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# Characterization of the Potent 5-HT$_{1A/B}$ Receptor Antagonist and Serotonin Reuptake Inhibitor SB-649915: Preclinical Evidence for Hastened Onset of Antidepressant/Anxiolytic Efficacy

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## ABSTRACT

An increase in brain serotonin (5-HT) levels is thought to be a key mechanism of action responsible for generating antidepressant efficacy. It has been proven that selective serotonin reuptake inhibitors are effective antidepressants, but the delay to therapeutic onset of these agents is thought to be due to the time required for 5-HT$_{1A,}$ and possibly 5-HT$_{1B}$, autoreceptors to desensitize. Therefore, an agent incorporating 5-HT reuptake inhibition coupled with 5-HT$_{1A}$ and/or 5-HT$_{1B}$ autoreceptor antagonism may provide a fast-acting clinical agent. The current studies review the profile of SB-649915 (6-[(1-{2-[(2-methylquinolin-5-yl)oxy]ethyl}piperidin-4-yl)methyl]-2*H*-1,4-benzoxazin-3(4*H*)-one), a novel compound with high affinity for human (h) 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (p$K_i$ values of 8.6 and 8.0, respectively) as well as the (h) 5-HT transporter (SERT) (p$K_i$ value of 9.3). SB-649915 behaved as an antagonist at both 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors *in vitro* and *in vivo,* reversing 5-HT, (+)8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and SKF99101-induced functional/behavioral responses. Furthermore, it inhibited [$^3$H]5-HT reuptake in rat cortical synaptosomes, *in vitro* and *ex vivo*. In electrophysiological studies SB-649915 had no effect on rat dorsal raphe neuronal cell firing *per se,* but reversed 8-OH-DPAT–induced inhibition of firing both *in vitro* and *in vivo*. In addition, in a microdialysis study, it produced an acute increase in extracellular 5-HT in forebrain structures of the rat. Finally, SB-649915 demonstrated acute anxiolytic activity in both rodent and non-human primate

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Conflict of interest. The authors are employed by GlaxoSmith Kline, the developer of SB-649915.

and reduced the latency to onset of anxiolytic behavior, compared to paroxetine, in the rat social interaction paradigm. In summary, SB-649915 is a novel, potent 5-$HT_{1A/1B}$ autoreceptor antagonist, and 5-HT reuptake inhibitor. This particular pharmacological profile provides a novel mechanism that could offer fast-acting antidepressant activity.

## INTRODUCTION

Several lines of evidence have led to the hypothesis that improved 5-HT neurotransmission underlies the therapeutic effects of current antidepressants regardless of their mode of action (Shopsin 1978; Young et al. 1985). Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, citalopram, sertraline, and paroxetine are now the first-line pharmacotherapy for the treatment of both depression and anxiety, and have provided significant support for the hypothesis that affective disorders result from a deficit in serotonergic neurotransmission (Delgado et al. 1990). However, clinical efficacy is only achieved following prolonged treatment with an SSRI (Artigas et al. 1994; Blier and Bergeron 1995), and there is a significant proportion of patients who remain unresponsive to treatment. The reason for the delay to onset of action of SSRIs has been the subject of many years of research and is thought to be due, in part, to the time required for the downregulation/desensitization of 5-$HT_1$ autoreceptors. Indeed, *in vitro* autoradiographic [$^{35}$S]GTP$\gamma$S binding and *in vivo* electrophysiological, neurochemical, and neuroendocrine studies in rodents suggest that prolonged treatment with SSRIs causes desensitization of 5-$HT_{1A}$ autoreceptors (Blier and DeMontigny 1983; Blier et al. 1988; Kreiss and Lucki 1995; Raap et al. 1999; Dawson et al. 2000, 2002; Hensler 2002; Shen et al. 2002).

Augmentation of SSRI action using a variety of pharmacological tools has been explored clinically, and combination treatment with the $\beta$-adrenergic/5-$HT_{1A}$ receptor partial agonist, pindolol, has shown some promise. In this respect, in the first open-label clinical report Artigas and colleagues (1994) demonstrated that combination of (±)pindolol with paroxetine shortened the onset to therapeutic response to a period of 3–7 days, which contrasts with 2–3 weeks that is commonly observed for patients treated with an SSRI alone. A number of subsequent trials have attempted to replicate this effect and to establish whether a pindolol add-on strategy could elicit a response in treatment-resistant patients. Despite the difficulties associated with measuring antidepressant efficacy in the clinic, pindolol was shown to accelerate the onset to efficacy in some, but not all, trials. Furthermore, in treatment-resistant depressed patients, combination of SSRIs with pindolol resulted in significant clinical efficacy in three of five trials (for reviews see McAskill and Taylor 1998; Artigas et al. 2001). The discrepancies in outcome between trials could be due, at least in part, to the fact that pindolol is a partial agonist at 5-$HT_{1A}$ receptors (Clifford et al. 1998) and, as such, may actually stimulate, not block 5-$HT_{1A}$ autoreceptors depending on the degree of receptor reserve in the system. Furthermore, suboptimal occupancy of 5-$HT_{1A}$ autoreceptors, due to the dose-limiting side effects of the $\beta$-adrenergic activity of pindolol, may contribute to the conflicting data reported in the clinic (Rabiner et al. 2001).

Although many studies have highlighted the SSRI-induced plastic changes in 5-$HT_{1A}$ autoreceptor-mediated serotonergic neurotransmission, other 5-$HT_1$ autoreceptors have also been shown to regulate serotonin release. It is well established that terminal 5-$HT_{1B}$ autoreceptors control 5-HT release into the synapse (Roberts et al. 1996, 2000; Selkirk et al. 1998; Middlemiss et al. 1999). There is also *in vitro* and *in vivo* evidence

demonstrating that chronic administration of SSRIs desensitizes terminal 5-$HT_{1B}$ autoreceptors (Blier and Bouchard 1994; Newman et al. 2004; Shalom et al. 2004), suggesting that plasticity in 5-$HT_{1B}$ receptor function may also be important for the clinical efficacy of these agents. This hypothesis has been substantiated by several neurochemical studies, which demonstrate that SSRI-induced effects on extracellular brain 5-HT levels, following acute administration, can be augmented by 5-$HT_{1B}$ receptor antagonism (Roberts et al. 1999; Sharp et al. 1997; Dawson and Nguyen 2000). Furthermore, co-administration of 5-$HT_{1A}$ and 5-$HT_{1B}$ (or 5-$HT_{1B/D}$) receptor antagonists can produce an additive augmentation of SSRI-induced neurochemical effects (Sharp et al. 1997; Gobert et al. 2000; Dawson and Nguyen 2000). Thus, based on preclinical data, a compound that incorporated SSRI and 5-$HT_{1A/1B}$ autoreceptor antagonist properties could have the potential to be an effective, rapid-onset antidepressant/anxiolytic drug.

