a soundproof box using a microphone. The signal was filtered and transformed into digital block pulse and was processed by a dedicated software (Ultravox system, Noldus, The Netherlands). Only subjects with duration of vocalization longer than 20 s over the 1 min training session were included in the test session. Immediately following the training session, animals were returned to their home cages and randomly assigned to treatment groups ($n = 6$–8). The test and training sessions occurred on the same day and vocalizations were recorded over a 5 min test period.

Human Threat Test in Marmosets

Both marmosets in each housing pair were involved in the test, which was carried out with the animals situated in their home cage. The behavioral response (number of postures, see Costall et al, 1988a) to a human observer standing in

close proximity to the home cage was recorded. Only animals that in the week before the test met the criterion of >10 postures over a 2 min session were included in the experimental design. Drug treatments were assigned according to a blind crossover design. The number of postures and jumps from the back of the cage to the cage front was measured during the 2 min test to assess potential anxiolytic-like activity and exclude sedation or locomotor stimulation ($n = 4$). After a washout period of at least 3 days, treatments were reassigned and the study was completed when all animals had received all treatments.

Social Interaction Test

Rats were allocated to a test pair on the basis of weight (max ± 21 g) and both received the same treatment ($n = 10$ or 12 individuals). SI was determined in a white perspex test arena (54 (width) × 36 (depth) × 29 (height) cm), with a solid white floor divided into 24 squares (9 × 9 cm). A transparent perspex front allowed behavior to be monitored using a video camera positioned in front of the arena and linked to a video recorder and monitor in an adjoining room. Lighting was provided using normal lighting levels augmented by a lamp with a 60 W bulb placed directly above the arena. All behavior was observed between 0900 and 1700 in 5 pair blocks with each treatment represented once within each block. Behavior was monitored from videotapes at a later date by an observer blind to treatment. Total time spent in interaction and amount of locomotor activity for each animal was scored over a 15 min test period. Duration of SI (interaction time) was defined as seconds spent sniffing, grooming, boxing, and climbing over and under partner. Locomotion (zone transitions) was defined as number of whole squares transversed on the base of the arena.

Ex Vivo Inhibition of [³H]5-HT Uptake

Immediately after SI testing (2 h 15 min post-dose) some animals ($n = 3-4$ per group) were sacrificed and tissue removed for *ex vivo* analysis (Thomas *et al*, 1987). Cortical tissue from each test animal was resuspended in 30 volumes (w/v) of ice-cold tissue buffer (50 mM TRIS Pre-Set, pH 7.7, 10 mM MgCl₂) and was homogenized using an Ultra-Turrux. Each tissue sample was then used to generate both total binding and nonspecific binding values in triplicate against [³H]5-HT. Nonspecific binding was determined using 1 μM paroxetine. Samples were incubated at 37 °C for 45 min before the assay was terminated by rapid filtration through GF/B filters presoaked in 0.3% PEI. This was followed by 5 × 1 ml washes with ice-cold harvest buffer (50 mM TRIS Pre-Set, pH 7.7, 10 mM MgCl₂). Filters were left to dry before scintillation fluid was added and bound radioactivity determined by scintillation spectrometry.

Drugs and Dosing

SB-649915-B (6-[(1-{2-[(2-methyl-5-quinolinyl)oxy]ethyl}-4-piperidinyl)methyl]-2*H*-1,4-benzoxazin-3(4*H*)-1) synthesized by the Department of Medicinal Chemistry, GlaxoSmithKline was administered as mg/kg free base (except to rat pups where doses were expressed as mg/kg salt).

Paroxetine (GlaxoSmithKline) and fluoxetine (Medicinal Chemistry, GlaxoSmithKline) were administered as mg/kg salt. All compounds were administered in 1% methylcellulose (Sigma-Aldrich, Poole, UK). Drugs and reagents for *ex vivo* binding were purchased from Sigma-Aldrich (Poole, UK), Research Biochemicals International (Poole, UK), and Tocris Cookson Ltd (Bristol, UK). [³H]5-HT was supplied by Amersham International (Little Chalfont, UK).

In the vocalization tests pups were treated intraperitoneally (i.p.) at 10 ml/kg with 1% methylcellulose, SB-649915-B (0.1, 0.3, and 1.0 mg/kg) or fluoxetine (10 mg/kg) 30 min before testing. Six hours before testing in the human threat test marmosets were treated subcutaneously (s.c.) at 1 ml/kg with 1% methylcellulose or SB-649915-B (1.0, 3.0, and 10 mg/kg).

