Selective Blockade of 5-Hydroxytryptamine (5-HT)\textsubscript{7} Receptors Enhances 5-HT Transmission, Antidepressant-Like Behavior, and Rapid Eye Movement Sleep Suppression Induced by Citalopram in Rodents

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ABSTRACT

Evidence has accumulated supporting a role for 5-hydroxytryptamine (5-HT)\textsubscript{7} receptors in circadian rhythms, sleep, and mood disorders, presumably as a consequence of the modulation of 5-HT-mediated neuronal activity. We hypothesized that a selective 5-HT\textsubscript{7} receptor antagonist, (2\textsuperscript{R})-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]-pyrrolidine (SB-269970), should increase activity of 5-HT neurons and potentiate the effect of selective serotonin reuptake inhibitors (citalopram). In rats, administration of 3 mg/kg s.c. citalopram alone increased the extracellular concentration of 5-HT. This effect of citalopram on extracellular 5-HT concentration was significantly enhanced by an ineffective dose of SB-269970. Combining this dose of SB-269970 with a low dose of citalopram also resulted in a significant increase in extracellular concentration of 5-HT, suggesting a potentiation of neurochemical effects. In mice, citalopram and SB-269970 dose-dependently decreased immobility time in the tail suspension test. The dose-effect curve of citalopram was shifted leftward by coadministration of an effective dose of SB-269970. Furthermore, combining ineffective doses of citalopram and SB-269970 also resulted in a significant decrease of immobility time in the tail suspension test, suggesting potentiation of antidepressant-like effects. In rats, SB-269970 potentiated the increase of rapid eye movement (REM) latency and the REM sleep decrease induced by citalopram. SB-269970 also reversed the increase in sleep fragmentation induced by citalopram. Rat plasma and brain concentrations of citalopram were not affected by coadministration of SB-269970, arguing for a pharmacodynamic rather than a pharmacokinetic mechanism. Overall, these results indicate that selective blockade of 5-HT\textsubscript{7} receptors may enhance the antidepressant efficacy of citalopram and may provide a novel therapy to alleviate sleep disturbances associated with depression.

The 5-HT\textsubscript{7} receptor is the most recently described member of the large family of serotonin receptors of which 14 members have been described to date (Bard et al., 1993; Lovenberg et al., 1993; Hedlund and Sutcliffe, 2004). Functionally, in vitro activation of the 5-HT\textsubscript{7} receptor has been shown to increase cyclic AMP formation. In the rodent and human brain, the highest receptor densities were found in the thalamus, hypothalamus (including the suprachiasmatic nucleus), amygdala, hippocampus, cortex, and dorsal raphe (To et al., 1995; Thomas et al., 2002; Varnas et al., 2004). The 5-HT\textsubscript{7} receptor has also been detected in the periphery where it is found primarily in smooth muscle cells of blood vessels (Bard et al., 1993) but also in the gastrointestinal tract where it is involved in peristalsis (Tuladhar et al., 2003).

In the central nervous system, important physiological roles for the 5-HT\textsubscript{7} receptor have been established in thermoregulation and circadian rhythmicity (Lovenberg et al., 1993; Hagan et al., 2000; Glass et al., 2003; Guscott et al., 2003; Hedlund et al., 2003). Recent data suggest that there is a complex interaction between 5-HT\textsubscript{7} receptor and glutamatergic neurons in the raphe nuclei that influences the activity of the 5-HT\textsubscript{7} neurons (Harsing et al., 2004). It has been speculated that the axon terminals of the glutamatergic corticoraphe neurons may possess 5-HT\textsubscript{7} receptors. Activation of

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); SB-269970, (2\textsuperscript{R})-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]-pyrrolidine; SSRI, selective serotonin reuptake inhibitor; EEG, electroencephalogram; DA, dopamine; AUC, area under the curve; ANOVA, analysis of variance; EMG, electromyogram; NREM, nonrapid eye movement; CV%, coefficient of variation; NE, norepinephrine; Veh, vehicle; AS19, (2S)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin; GR205171, 2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl[2(S)-phenyl-piperidin-3S-y]-amine HCl; M100907, (+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol.
these 5-HT\textsubscript{7} receptors inhibits glutamate release, which consequently leads to decreased activity of serotonergic neurons. Therefore, blockade of 5-HT\textsubscript{7} receptor might lead to an enhanced release of glutamate in the raphe with a consequent increase in the activity of 5-HT neurons. This is supported by preliminary electrophysiological data showing that AS19, a 5-HT\textsubscript{7} agonist, inhibited the firing frequency of dorsal raphe 5-HT neurons (Faure et al., 2006). This effect was prevented by SB-269970, a selective 5-HT\textsubscript{7} antagonist (Hagan et al., 2000).