To this end, we now review the pharmacological properties of SB-649915-B (6-[(1-{2-[(2-methylquinolin-5-yl)oxy]ethyl}piperidin-4-yl)methyl]-2*H*-1,4-benzoxazin-3(4*H*)-one, diHCl; Atkinson et al. 2005), the first compound in its class to act as a combined SSRI and 5-$HT_{1A/1B}$ autoreceptor antagonist *in vitro* and *in vivo*. These pharmacological attributes produce acute increases in extracellular levels of serotonin in multiple brain structures, acute anxiolytic/antidepressant efficacy, and a more rapid onset of efficacy than an SSRI.

## *IN VITRO* PHARMACOLOGY

### Radioligand Binding Studies

SB-649915 displayed high affinity for human (h) 5-$HT_{1A}$ and 5-$HT_{1B}$ receptors, with $pK_i$ values of 8.6 and 8.0, respectively (Scott et al. 2006; Table 1). Cross-species affinity determinations, in rodent and non-human primate native tissue membrane preparations, demonstrated that SB-649915 displaced the 5-$HT_{1A}$ receptor radioligand [$^3$H]WAY-100635 with $pK_i$ values of $\geq$8.5 and the 5-$HT_{1B/D}$ receptor radioligand [$^3$H]5-CT with $pK_i$ values $\sim$7.5 (Table 2). Interestingly, although the affinity for the species orthologs of the 5-$HT_{1A}$ receptor appear to be similar to human, the affinity for rat native 5-$HT_{1B}$ receptors was significantly lower, indicative of species differences. Indeed, it is well established that, despite a high sequence homology, the pharmacology of rodent versus non-rodent 5-$HT_{1B}$ receptors can differ for certain classes of compounds and this difference is attributed to the single amino acid substitution, T355N (Adham et al. 1992; Oksenberg et al. 1992). At the recombinant human and native rodent serotonin transporter (SERT), SB-649915 displaced [$^3$H] citalopram with $pK_i$ values of 9.9 and 9.3, respectively (Table 2). Cross-screening binding studies revealed SB-649915 to be 100-fold selective over a range of monoaminergic receptors (h5-$HT_{1E}$, h5-$HT_{1F}$, h5-$HT_{2A}$, h5-$HT_{2B}$, h5-$HT_{2C}$, h5-$HT_4$, h5-$HT_{5A}$, h5-$HT_6$, h$D_2$, h$D_4$, h$\alpha_{1B}$-adrenergic, h$\beta_2$-adrenergic) and transporters (NET and DAT) with the exception of h5-$HT_{1D}$, h5-$HT_7$, and h$D_3$ receptors ($pK_i$ values of 8.8, 6.7, and 6.2, respectively) (Scott et al. 2006). Although 5-HT-$_{1D}$ receptors are reported to be present on somatodentritic regions of 5-HT neurons and can modulate local 5-HT efflux (Davidson and Stamford 1995; Roberts and Price 2001), the current lack of selective tool compounds or assay systems render the full interpretation of the functional consequence of the affinity of SB-649915 for this receptor unknown.

*TABLE 1. Affinity/potency of SB-649915 at h5-HT$_{1A}$ and h5-HT$_{1B}$ receptors and hSERT. Data represent mean ± S.E.M. from at least three individual experiments. Radioligand binding studies were performed in CHO cells expressing h5-HT$_{1A}$ or h5-HT$_{1B}$ receptors and LLCPK cells expressing hSERT. The radioligands employed were [$^3$H]WAY100635, [$^3$H]5-HT and [$^3$H]citalopram, respectively. [$^{35}$S]GTPγS binding studies were performed in HEK293 cells expressing h5-HT$_{1A}$ receptors and Chinese Hamster Ovary (CHO) cells expressing h5-HT$_{1B}$ receptors. I.A. = intrinsic activity as compared to the full agonist, 5-HT. SERT potency was determined by [$^3$H]5-HT uptake studies in LLCPK cells expressing hSERT (see Scott et al. 2006 for details).*

| h5-HT$_{1A}$ | | | h5-HT$_{1B}$ | | | hSERT | | |
|---|---|---|---|---|---|---|---|---|
| $pK_i$ | I.A. | $pA_2$ | $pK_i$ | $pEC_{50}$ | I.A. | $pA_2$ | $pK_i$ | $pEC_{50}$ |
| 8.6 ± 0.1 | 0.0 | 9.0 ± 0.2 | 8.0 ± 0.1 | 7.8 ± 0.1 | 0.3 ± 0.05 | 7.9 ± 0.1 | 9.3 ± 0.1 | 7.9 ± 0.1 |

Data published previously by Scott et al. (2006).

*TABLE 2. Affinity of SB-649915 for native tissue 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors and SERT. Data represent mean ± S.E.M. of at least three individual experiments, each performed in duplicate. [$^3$H]WAY100635 and [$^3$H]5-CT were employed in these studies to determine the affinity of SB-649915 at non-human native 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, respectively. In [$^3$H]WAY100635 binding studies, rat and guinea pig hippocampus was used, whereas rat and guinea pig striatum was the preparation used for [$^3$H]5-CT binding studies. Affinity for SERT was determined by [$^3$H]citalopram binding studies (see Scott et al. 2006 for details).*

| | Rat | Guinea pig | Mouse whole brain | Marmoset cortex |
|---|---|---|---|---|
| $pK_i$ (5-HT$_{1A}$) | 9.0 ± 0.1 | 8.8 ± 0.1 | 8.5 ± 0.5 | 8.8 ± 0.1 |
| $pK_i$ (5-HT$_{1B/1D}$) | 7.5 ± 0.1 | 8.3 ± 0.1 | 8.4 ± 0.1 | 8.5 ± 0.1 |
| $pK_i$ (SERT) | 9.7 ± 0.2 | ND | ND | ND |

ND (not determined). Data published previously by Scott et al. (2006).