In the SI studies 1% methylcellulose or SB-649915-B (1.0, 3.0, or 7.5 mg/kg) was administered by oral gavage (p.o.), at 2 ml/kg, three times daily (t.i.d.; 0800, 1400, and 2000). Animals were dosed using this regiment for 4, 7, or 21 days in three separate experiments (each time point was investigated in an individual experiment). On the final day of treatment (day 4, 7, or 21 of dosing), animals were dosed once 2 h before testing. Paroxetine was administered p.o. once daily (u.i.d.) at the 0800 dosing point and 1 h before testing. On the two subsequent daily dosing occasions these animals received vehicle only. This dosing regimen was based on pharmacokinetic data generated in-house to model the systemic exposure levels in a 24 h period with SB-649915-B (data not shown).

Data Analysis and Statistics

Rat pup ultrasonic vocalization data are expressed as a mean duration of vocalization ($\pm$ standard error of the mean (SEM)). Data were subjected to a paired *t*-test, comparing each compound dose with the related vehicle treatment (GB-STAT statistical software, Dynamic Microsystems). ED₅₀ values and 95% confidential limits were calculated using a linear fit procedure using RS1 software (version 4.21, BBN Software Product Corporation) (Draper and Smith, 1966).

Human threat test data are expressed as mean number of postures or jumps $\pm$ SEM. Data were subjected to a one-way ANOVA, followed by a *post hoc* (Dunnett's) test (GB-STAT statistical software, Dynamic Microsystems).

SI data were captured as interaction time (seconds) and locomotor activity (number of zone transitions) and presented as mean $\pm$ SEM for each treatment group. The effects of SB-649915-B and paroxetine *vs* vehicle control animals were assessed by one-way ANOVA followed by a *post hoc* *t*-test (Duncan's) when appropriate (Statistica, SatSoft Inc., OK, USA).

[³H]5-HT uptake was calculated from the specific d.p.m. per treatment group and presented normalized to vehicle $\pm$ SEM. The effects of SB-649915-B and paroxetine were compared to vehicle controls by one-way ANOVA followed by Duncan's *t*-test when appropriate (Statistica, SatSoft Inc., OK, USA).

RESULTS

SB-649915-B in the Rat Pup Vocalization Model

SB-649915-B significantly decreased duration of vocalization in rat pups compared to vehicle at 0.1 ($p < 0.05$), 0.3,

and 1.0 mg/kg ($p<0.01$) with an estimated ED_{50} of 0.17 mg/kg (95% confidence limits, 0.09–0.26 mg/kg), consistent with an anxiolytic-like activity (Figure 1). The positive control fluoxetine (10 mg/kg) also significantly ($p<0.01$) reduced duration of vocalization.

SB-649915-B in the Marmoset Human Threat Test

Acute administration of SB-649915-B significantly ($p<0.01$). reduced the number of postures compared to vehicle control by 45% and 47% at 3.0 and 10 mg/kg s.c. respectively ($F_{3,12}=10.2$, $p=0.001$, Figure 2a). SB-649915 did not affect the number of jumps, suggesting the reduction in postures was not confounded by sedative effects over this dose range ($F_{3,12}=18.7$, $p=0.23$; Figure 2b).

SB-649915-B on Rat Social Interaction Behavior

(i) Subchronic (4 days) SB-649915-B on rat social interaction behavior. Administration of SB-649915-B
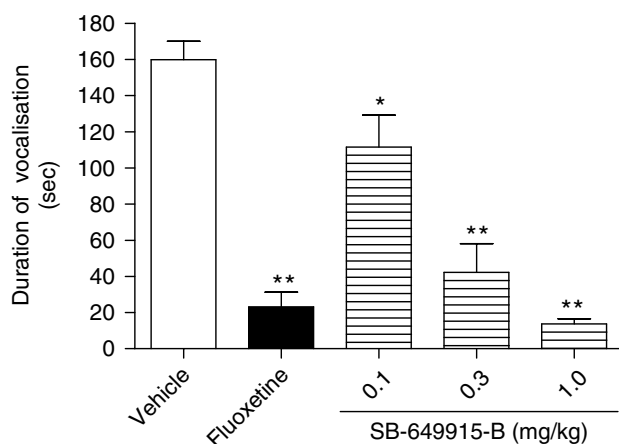