Early indications of an involvement of 5-HT\textsubscript{7} receptors in mood disorders came from a study showing down-regulation of 5-HT\textsubscript{7} receptor expression after chronic treatment with various antidepressants (Mullins et al., 1999). Recent studies further support a role for 5-HT\textsubscript{7} receptors in depression. Hence, SB-269970, like classic selective serotonin reuptake inhibitors (SSRIs), decreased immobility in both tail suspension and forced swim test, two tests widely used as predictor of antidepressant activity (Guscott et al., 2005; Hedlund et al., 2005; Faure et al., 2006; Wesolowska et al., 2006a,b). In agreement with these pharmacological data, 5-HT\textsubscript{7} knockout mice showed reduced immobility in both the forced swim and the tail suspension tests (Hedlund et al., 2005).

Interestingly, both SSRIs and 5-HT\textsubscript{7} antagonists have been shown to induce changes in sleep parameters in rats in a pattern opposite from those in patients with clinical depression. More specifically, in rats, selective 5-HT\textsubscript{7} receptor-selective antagonists and SSRIs when administered at the beginning of the sleep phase increased the latency to rapid eye movement (REM) sleep and decreased the amount of time spent in REM sleep (Hagan et al., 2000; Thomas et al., 2003; Hedlund et al., 2005). Consistent with these observations, 5-HT\textsubscript{7} knockout mice spent less time in and had less frequent episodes of REM sleep (Hedlund et al., 2005).

A delayed onset of action and high percentage of nonresponders are the major drawbacks of SSRIs (Hamon and Bourgon, 2006; Kennedy, 2006). A number of combination therapies have been tested with SSRIs, including, for example, 5-HT\textsubscript{1A} antagonists, with mixed clinical results (Adell et al., 2005; Hamon and Bourgon, 2006).

Combining a lower dose of citalopram with a 5-HT\textsubscript{7} antagonist might accelerate the onset of action and minimize the side effect profile. The combination might also be very useful for patients who either do not respond to classic antidepressant treatment or in whom SSRI monotherapy provides insufficient efficacy. Moreover, blockade of 5-HT\textsubscript{7} receptor might help restore altered sleep pattern and circadian rhythms. In the present study, we investigated the ability of a 5-HT\textsubscript{7} receptor antagonist, SB-269970, to augment the effect of a selective serotonin reuptake inhibitor, citalopram, on 5-HT transmission, antidepressant-like behavior, and REM sleep suppression in rodents.

Materials and Methods

Animals. All the studies have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The tail suspension test and locomotor activity measurements were performed in male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) weighing from 22 to 30 g. The microdialysis, blood-brain barrier penetration, and sleep/electroencephalogram (EEG) experiments were performed in male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA for microdialysis; Harlan, Indianapolis, IN for sleep EEG) weighing from 280 to 320 g (microdialysis) or from 400 to 500 g (sleep EEG).

Animals were allowed to acclimate for at least 7 days before investigations after receipt in the facility. They were housed in accordance with institutional standards, and they were provided food and water ad libitum and maintained on a 12-h light dark cycle (lights on from 6:00 AM to 6:00 PM).

Microdialysis. Each rat was given a 0.05-ml s.c. injection of Buprenex (buprenorphine hydrochloride) (Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA) at 0.06 mg/kg 5 min before anesthesia. Animals were anesthetized with an isoflurane/air mixture, and then they were stereotaxically implanted with a guide cannula (Eicom, Kyoto, Japan) in the prefrontal cortex (incisor bar, −3.5 mm, +3.2 mm anterior, 0.8 mm lateral, and 1 mm ventral to bregma) (Paxinos and Watson, 1997). The guide cannula was secured in place with skull screws and dental cement. Animals were allowed at least 4 days to recover from surgery before experimentation.