## Functional Studies

In [$^{35}$S]GTP$\gamma$S binding assays (Watson et al. 1996, 2000), SB-649915 displayed no intrinsic functional activity at the h5-HT$_{1A}$ receptor up to concentrations of 1 $\mu$M, but stimulated h5-HT$_{1B}$ receptors with a low degree of intrinsic activity (~0.3 relative to 5-HT (Scott et al. 2006; Table 1). However, SB-649915 inhibited 5-HT-induced responses, at both h5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, in a concentration-dependent, competitive manner with $pA_2$ values of 9.0 ± 0.2 and 7.9 ± 0.1, respectively (Table 1), comparable to its binding affinity at these receptors. Taken together these data demonstrate SB-649915 to be a potent, selective h5-HT$_{1A/B}$ receptor antagonist.

The modulatory action of 5-HT$_1$ autoreceptors on the activity of the serotonergic cell bodies has been well characterized and is attributed to the 5-HT$_{1A}$ receptor subtype. Thus, extracellular single unit recordings in rat dorsal raphe nucleus (DRN) were used to assess the effect of SB-649915 on serotonergic cell body activity. In an *in vitro* study using coronal rat brain slices (containing the DRN) (Corradetti et al. 1998), SB-649915 (1 $\mu$M) was without effect on DRN cell firing (Scott et al. 2006). Given that SSRIs produce a reduction in neuronal activity in this preparation (data not shown), it may have been anticipated that SB-649915 would inhibit cell firing. However, the lack of effect of SB-649915 *per se* may be due to the simultaneous blockade of 5-HT$_1$ autoreceptors and, in particular, the 5-HT$_{1A}$ receptor subtype. In support of this hypothesis, 5-HT$_{1A}$ receptor agonist (+)8-hydroxy-2-(di-n-propylamino) tetralin ((+)8-OH-DPAT)–induced inhibition of cell firing in the DRN is reversed by the 5-HT$_{1A}$ receptor antagonist, WAY100635 (Watson et al. 2000). Consistent with its 5-HT$_{1A}$ receptor antagonist activity, SB-649915 (1 $\mu$M) produced a rightward shift of the concentration response curve to (+)8-OH-DPAT, yielding an apparent $pK_B$ of 9.5 ± 0.2, which was again comparable to its binding affinity for rat native 5-HT$_{1A}$ receptors (Fig. 1, Table 2).

With respect to function of other 5-HT$_1$ receptor subtypes, 5-HT-mediated inhibition of electrically stimulated [$^3$H]5-HT release from guinea pig cortex is reported to be mediated via activation of 5-HT$_{1B}$ autoreceptors (Roberts et al. 1996; Selkirk et al. 1998). Using this experimental paradigm, SB-649915 (1 $\mu$M) significantly attenuated exogenous 5-HT-induced inhibition of release (in a manner similar to that produced by the 5-HT$_{1B/1D}$ receptor

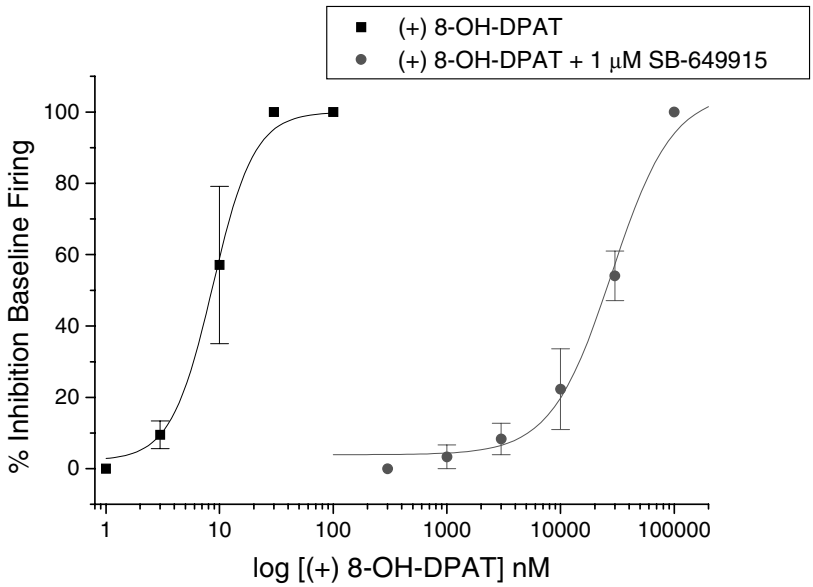


**FIG. 1.** Effect of SB-649915-B on (+)8-OH-DPAT-induced inhibition of cell firing in rat mid-brain slices containing the dorsal raphe nucleus. Data are mean ± S.E.M. from three or more slices. Data published previously by Scott et al. (2006).

partial agonist, GR127935; Watson et al. 1996). Furthermore, under conditions whereby endogenous 5-HT was enhanced via electrical stimulation, SB-649915 potentiated [$^3$H]5-HT release, thus confirming its antagonist activity at guinea pig native terminal 5-HT$_{1B}$ autoreceptors (Fig. 2).

In terms of its inhibitory effect on serotonin uptake, functional studies showed that SB-649915 inhibited [$^3$H]5-HT uptake in a monophasic manner, both in Lewis Lung Carcinoma Porcine tubule epithelial (LLCPK) cells, expressing recombinant hSERT and in a rat cortical synaptosomes, exhibiting pIC$_{50}$s of 7.9 ± 0.1 and 9.7 ± 0.2 (Tables 1 and 2), respectively, demonstrating SB-649915 to be a competitive functional inhibitor of SERT. The difference in potency at human recombinant and native SERT is likely due to differences in parameters such as cell density between the recombinant and native preparations.

Taken together, these *in vitro* functional assays demonstrate SB-649915 to be a highly selective, potent, competitive antagonist at the human and rodent 5-HT$_{1A/B}$ receptors and a potent inhibitor of 5-HT uptake at SERTs.