Figure 1 Effects of SB-649915-B on ultrasonic vocalizations in rat pups. SB-649915-B (0.1, 0.3, and 1.0 mg/kg) was administered i.p. 30 min before test. Data are expressed as mean duration of vocalization(s) ± SEM ($n = 6$–8 per group). SB-649915-B (0.1, 0.3, and 1.0 mg/kg, i.p.) and fluoxetine (10 mg/kg, i.p.) significantly reduced vocalization compared to vehicle. *$p<0.05$, **$p<0.01$ from vehicle-treated animals (ANOVA followed by Dunnett's test).

(1.0–7.5 mg/kg, p.o., t.i.d.) or paroxetine (3.0 mg/kg, p.o., u.i.d.), for 4 days did not influence either time spent in SI or locomotor activity ($F_{4,55}=1.4$, $p=0.88$ interaction; $F_{4,55}=1.9$, $p=0.13$ locomotion; Figure 3a) in any of the treatment groups. In a subset of behavioral animals, paroxetine (3.0 mg/kg, p.o., u.i.d.) and SB-649915-B (3.0 and 7.5 mg/kg, p.o., t.i.d.) significantly ($p<0.05$ and $p<0.001$ respectively) reduced [³H]5-HT uptake after 4 days administration ($F_{4,10}=18.7$, $p=0.001$, 4 days; Figure 3a).

(ii) Subchronic (7 days) SB-649915-B on rat social interaction behavior. Administration of SB-649915-B (1.0–7.5 mg/kg, p.o. t.i.d.) or paroxetine (3.0 mg/kg, p.o., u.i.d.) for 7 days was assessed compared to vehicle-treated controls. Paroxetine (3.0 mg/kg, p.o., u.i.d., 7 days) did not affect interaction time or locomotor activity (Figure 3b). SB-649915-B (1.0 and 3.0 mg/kg, p.o., t.i.d., 7 days) significantly ($p<0.01$) increased interaction time ($F_{4,55}=6.4$, $p=0.0003$), but did not influence locomotor activity ($F_{4,55}=0.7$, $p=0.59$) (Figure 3b). In contrast, the top dose of SB-649915-B tested (7.5 mg/kg, p.o., t.i.d., 7 days) failed to show effect either on interaction time or locomotor activity. [³H]5-HT uptake was assessed in a group of animals that had undergone behavioral testing and was significantly reduced ($F_{4,15}=40.7$, $p=0.0001$, 7 days; Figure 3b) following 7 days administration of paroxetine ($p<0.001$) and SB-649915-B 1.0 ($p<0.05$), 3.0 ($p<0.001$), and 7.5 mg/kg ($p<0.001$).

(iii) Chronic (21 days) SB-649915-B on rat social interaction behavior. Administration of SB-649915-B (1.0–7.5 mg/kg, p.o., t.i.d.) for 21 days significantly ($p<0.01$) increased interaction time with no significant concurrent increase in locomotor activity compared to vehicle-treated controls. Paroxetine (3 mg/kg, p.o., u.i.d.)-treated animals also significantly increased interaction time with no effect on locomotor activity ($F_{4,53}=5.5$, $p=0.003$ interaction; $F_{4,53}=2.4$, $p=0.06$ locomotion; Figure 3c). Following 21 days dosing, [³H]5-HT uptake was significantly reduced ($F_{4,15}=7.3$, $p=0.002$, 21 days; Figure 3c) in a subset of the behavioral animals following paroxetine ($p<0.001$). SB-649915-B also significantly ($p<0.001$) inhibited [³H]5-HT uptake in a dose-dependent manner at 3.0 and 7.5 mg/kg. The effect at 1.0 mg/kg was also not significant; however the magnitude of the effect was as that at 7 days.
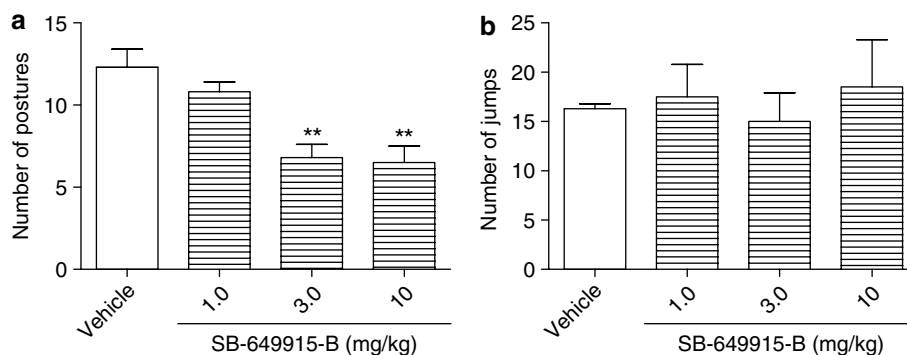