Dialysis experiments were conducted between 8:00 AM and 3:00 PM in a controlled environment with animals remaining in their home cage throughout experimentation. Dialysis probes (4-mm active membrane length; Eicom) were perfused with arterial cerebrospinal fluid (147 mM NaCl, 4 mM KCl, 0.85 mM MgCl\textsubscript{2}, and 2.3 mM CaCl\textsubscript{2}, pH 7.4) at a flow rate of 1 μl/min, and probes were implanted the afternoon before sample collection. The probe was connected via fluorinated ethylene-propylene tubing to a liquid swirl (QM; Insteck Laboratories, Plymouth Meeting, PA) mounted on a counterbalance arm. The following morning, 2 h of baseline samples were collected into a 96-well plate (96-well multiply polymerase chain reaction; Sarstedt, Newton, NC) via a four-channel fraction collector (Eicom). Samples were collected every 30 min for 6 h into the 96-well plate maintained at 4°C containing 7.5 μl of the antioxidant (0.1 M acetic acid, 1 mM oxalic acid, and 3 mM L-cysteine in sterile water). Samples were split into two plates, and they were analyzed immediately after each experiment.

Dialysis samples were analyzed for 5-HT and DA by high-performance liquid chromatography with electrochemical detection. Ten-microliter sample aliquots were injected by a refrigerated autosampler (model 540; ESA, Chelmsford, MA), and separation was achieved using an Eicompak PP-OĐS column (4.6 mm i.d. × 30 mm; Eicom) with the potential of the graphite electrode set to +400 mV against the Ag/AgCl reference electrode. The mobile phase consisted of 100 mM sodium phosphate buffer, pH 6.0, 500 mg/l decanesulfonic acid, 50 mg/l EDTA, and 1% (v/v) methanol. The limit of detection for both 5-HT and DA was 0.01 pg/μl.

Norepinephrine detection was achieved by high-performance liquid chromatography-electrochemical detection using an Eicom pack CA-50DS column (2.1 mm i.d. × 150 mm; Eicom) with the potential of the graphite electrode (Eicom) set to +450 mV against the Ag/AgCl reference electrode. Ten-microliter sample aliquots were injected by a refrigerated autosampler (model 540; ESA). The mobile phase consisted of 100 mM sodium phosphate buffer, pH 6.0, 400 mg/l octanesulfonic acid, 50 mg/l EDTA, and 5% (v/v) methanol. The limit of detection for NE was 0.01 pg/μl.

The concentration for each sample was calculated from the peak area of the chromatographic signal and the slope from the corresponding standard curve. The percentage of change from baseline values was calculated from the mean basal value of each neurotransmitter for each animal and is presented in the figures as mean ± S.E.M. The area under the curve (AUC) values were calculated by the summation of the difference between each neurotransmitter postdrug administration and the mean percentage of basal release value (100%). Statistical analyses were performed on the AUC values by a one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. The level of significance was P < 0.05. Data were graphed, and statistics were calculated using Prism software (GraphPad Software Inc., San Diego, CA).
Tail Suspension Test. Mice were dosed 30 min before testing. A piece of tape was adhered to the upper middle of the tail of each animal, creating a flap with the overlap of tape. One strand of size 0 polyester suture with attached needle was tied to a previously calibrated force transducer (MLT500/D; ADInstruments, Colorado Springs, CO). The needle end of the suture hung down from the force transducer, and it was inserted through the tape flap of each animal. All mice were hung face down from the force transducer in this manner for 6 min. During the test, a Power Lab (ADInstruments) recorded the data from the force transducer via a bridge amplifier. The data were accessible using Chart 4 (ADInstruments) software. Time struggling and time immobile were differentiated by hand scoring of the force versus time trace using the Chart 4 software. The time spent immobile was totaled for the last 4 min of the 6-min test for each animal, averaged for the dose group, and then compared. Statistics were calculated using Prism software (GraphPad Software Inc.). The data were presented as the means ± S.E.M. and evaluated by one-way ANOVA followed by Newman-Keuls multiple comparison test. The level of significance was P < 0.05.

Locomotor Activity. Locomotor activity was measured with a Motor Monitor infrared beam system equipped with a SmartFrame cage rack (Hamilton Kinder, Poway, CA). Each cage rack is equipped with a two-dimensional 4 × 8 beam grid that divides the frame in 32 areas. Fine movements (i.e., beams breaks within each area), horizontal activity (i.e., X and Y beam breaks between areas), and vertical activity (rearing or Z beam breaks between areas) can be recorded. Clean test cages are positioned into the apparatus. The dependent variable was basic movements, defined as the sum of fine movements and horizontal activity.