## *IN VIVO* PHARMACOLOGY

In *ex vivo* binding studies, SB-649915, following oral dosing, caused a dose-dependent inhibition of [$^3$H]WAY100635 binding in rat and guinea pig cortex (Fig. 3). Likewise, SB-649915 inhibited *ex vivo* [$^3$H]5-HT uptake, within the same dose range, in both rat and guinea pig cortex (Fig. 4). These data, therefore, demonstrate that SB-649915 simultaneously occupies 5-HT$_{1A}$ receptors and functionally blocks 5-HT transporters *in vivo*

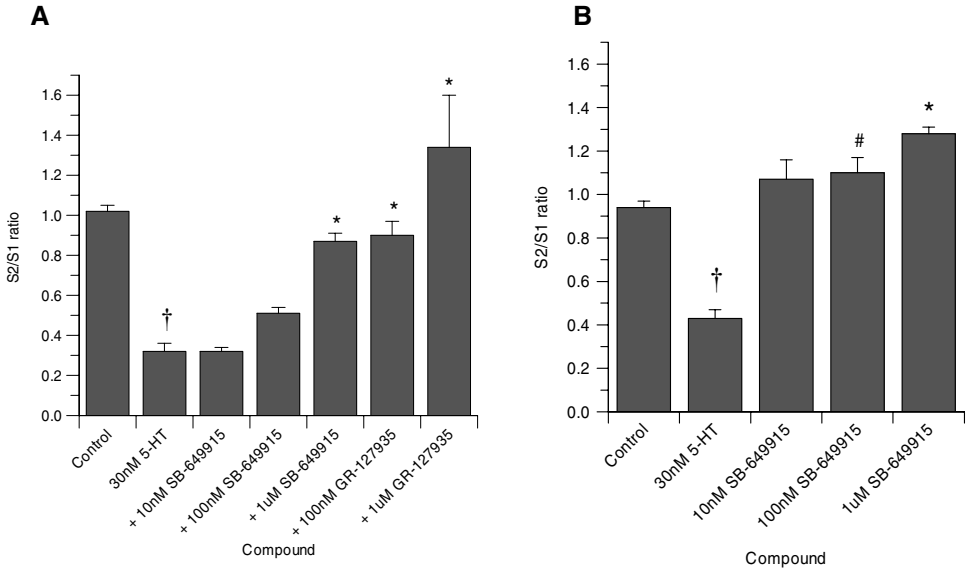


**FIG. 2.** Electrically stimulated [$^3$H]5-HT release from guinea pig cortical slices. (A) Attenuation of exogenous 5-HT-induced inhibition of release (1 Hz, 3 min, 20 mA) $^{\dagger}P < 0.01$ vs. control; $^{*}P < 0.01$ vs. 5-HT, ANOVA with post hoc t-test. After 30 min equilibration (t = 0), slices were stimulated at t = 12 (S1) and t = 56 (S2) min and results expressed as the S2/S1 ratio. Data are the mean of three individual experiments, each performed in duplicate. GR-127935 is a standard 5-HT$_{1B/1D}$ receptor partial agonist. (B) Potentiation (3 Hz, 1 min, 20 mA) of [$^3$H]5-HT release by increasing concentrations of SB-649915 $^{\dagger}P < 0.01$; $^{*}P < 0.01$; $^{\#}P = 0.07$ vs. control, ANOVA with post hoc t-test. Data published previously by Scott et al. (2006).

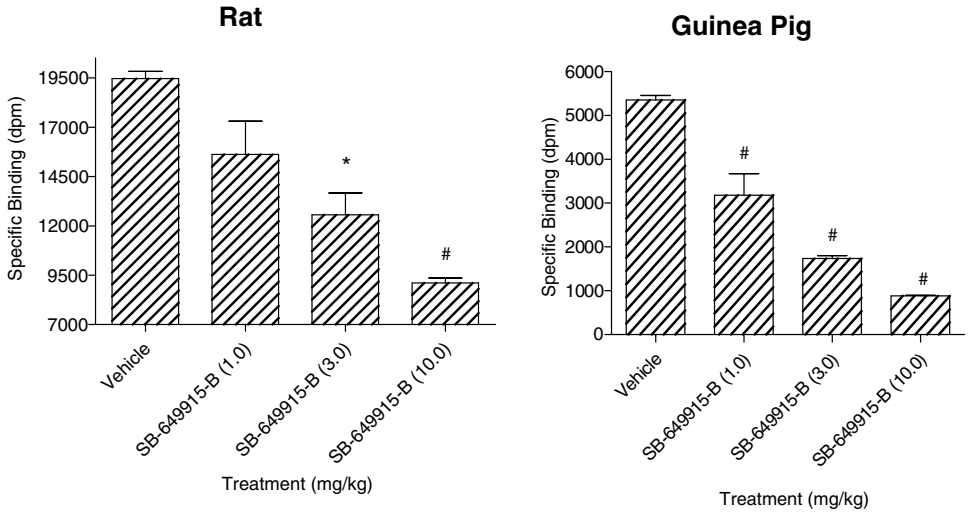


**FIG. 3.** Evaluation of 5-HT$_{1A}$ receptor occupancy by SB-649915: *ex vivo* occupancy measured by displacement of [$^3$H]WAY-100635 from rat and guinea pig cortical homogenates. Following p.o. administration of SB-649915-B (2 h pretreatment), animals were sacrificed and cortical tissue removed for use in [$^3$H]WAY-100635 binding studies (see Scott et al. 2006 for methodology). Data are expressed as mean ± S.E.M. (n = 3–4 per study group). $^{*}P < 0.05$ $^{\#\#}P < 0.01$ vs. vehicle treated animals. ANOVA followed by Duncan's t-test. Data published previously by Hughes et al. (2007).