Figure 2 Effects of SB-649915-B in the human threat test in marmosets. SB-649915-B (1.0, 3.0, and 10 mg/kg) was administered s.c. 60 min before test. Data are expressed as mean number of postures and jumps ± SEM ($n = 4$ per group). SB-649915-B significantly reduced postures with no effect on the number of jumps. **$p<0.01$ compared to vehicle-treated animals (Dunnett's test).
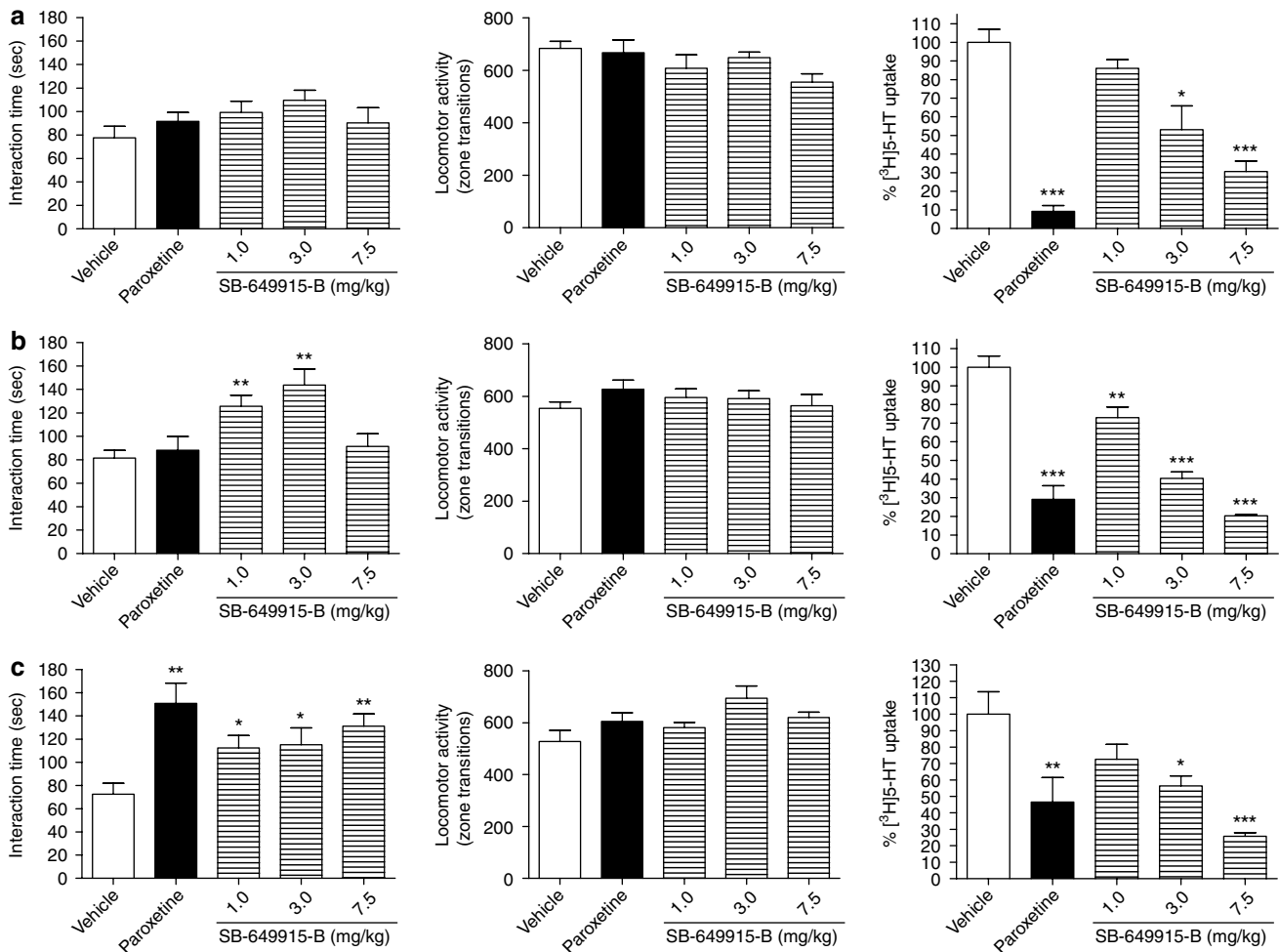
Figure 3 The effect of subchronic (4 and 7 days) and chronic (21 days) SB-649915-B (1.0–7.5 mg/kg, p.o., t.i.d.) or paroxetine (3.0 mg/kg, p.o., u.i.d.) on rat behavior in the high light SI test and SERT occupancy as determined by inhibition of [^{3}H]5-HT uptake. Data are presented as mean interaction time(s) $\pm$ SEM, mean locomotion (zone transitions) $\pm$ SEM ($n = 10–12$), and mean % occupancy $\pm$ SEM ($n = 3–4$). *, **, and *** denote significant change ($p < 0.05$, $p < 0.01$, or $p < 0.001$, respectively) from vehicle-treated animals (Duncan's t-test following ANOVA SI; Dunnett's test following ANOVA SERT occupancy). A significant increase in interaction time was observed with SB-649915-B on day 7 (1.0 and 3.0 mg/kg) and 21 (1.0, 3.0, and 7.5 mg/kg) and paroxetine on day 21 with no concurrent effect on locomotion (b and c). SB-649915-B significantly occupied SERT compared to vehicle. *$p < 0.05$, **$p < 0.01$ from vehicle-treated controls.