Locomotor activity (i.e., basic movements) data were collected using Motor Monitor System software (Hamilton Kinder) and exported into Microsoft Excel (Microsoft, Redmond, WA) for further analysis. Spontaneous locomotor activity data were expressed as mean locomotor activity ± S.E.M. To quantify any statistical difference, one-way ANOVA followed by Dunnett’s test was conducted using Prism software (GraphPad Software Inc.). The level of significance was P < 0.05.

Sleep EEG. For the determination of EEG/electromyogram (EMG) waveforms, two stainless steel screw electrodes for EEG (frontal and parietal cortex) and wire electrodes for EMG (dorsal neck muscles) were implanted in each animal under isofluorane anesthesia. Electrodes were connected to a sterile two-channel telemetry device (TL10M3-F50-EET; Data Sciences International, St. Paul, MN) that had been implanted in the intraperitoneal cavity. The animals were allowed to recover for 2 weeks after surgery, and then they were moved to their designated housing/procedure room to allow for adaptation to the recording chamber and environment. On the designated day the animals were to receive vehicle or compound administration, telemetry devices were activated in each animal 10 min before injection. EEG/EMG traces were recorded on an IBM PC-compatible computer using Dataquest A.R.T software (Data Sciences International) at a sampling rate of 100 Hz. Polysomnographic waveforms were recorded for 6 h postinjection.

Using the computer program SleepSign (Kissié Comte, Nagano, Japan), consecutive EEG/EMG recordings were divided into 10-s epochs. Vigilance states were visually assigned to these individual epochs using the conventional criteria for wake (less regular low-amplitude EEG, EMG activation), nonrapid eye movement (NREM) sleep [high-amplitude EEG waves with predominant frequency in the delta range (0.5–5 Hz)] and lack of body movement, and REM sleep [stable low-amplitude EEG, dominance of theta activity (5.1- to 8-Hz frequency range), with general EMG atonia].

After scoring the EEG/EMG traces, analysis and compilation of the raw data files were executed by an in-house sleep analysis program. Using a fully customizable computer script in conjunction with the software program R, several parameters relating to the architecture (time spent in wake, NREM, or REM), continuity, and consolidation of the sleep/wake cycle (i.e., bout analysis) were evaluated. A bout was defined as at least two consecutive epochs, 20 s, of wake, NREM, or REM. Microarousals from sleep (NREM or REM sleep bout interrupted by a 10-s epoch of wake) and awakenings from NREM (number of transitions between a NREM bout to a bout of wake) were also measured.

Results were averaged, and they are expressed as mean ± S.E.M. in defined time intervals. To determine whether differences were significant at a given interval, either a one-way ANOVA with Newman-Keuls post hoc analysis or two-way repeated measures ANOVA followed by a Bonferroni post hoc test was executed. Differences were determined to be significant if P < 0.05.

Pharmacokinetics and Bioanalysis. Dosing was followed by blood sampling via cardiac puncture over a time course. Brains were removed from the animals and homogenized for liquid chromatography-tandem mass spectrometry analysis. All blood samples were deproteinized with 1:4 dilution of the sample with acetonitrile with vigorous mixing. These samples were incubated for 5 min, and then they were centrifuged at 14,000 rpm in a microcentrifuge for 4 min. The supernatant was recovered into autosampler vials and diluted 1:1 with sterile water. Samples were analyzed by liquid chromatography-tandem mass spectrometry analysis. A Vydac SP C18 (2.1- × 50-mm) analytical column was used for separation.

Statistics (paired r test) were calculated using Prism software (GraphPad Software Inc.). The level of significance was P < 0.05. A one-compartmental pharmacokinetic model was also applied to these data using the software package WinNonlin version 4.0.1 (Pharsight, Palo Alto, CA). The model that was used was a one-compartment first-order, no lag time, first-order elimination model (model 3). The parameters of the model were optimized using least-squares nonlinear regression. Pharmacokinetic and blood-brain barrier penetration parameters are given as the means ± coefficient of variation (CV%). The CV% is a measure of dispersion of a probability distribution. It is defined as the ratio of the standard deviation to the mean. The CV% was calculated as the ratio of the standard error for each parameter to its estimated value.