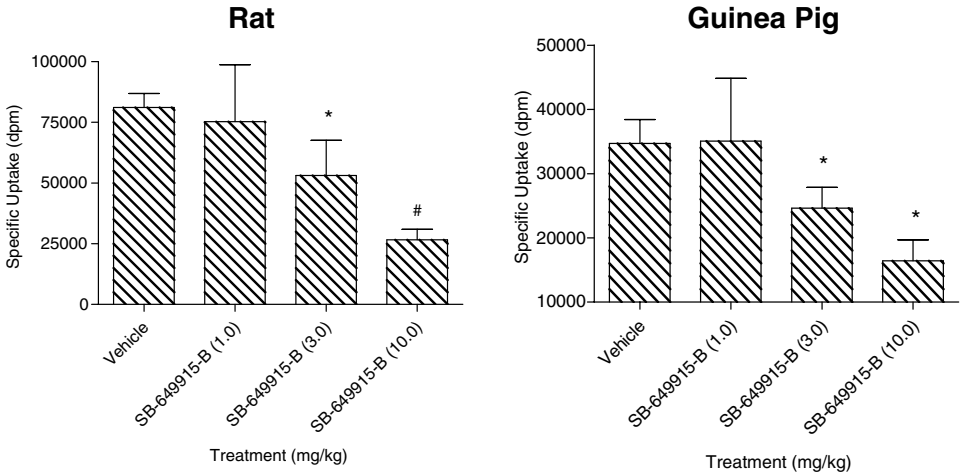


**FIG. 4.** Inhibition of [$^3$H]-5-HT uptake into rat and guinea pig cortical synaptosomes by SB-649915-B (1, 3, and 10 mg/kg p.o.): *ex vivo* assessment of SSRI occupancy. Following p.o. administration of SB-649915-B (2 h, 15 min pretreatment), animals were sacrificed and cortical tissue removed for use in [$^3$H]5-HT studies (see Scott et al. 2006 for methodology). Data are expressed as mean ± S.E.M. (n = 3–4 per study group). $^{*}P < 0.05$ $^{\#\#}P <$ 0.01 vs. vehicle treated animals. ANOVA followed by Duncan's t-test. Data published previously by Hughes et al. (2007).

following oral administration. Unfortunately, a selective 5-HT$_{1B}$ receptor radioligand was not available to ascertain the concurrent 5-HT$_{1B}$ receptor occupancy. Although, based on comparative affinity estimates, one may speculate that 5-HT$_{1B}$ receptor occupancy should be approximately equivalent to that seen at the 5-HT$_{1A}$ receptor in the guinea pig.

To demonstrate functional blockade of 5-HT receptors, *in vivo* SB-649915 was assessed in both 8-OH-DPAT–induced hyperlocomotion in rat, a behavioral model of post-synaptic 5-HT$_{1A}$ receptor function (Forster et al. 1995), and SKF99101 induced increase in maximal electroshock seizure threshold, a pharmacodynamic model of 5-HT$_{1B}$ receptor function in the rat (Stean et al. 2005). SB-649915 reversed both the 8-OH-DPAT–induced hyperlocomotion (Fig. 5A) and inhibited the SKF99101 induced elevations in seizure threshold (Fig. 5B). These data demonstrate, therefore, that SB-649915 is a centrally active 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor antagonist in rat following p.o. administration. ED$_{50}$s for both assays were approximately 3 mg/kg p.o., demonstrating equipotency at both 5-HT$_1$ receptor subtypes.

## Electrophysiological Evaluations

*In vivo* extracellular recordings in the rat DRN revealed SB-649915 to possess a profile of activity similar to that seen *in vitro,* that is, no effect of the compound *per se* but able to produce a dose-related rightward shift in 8-OH-DPAT–induced inhibition of serotonergic cell firing (Fig. 6). Although it appears that the rightward shift shown *in vivo* is less marked than that *in vitro*, factors such as drug exposure and concentration at the receptor will most likely explain this observation. In contrast, SSRIs produce a decrease in raphe cell firing, which can be blocked by 5-HT$_{1A}$ receptor antagonists (Gartside et al. 1995). On the basis of these results it is tempting to speculate that the effects of SSRIs and 8-OH-DPAT are mediated via indirect (as a result of increased local 5-HT) and direct stimulation of

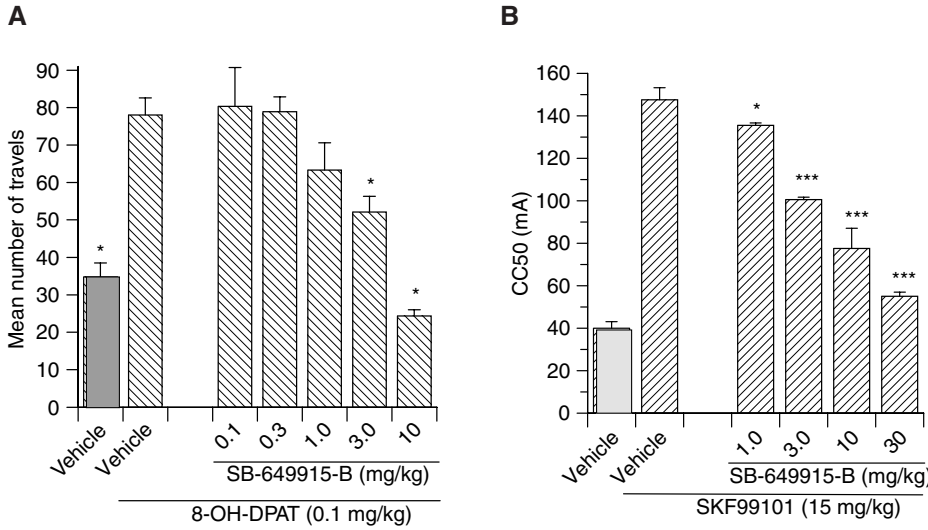


**FIG. 5.** Effect of SB-649915-B on (A) 8-OH-DPAT–induced hyperlocomotion in rat and (B) SKF99101-induced elevation of seizure threshold in the rat maximal electroshock seizure threshold test. SB-649915-B was administered p.o.. Data (A) are expressed as mean ± S.E.M. over a 30-min period (n = 8 per study group), $^{*}P < 0.05$ vs. 8-OH-DPAT alone. ANOVA followed by Duncan's t-test. Data (B) are expressed as current producing tonic hindlimb extensor seizure in 50% of animals ($CC_{50}$) ± S.E.M. values (n = 12 per treatment group). ~ $P < 0.001$ vs. vehicle control; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. SKF99101 alone group. ANOVA followed by Wilcoxon post hoc analysis. Data published previously by Hughes et al. (2007).

somatodendritic 5-$HT_{1A}$ autoreceptors, respectively. In this respect, SB-649915 appears to act as a 5-$HT_{1A}$ autoreceptor antagonist, whereby this activity not only attenuates 8-OH-DPAT–induced inhibition of cell firing but also occludes the increased 5-HT-induced inhibitory effect (resulting from the SERT inhibitory activity of SB-649915). However, it is possible that 5-$HT_{1A}$ receptors, postsynaptic to the raphe neuron, may also contribute to