DISCUSSION

SSRIs are well established in the treatment of anxiety disorders (such as panic disorder, obsessive compulsive disorder, generalized anxiety disorder, and posttraumatic stress disorder) (for review see Nutt, 2005) and depression. However, it is widely established that it can take several weeks for clinical efficacy to be seen. It has been suggested that this delayed onset of clinical effect may be due, at least in part, to the need for desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe (Le Poul et al, 1995). Hence, it has been hypothesized that concomitant therapy with an SSRI increasing 5-HT in the forebrain and a 5-HT$_{1A}$ receptor antagonist preventing feedback via blockade of the autoreceptors may result in a reduction of the time to onset of efficacy in the clinic. In support of this hypothesis, clinical data have indicated that the coadministration of an SSRI with the mixed β-adrenergic and 5-HT$_{1A}$ receptor antagonist ($\pm$) pindolol can hasten the commencement of

antidepressant efficacy from several weeks to between 3 and 7 days (Artigas et al, 1994; Blier and Bergeron, 1995), although there has been evidence to the contrary in depression (Berman et al, 1997, 1999) and anxiety (Stein et al, 2001). There are also a substantial number of preclinical rodent neurochemistry studies that support the proposal that the increase in forebrain 5-HT seen with SSRIs after chronic administration can been seen acutely with concurrent blockade of the 5-HT$_{1A}$ autoreceptors (Dawson and Nguyen, 1998; Gartside et al, 1995). In addition, 5-HT1$_{B/D}$ antagonists augment an SSRI-induced increase in 5-HT (Dawson and Nguyen, 2000) and further potentiate the increase in 5-HT following WAY100635 and fluoxetine (Gobert et al, 2000).

With this in mind, a novel 5-HT$_{1A/B}$ autoreceptor antagonist and 5-HT reuptake inhibitor SB-649915-B has been developed (Atkinson et al, 2005). SB-649915-B occupies and fully blocks the serotonin transporter and 5-HT$_{1A/B}$ receptors whilst inducing an increase in extracellular 5-HT

in the forebrains of guinea pigs and rats following acute administration (Hughes *et al*, 2007). Here, we evaluate the effects of SB-649915-B following acute and chronic administration in preclinical tests of anxiety-like behavior.

SSRIs have been shown to have acute behavioral effects in a number of preclinical assays of anxiety (for review, see Borsini *et al*, 2002). When rat pups are separated from their mothers they emit ultrasonic vocalizations which can be attenuated by acutely administered anxiolytic agents such as benzodiazepines and SSRIs (Gardner, 1985; Olivier *et al*, 1998). In the rat pup, vocalization was reduced in a dose-dependent manner by SB-649915-B, and at the top dose (1.0 mg/kg) this was to an equivalent degree as the SSRI, fluoxetine (10 mg/kg). Based on these acute findings in the rodent model, the studies were extended to a primate species, the common marmoset, in the human threat test. When confronted by a human in close proximity to their home cage, marmosets show a series of behavioral postures that are considered to be related to their level of anxiety (Costall *et al*, 1988a). These postures can be reduced by administration of anxiolytic agents at doses that have no sedative effects as measured by a decrease in the number of jumps (Costall *et al*, 1988a, b). SB-649915-B significantly reduced the number of postures at 3.0 and 10 mg/kg with no effect on the number of jumps, demonstrating a specific anxiolytic effect in a nonhuman primate.

The mechanism of action in the pup vocalization model is thought to be 5-HT related as only compounds with serotonergic activity (eg SSRIs, clomipramine, and impiramine) show efficacy in this assay (for review, see Borsini *et al*, 2002). However, a small or no increase in 5-HT has been measured using microdialysis following acute SSRI administration (Sharp *et al*, 1997; Roberts *et al*, 1999; Dawson and Nguyen, 2000) questioning whether the acute anxiolytic effects are due to changes in 5-HT levels or whether other neurotransmitter systems play a role. It could be postulated that an overflow technique such as microdialysis simply lacks the sensitivity to measure small changes in 5-HT, and increases in synaptic 5-HT are sufficient to mediate the observed acute anxiolytic effect. Alternatively, SSRIs and $5\text{-HT}_{1A/B}$ receptor antagonists have been shown to modulate several other neurotransmitter systems including glutamate (Marcoli *et al*, 1999; Schechter *et al*, 2002), acetylcholine (Consolo *et al*, 1996; Nakai *et al*, 1998; Koyama *et al*, 1999), dopamine (Boulenguez *et al*, 1996; Nomikos *et al*, 1996; Iyer and Bradberry, 1996), and noradrenaline (Hajos-Korcsok *et al*, 1999) which may contribute to or mediate the SB-649915-B-induced effect. However, the studies to address the effects on systems other than the 5-HT and monoaminergic (Hughes *et al*, 2007), have, as yet, not been performed.

The data from the rat pup vocalization and marmoset human threat test studies suggest that SB-649915-B has an anxiolytic-like profile in a rodent and primate species following acute dosing. These acute data are encouraging and indicate that the presence of $5\text{-HT}_{1A/B}$ antagonism does not adversely affect the SSRI response in these anxiety models. However, as SSRIs may take several weeks to produce therapeutically beneficial effects, there is a need to introduce novel agents that are equally efficacious against the symptoms of depression and anxiety, as established in acute models, yet exhibit a faster onset of action. Several

preclinical models (eg elevated plus maze, social defeat model, reversal of cholecystokinin, or CRH-induced anxiety in the SI) are able to model the delay in onset to activity that is evident in the clinic (Griebel *et al*, 1994, 1995; Berton *et al*, 1999; To and Bagdy, 1999; To *et al*, 1999). The studies by Lightowler *et al* (1994) and Duxon *et al* (2000) demonstrated that the rat high light SI could be used to predict the onset of anxiolytic activity of paroxetine after chronic (21 days) dosing. Further, Duxon *et al* (2000) demonstrated that the concomitant administration of paroxetine with WAY100635 reduced the latency to onset of anxiolytic behavior from 21 to 7 days. In the light of this data we have chosen to use the SI test of anxiety-like behavior to determine the rate of anxiolytic onset of the combined SSRI and $5\text{-HT}_{1A/B}$ receptor antagonist SB-649915-B.