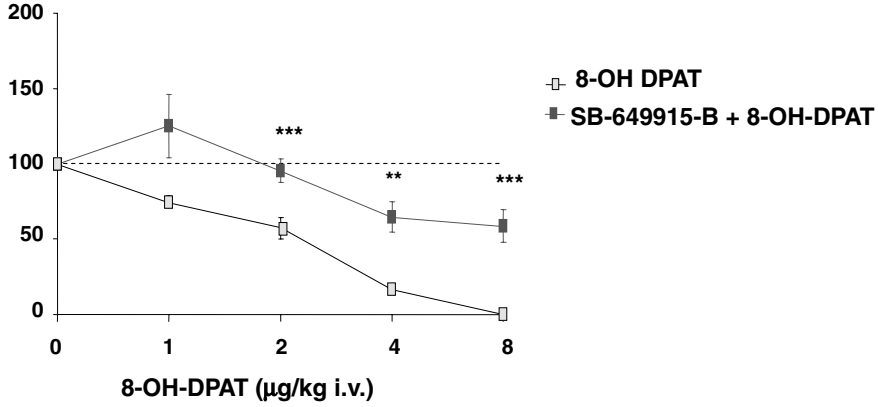


**FIG. 6.** Effect of SB-649915-B on (+)8-OH-DPAT–induced inhibition of cell firing in rat dorsal raphe nucleus *in vivo*. SB-649915-B was administered p.o. Data expressed as a% pre-drug firing rate (mean ± S.E.M., n = 6–8 per group). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle. ANOVA followed by Dunnet's post hoc analysis. Data published previously by Hughes et al. (2007).

the 8-OH-DPAT/SERT–induced effects via an inhibitory feed-back loop (Casanovas et al. 1999) and preclude a definitive identification of the subpopulations of 5-$HT_{1A}$ receptors, which are involved in the effects of SSRIs and SB-649915 *in vivo*.

## Neurochemical Evaluations

*In vivo* microdialysis in the rat revealed that SB-649915 dose-dependently increased extracellular levels of 5-HT in the rat frontal cortex, whereas the SSRI paroxetine had no effect (Fig. 7). Furthermore, SB-649915 did not influence brain norepinephrine levels, consistent with the *in vitro* selectivity profile, over NET, of this molecule. The effects of paroxetine were also in keeping with published data demonstrating that SSRI administration produces no (Bel and Artigas 1993; Gartside et al. 1995; Dawson and Nguyen 1998; Beyer et al. 2002) or moderate (Malagie et al. 1995; Romero and Artigas 1997; Gobert et al. 1997) changes in forebrain 5-HT levels, presumably because of activation of the negative feedback circuitry mediated through terminal and somatodendritic 5-$HT_1$ autoreceptors. Co-administration of an SSRI plus 5-$HT_{1A}$ and 5-$HT_{1B}$ antagonists or a 5-$HT_{1B/D}$ receptor antagonist also produced an augmented increase in 5-HT when compared to the effect of an SSRI alone (Sharp et al. 1997; Roberts et al. 1999; Dawson and Nguyen 1998; Gobert et al. 2000) most likely as a result of blocking 5-$HT_1$ receptor mediated negative feedback. Thus, these data provide neurochemical evidence that SB-649915 acts as a 5-$HT_{1A/B}$ receptor antagonist/SERT inhibitor *in vivo*. Notably, the magnitude of the effects of acute SB-649915

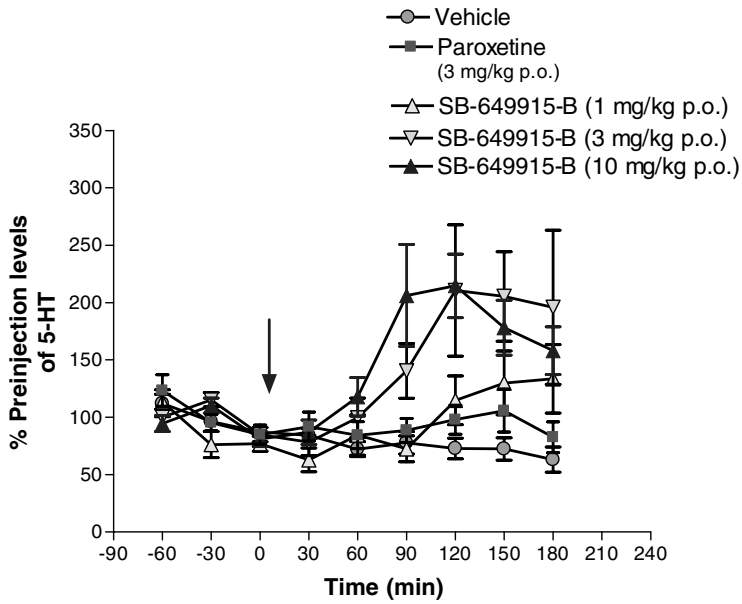


**FIG. 7. Effect of acute administration of SB-649915-B on extracellular 5-HT levels in rat frontal cortex.** SB-649915-B was administered p.o. Data are expressed as mean% preinjection levels ± SEM (n = 9–11 per study group). Arrow denotes time of drug administration. Increases attained significance at 3 ($P < 0.001$) and 10 mg/kg ($P < 0.0001$) vs. vehicle. Two-way ANOVA with repeated measures. Data published previously by Hughes et al. (2007).

on extracellular 5-HT was comparable to that produced by an SSRI following a period of chronic treatment (Roberts et al. 2000; Hughes et al. 2007). This SSRI treatment regimen has been shown to produce a functional desensitization of 5-$HT_{1A}$ (Chaput et al. 1986; Blier et al. 1987; Le Poul et al. 1995; Dawson et al. 2000) and also possibly 5-$HT_{1B}$ receptors (Shalom et al. 2004), although the data for the latter are controversial (Gobbi et al. 1997; for review on both receptors see Hjorth et al. 2000). Thus, a combination of 5-$HT_{1A/B}$ receptor antagonism with SERT inhibition (either as separate molecules or as a single molecule, such as SB-649915) circumvents the need for 5-$HT_1$-receptor desensitization in order to achieve an increase in 5-HT release (Dawson et al. 2000; Hervas et al. 2001) and thus produces acute increases in forebrain 5-HT.

## Animal Models of Acute and Chronic Anxiety

Several acute models of anxiety/despair, such as the mouse forced swim test (Porsolt et al. 1977a, 1997b), Vogel conflict (Dekeyne et al. 2000), pup maternal separation (Gardner 1985) and social interaction (Dekeyne et al. 2000; Duxon et al. 2000), have been shown to be predictive for clinical efficacy in affective disorders. Of these, the rat pup vocalization model was chosen to assess the acute anxiolytic-like efficacy of SB-649915. Following separation from their mothers, rat pups emit ultrasonic vocalizations, which can be attenuated by acutely administered anxiolytic agents, such as benzodiazepines and SSRIs (Gardner 1985; Olivier et al. 1998). SB-649915 reduced vocalizations in a dose-related manner to a degree comparable to that of the SSRI, fluoxetine (Fig. 8; Starr et al. 2007).