Consistent with previous findings chronic (21 days), but not subchronic (4 and 7 days) treatment with the SSRI paroxetine increased SI with no concurrent effect on locomotor activity, indicative of a delayed onset of anxiolytic effect (Lightowler *et al*, 1994; Duxon *et al*, 2000). In contrast to paroxetine, SB-649915-B dosed for 7 days significantly increased SI with no effect on locomotor activity and this effect was still evident after 21 days dosing. SI was also monitored after 4 days administration, but there was no significant effect of SB-649915-B at this earlier time point. *Ex vivo* uptake in cortical tissue isolated from behavioral animals showed a decrease in [^{3}H]5-HT uptake at 4, 7, and 21 days dosing following paroxetine and SB-649915-B. The lack of behavioral efficacy of paroxetine until day 21, therefore, is not owing to a lack of SERT activity. Because SB-649915-B also produced a robust dose-related occupancy of SERT at all time points, the apparent hastened onset of efficacy is therefore likely to be due to the combination of an SSRI and $5\text{-HT}_{1A/B}$ receptor antagonism in this molecule. The adaptive changes required for SB-649915-B to show anxiolytic behavior potentially occur between days 4 and 7 in this assay, whereas the paroxetine effects have been postulated to occur between days 14 and 21 of administration (Lightowler *et al*, 1994). There are also a substantial number of preclinical rodent neurochemistry studies that support the proposal that the increase in forebrain 5-HT seen with SSRIs alone after chronic administration can be seen acutely with concurrent blockade of the 5-HT_{1A} autoreceptors using ($\pm$) pindolol (Dreshfield *et al*, 1996; Romero *et al*, 1996a; Hjorth, 1996; Hjorth and Auerbach, 1996; Dawson and Nguyen, 2000) or more selective 5-HT_{1A} receptor antagonist compounds such as WAY100635 (Romero *et al*, 1996a, b; Hjorth *et al*, 1997; Dawson and Nguyen, 1998). A combination of 5-HT_{1A} and 5-HT_{1B} (or $5\text{-HT}_{1B/D}$) receptor antagonists and SSRIs (Sharp *et al*, 1997; Gobert *et al*, 2000; Dawson and Nguyen, 2000) augments extracellular 5-HT even further. SB-649915-B significantly increased 5-HT in the frontal cortex of rats and guinea pigs after acute administration while, in contrast, an acute dose of paroxetine had no effect (Hughes *et al*, 2007). Despite the increase in 5-HT levels following acute SB-649915-B administration, the behavioral effect in the SI paradigm was not observed until day 7 of dosing. Hence, presumably there are downstream effects as a result of the increase in extracellular 5-HT that mediate this response.

It has previously been reported that chronic administration of SSRIs, including paroxetine, to rodents (Kennett *et al*, 1994b; Maj *et al*, 1996; Yamauchi *et al*, 2004) and man (Zohar *et al*, 1988; Hollander *et al*, 1991; Quested *et al*, 1997) cause 5-HT$_{2C}$ receptor desensitization. In the SI test, the anxiogenic effects of the 5-HT$_{2C}$ receptor agonist mCPP (see Kennett *et al*, 1989) are inhibited by the coadministration of WAY100635 and fluoxetine for 7 days (Bristow *et al*, 2000) while no effect is seen with an SSRI alone. Moreover, 5-HT$_{2C}$ receptor antagonists and inverse agonists have been shown to be acutely anxiolytic in rodent models including the SI (Kennett *et al*, 1997, 2000; Wood *et al*, 2001). Therefore, it is possible that the acute increase in 5-HT seen following SB-649915-B (Hughes *et al*, 2007) results in a rapid desensitization of 5-HT$_{2C}$ receptors which are, at least in part, mediating the effect. However, further work is needed to investigate this hypothesis.