Based on these positive findings, the anxiolytic activity of SB-649915 was subsequently assessed in a primate species, the common marmoset, in the human threat test model. When confronted by a human in proximity to their home cage, marmosets show a series of

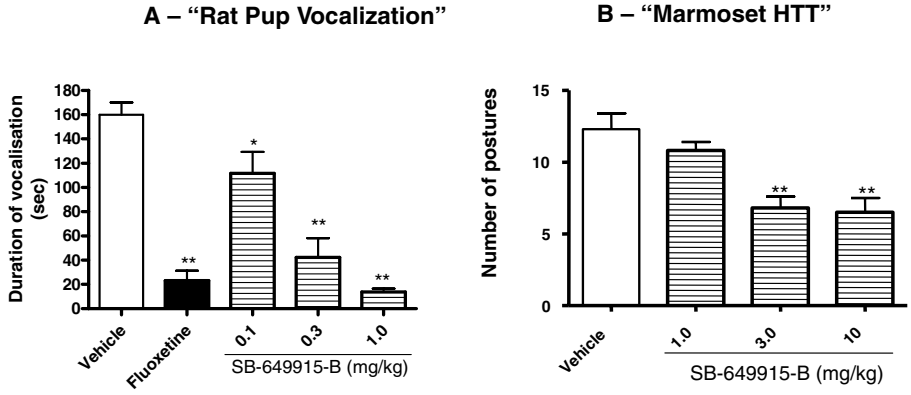


**FIG. 8.** Effects of SB-649915-B on ultrasonic vocalizations in rat pups (A) and in the human threat test (HTT) in marmosets (B). (A) SB-649915-B (0.1, 0.3, and 1.0 mg/kg) was administered i.p. 30 min before test. Data are expressed as mean duration of vocalization (sec) ± S.E.M. (n = 6–8 per group). SB-649915-B (0.3 and 1.0 mg/kg, i.p.) and fluoxetine significantly reduced vocalization compared to Vehicle. $^{**}P < 0.01$ from vehicle-treated animals (ANOVA followed by Dunnett's). (B) SB-649915-B (1.0, 3.0, and 10 mg/kg) was administered s.c. 360 min before test. Data are expressed as mean number of postures and jumps ± S.E.M. (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 (Student's t-test for paired group). Data published previously by Starr et al. (2007).

behavioral postures that are considered to be directly related to the level of anxiety experienced (Costall et al. 1988a). These postures can be reduced by administration of anxiolytic agents at doses that have no sedative effects, as assessed by decreases in the number of jumps made by the marmoset (Costall et al. 1988a, 1988b). SB-649915 significantly reduced the number of postures (Fig. 8) without producing any confounding effects on locomotor activity (data not shown; Starr et al. 2007). These data suggest that SB-649915 possesses an anxiolytic profile in both a rodent and primate following acute dosing and encouragingly indicate that the presence of 5-$HT_{1A}$-receptor antagonism does not adversely affect the SSRI response in these paradigms. However, these acute models are limited in that they do not temporally represent the clinical scenario where SSRIs take several weeks to achieve therapeutic efficacy.

Several preclinical models [e.g., elevated plus maze, social defeat model, reversal of cholecystokinin, or corticotropin releasing hormone (CRH)-induced anxiety in the social interaction (SI)], are, however, able to model the delay in onset to anxiolytic/antidepressant activity that is evident in the clinic (Griebel et al. 1994, 1995; Berton et al. 1999; To and Bagdy 1999; To et al. 1999). Notably, 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. Subsequent studies (Duxon et al. 2000) further demonstrated that the concomitant administration of paroxetine with WAY100635 reduced the latency to onset of anxiolytic behavior from 21 to 7 days. In light of these data, the SI model was used to examine the temporal profile of SB-649915–induced anxiolysis. SB-649915 dosed for 7 days significantly increased SI with no effect on locomotor activity (data not shown; Starr et al. 2007); an effect that was still evident following 21 days dosing (Fig. 9). SI was also monitored following 4 days of dosing, but there was no significant effect of SB-649915 at this earlier time-point. In comparison chronic (21 days), but not subchronic (4 and 7 days), treatment with the SSRI, paroxetine, increased SI (Fig. 9); findings in accordance with previous data generated under the same experimental conditions (Lightowler et al. 1994; Duxon et al. 2000). Concurrent *ex vivo* estimates of SERT occupancy (Fig. 9) revealed a dose-related increase in occupancy with SB-649915 that did not change with duration of administration. Furthermore, paroxetine-induced SERT occupancy was approximately equivalent to that produced by SB-649915, suggesting that the different temporal profiles of anxiolytic activity of paroxetine and SB-649915 were not directly related to SERT occupancy.

If the hypothesis that antidepressant efficacy is a consequence of enhanced serotonergic neurotransmission (Delgado et al. 1990) is correct, and the same hypothesis holds for preclinical models, such as SI, then it is tempting to speculate that hastened onset of efficacy of SB-649915 results from a greater acute increase in serotonergic neurotransmission in forebrain structures compared to that produced by paroxetine. Interestingly, however, although SB-649915 produced an acute increase in serotonergic neurotransmission, it still did not produce efficacy following 4 days of repeated administration in the high light SI paradigm. Furthermore, although efficacy was observed in the rat pup vocalization model and the marmoset threat test following acute administration of SB-649915-B, these assay systems are also sensitive to acute SSRI treatment, which acutely produce no (Bel and Artigas 1993; Gartside et al. 1995; Dawson and Nguyen 1998; Beyer et al. 2002) or moderate (Malagie et al. 1995; Romero and Artigas 1997; Gobert et al. 1997) increases in extracellular levels of serotonin as measured by microdialysis. That said, since this technique is relatively insensitive to subtle changes in synaptic neurotransmission, it is plausible

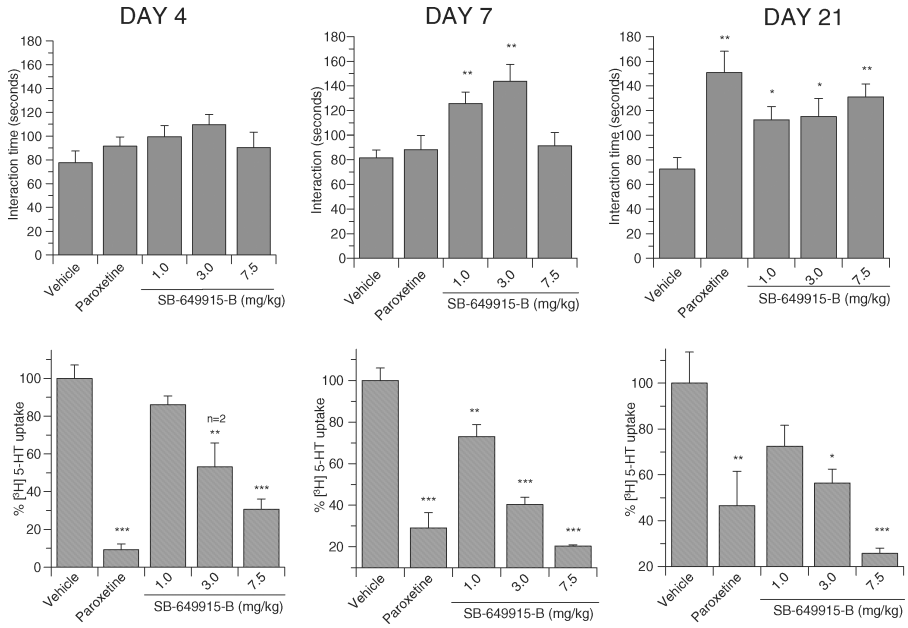


**FIG. 9.** Effect of subchronic (4 and 7 days) and chronic (21 days) treatment of SB-649915-B in the rat high light social interaction test vs. *ex vivo* SERT occupancy. SB-649915-B was administered p.o. Social interaction scores are shown in the upper panel (solid bars) and *ex vivo* SERT occupancy (determined by inhibition of [$^3$H] 5-HT uptake) are represented in the lower (striped bar) panel. Data are presented as mean interaction time (sec) ± S.E.M. and mean% occupancy ± S.E.M. (n = 12–14). *, ** 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 social interaction; Dunnett's 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 (data not shown). SB-649915-B significantly occupied SERT compared to vehicle. $^*P < 0.05$, $^{**}P < 0.01$ from vehicle-treated controls. Data published previously by Starr et al. (2007).

that subtle enhancement in synaptic transmission may be sufficient to overcome the relatively mild anxiogenic stimuli of these experimental models. The chronic nature of the anxiogenic stimuli in the SI paradigm (potentially more akin to anxiety/depression?) is clearly less easily overcome and thus requires a more sustained increase in serotonergic neurotransmission that presumably results in downstream plastic adaptation. The specific substrates of these 5-HT-mediated adaptive changes have been the topic of a number of published reports (Bianchi et al. 2005; Yamada et al. 2005; Celine et al. 2006), so they will not be reviewed here.

## THERAPEUTIC IMPLICATIONS

It is difficult to make specific clinical therapeutic claims based on preclinical behavioral models. However, assuming that antidepressant/anxiolytic efficacy is a consequence of enhanced serotonergic neurotransmission (Delgado et al. 1990), then the neurochemical profile of SB-649915 suggests that it should provide therapeutic utility in the treatment of affective disorders, such as depression and anxiety. Furthermore, since clinical

efficacy is delayed with current SSRI treatments, it is possible that the immediate nature of SB-649915–induced neurochemical effects (in multiple brain regions) would result in a faster onset of therapeutic activity; a suggestion that is supported by the data generated in the SI studies, a model thought to be predictive of the onset of anxiolytic/antidepressant activity (Duxon et al. 2000). Furthermore, an independent study using a different paradigm (i.e., scheduled-induced polydipsia [Hogg and Dalvi 2004]) also substantiates the concept that SSRI inhibition combined with 5-$HT_1$ autoreceptor blockade can produce a faster onset to anxiolytic/antidepressant-like efficacy. Importantly, this hypothesis is supported by numerous clinical studies that have demonstrated an acceleration to onset of antidepressant activity, and, potentially also augmentation of efficacy, with combined administration of (±)pindolol with various SSRIs (for reviews see McAskill et al. 1998; Artigas et al. 2001). It should be noted, however, that there are also a number of reports that contradict this combination strategy. These inconsistencies could be a direct result of the use of pindolol as the tool compound, which is not the ideal molecule to use clinically as a 5-$HT_{1A}$ receptor antagonist given it has 5-$HT_{1A}$ receptor partial agonist activity. More specifically the dose-limiting side effects of pindolol, likely mediated by its $\beta$-adrenergic receptor blocking activity, also limit the level of 5-$HT_{1A}$ receptor occupancy that can be achieved (Rabiner et al. 2001). A molecule, such as SB-649915, which has high affinity and selectivity for 5-$HT_1$ autoreceptor and SERT should not be hindered by such dose limitations. Selective 5-$HT_{1B}$ receptor antagonists, with potency similar to that of SB-649915, have been developed as anxiolytic/antidepressant agents (Hudzig et al. 2003; Dawson et al. 2006) and are currently undergoing clinical evaluation in the belief that this activity may contribute or even augment clinical efficacy. Thus, the hypothesis that simultaneous blockade of 5-$HT_1$ autoreceptors and SERT may result in a fast onset of therapeutic action is compelling and the preclinical evidence provides strong support; this hypothesis ultimately has to be proven clinically. To this end, we await clinical evaluation of this and potentially other molecules with similar pharmacology with interest.

## SUMMARY

In summary, SB-649915 is a novel and selective SERT inhibitor and 5-$HT_{1A/B}$ receptor antagonist. SB-649915 occupies 5-$HT_{1A}$ receptors and functionally blocks 5-$HT_{1A}$ and 5-$HT_{1B}$ receptors and the 5-HT transporter *in vivo* following oral administration. Acute administration of SB-649915 results in significant increases in extracellular 5-HT in forebrain structures of rodents. These increases were greater than acute SSRI-induced effects, but comparable to that induced by an SSRI following a period of chronic treatment. SB-649915 was acutely anxiolytic in both rat and non-human primates and produced evidence of hastened onset of efficacy in the rat highlight SI paradigm. Based on these preclinical data this dual activity molecule should have therapeutic efficacy in the treatment of affective disorders and a potential for a fast onset of action.

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