More long-term neuronal plastic processes such as neurogenesis have been implicated in antidepressant-mediated clinical efficacy (Duman, 2004). Furthermore, evidence suggests that the effect of antidepressants on neurogenesis is observed after chronic, but not acute administration (Malberg *et al*, 2000; Duman, 2004). The effects of increased 5-HT on cell proliferation are thought to be, at least in part, mediated via agonism of 5-HT$_{1A}$ receptors in the hippocampus (Gould, 1999). Indeed, 5-HT$_{1A}$ receptor antagonists have been shown to reduce cell proliferation in the hippocampus (Radley and Jacobs, 2002). It could be of concern that the 5-HT$_{1A}$ receptor antagonist properties of SB-649915-B may impact neurogenesis. Although this has not been specifically investigated, at 1.0 and 3.0 mg/kg, the postsynaptic 5-HT$_{1A}$ receptors in the hippocampus are, potentially, not saturated by SB-649915-B, as determined by an inability to block fully 8-OH-DPAT-induced hyperactivity (Hughes *et al*, 2007). The extracellular increase in 5-HT (Hughes *et al*, 2007) seen at these doses may be acting at available 5-HT$_{1A}$ receptors, inducing neurogenesis and thereby mediating the anxiolytic effect in the SI. This is supported by the lack of effect of 7.5 mg/kg at this early time point, assuming this dose completely blocks pre- and postsynaptic 5-HT$_{1A}$ receptors as seen at 10 mg/kg (Hughes *et al*, 2007). After 21 days administration, there is a significant increase in SI at 7.5 mg/kg indicating that, if neurogenesis is playing a role in the anxiolytic behavior, 5-HT$_{1A}$ receptor activation may have occurred at this time point, and a longer period of 5-HT elevation is required to overcome the SB-649915-B-mediated competitive 5-HT$_{1A}$ receptor block. It could be suggested that chronic blockade of postsynaptic 5-HT$_{1A}$ receptors will induce an increase in receptor population or sensitization, however, the literature dose not support a change in 5-HT$_{1A}$ receptor sensitivity (Dawson *et al*, 2002; Hervas *et al*, 2001). The Duxon *et al* (2000) data showing an accelerated onset of effect following coadministration of paroxetine (3.0 mg/kg) and WAY100635 (0.1 mg/kg) clearly indicate that 5-HT$_{1A}$ receptor antagonism does not have a negative impact on the SI responses. Clinical efficacy with pindolol in combination with SSRIs may also lend some support to this hypothesis although, the level of 5-HT$_{1A}$ receptor blockade achieved with this combination has been questioned (Rabiner *et al*, 2001).

It could be proposed that the differing dosing regimen used for paroxetine and SB-649915-B (once-daily dosing of paroxetine *vs* thrice-daily dosing of SB-649915-B) could have contributed, at least in part, to the improvement in latency to efficacy. The SB-649915-B dosing regimen was chosen to provide an appropriate level of exposure over a 24 h period (data not shown), and the paroxetine based on previous data showing efficacy in this test (Lightowler *et al*, 1994). Put into context with previous data, we suggest that a higher dose of paroxetine, or increased frequency of dosing would not drive a more rapid onset of effect, as at 10 mg/kg there was no efficacy in the SI following 21 days dosing (Lightowler *et al*, 1994). Duxon *et al*, (2000) showed an accelerated onset in the SI following a combination of paroxetine and the 5-HT$_{1A}$ receptor antagonist WAY100635, therefore it is likely that novel pharmacology of SB-649915-B is responsible for the accelerated action and not the differences in dosing regimen used. SB-649915-B clearly shows an advantage over paroxetine with regards to onset of anxiolytic activity in the SI paradigm. Although the top (7.5 mg/kg) dose of SB-649915-B failed to demonstrate an anxiolytic effect after 7 days administration, it is noteworthy that the efficacy of paroxetine alone after 21 days administration in this model also displays a bell-shaped dose–response curve with only 3.0 mg/kg (but not 1.0 or 10 mg/kg) significantly increasing SI time (Lightowler *et al*, 1994). These authors are not aware of any SI studies where administration of SSRIs beyond 21 days dosing has been investigated, but it is possible that, like SB-649915-B, prolonged dosing of paroxetine yields an anxiolytic effect at doses that have had no effect at earlier time points.

In conclusion, SB-649915-B is a novel compound showing SSRI activity together with selective 5-HT$_{1A/B}$ receptor blockade in a single molecule (Atkinson *et al*, 2005; Scott *et al*, 2006). The anxiolytic activity of this compound is shown in rodent and primate tests which also detect the activity of SSRIs after acute dosing. After chronic administration SB-649915-B clearly shows an advantage over paroxetine in reducing the latency of onset to anxiolytic activity from 21 to 7 days in the SI paradigm, a suggested surrogate marker of onset to SSRI activity in the clinic. The clear anxiolytic effect at both 7 and 21 days indicates a lack of tolerance to the compound. These data indicate that the augmentation of an SSRI with 5-HT$_{1A/B}$ receptor antagonism in a single compound reduces the latency to anxiolytic effect compared to a selective SSRI alone and this may be of therapeutic benefit.

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