Drugs. SB-269970 (HCI salt) and citalopram (HBr salt) were purchased from Sigma-Aldrich (St. Louis, MO). All doses are expressed as free base. For rat studies (microdialysis, sleep EEG, and pharmacokinetics studies), all compounds were formulated in 5% dextrose and delivered s.c. in a volume of 1 ml/kg body weight. For mouse studies (tail suspension and locomotor activity studies), all compounds were formulated in 5% dextrose and delivered i.p. in a volume of 10 ml/kg. Coadministration refers to two separate injections (one injection of SB-269970 and one injection of citalopram or vehicle) in immediate succession.

Results

Effect of SB-269970 on Citalopram-Induced 5-HT Release in Rat Frontal Cortex. Absolute basal levels of 5-HT, DA, and NE in dialysate from the rat frontal cortex (without adjusting for probe recovery) were 0.046 ± 0.002 (n = 30), 0.120 ± 0.008 (n = 30), and 0.215 ± 0.007 pg/μl (n = 30), respectively.

Subcutaneous administration of 10 mg/kg SB-269970 alone did not increase extracellular 5-HT concentration compared with vehicle-treated rats (Fig. 1, A and B). Administration of 3 mg/kg s.c. citalopram alone significantly increased the extracellular concentration of 5-HT in rat prefrontal cortex compared with vehicle-treated rats (P < 0.001 versus vehicle + vehicle; Fig. 1, A and B). This effect of citalopram on extracellular 5-HT concentration was significantly enhanced by coadministration of SB-269970 (P < 0.01 versus vehicle + citalopram; Fig. 1, A and B). An additional experiment was performed using a low dose of citalopram (0.05 mg/kg s.c.) that slightly increased (P < 0.05 versus vehicle + vehicle)
extracellular concentration of 5-HT (Fig. 2, A and B). Coadministration of a low dose of citalopram (0.05 mg/kg s.c.) with SB-269970 (10 mg/kg s.c.) also resulted in a significant increase in extracellular concentration of 5-HT (P < 0.05 versus vehicle + citalopram; Fig. 2, A and B). Neither citalopram (0.05 or 3 mg/kg s.c.), SB-269970 (10 mg/kg s.c.), nor coadministration of citalopram and SB-269970 induced significant change in extracellular concentration of DA or NE (data not shown).

Interaction of SB-269970 and Citalopram in Mouse Tail Suspension Test. SB-269970, administered i.p., at doses of 3, 10, and 30 mg/kg i.p. was not significantly different from that of citalopram (5 mg/kg i.p.) (Fig. 3A). Citalopram, administered at dose of 3, 5, and 10 mg/kg i.p., significantly decreased the immobility time compared with vehicle-treated mice by 35 (P < 0.01 versus vehicle), 54 (P < 0.001 versus vehicle + vehicle), and 79% (P < 0.001 versus vehicle + vehicle), respectively (Fig. 3B). The efficacy of SB-269970 used at doses of 3, 10, and 30 mg/kg i.p. was not significantly different from that of citalopram (5 mg/kg i.p.) (Fig. 3A). Citalopram, administered at dose of 3, 5, and 10 mg/kg i.p., significantly decreased the immobility time compared with vehicle-treated mice by 35 (P < 0.01 versus vehicle + vehicle), 54 (P < 0.001 versus vehicle + vehicle), and 74% (P < 0.001 versus vehicle + vehicle) (Fig. 3B). Coadministration of SB-269970 (10 mg/kg i.p.) and citalopram (3, 5, and 10 mg/kg i.p.) significantly decreased the immobility time compared with vehicle-treated mice by 55 (P < 0.001 versus vehicle + vehicle), 58 (P < 0.001 versus vehicle + vehicle), 68
At 1 and 3 mg/kg, the effect of citalopram on immobility time was significantly enhanced by coadministration of 10 mg/kg SB-269970 (20% versus 55%, \( P < 0.001 \) versus vehicle citalopram and 35% versus 58%, \( P < 0.05 \) versus vehicle citalopram, respectively) (Fig. 3B).

An additional experiment was performed with a combination of ineffective doses of citalopram and SB-269970 (Fig. 3C). Citalopram (1 mg/kg i.p.) or SB-269970 (1 mg/kg i.p.) did not significantly change immobility time compared with vehicle (Fig. 3C). In contrast, coadministration of citalopram (1 mg/kg i.p.) and SB-269970 (1 mg/kg i.p.) significantly decreased the immobility time versus vehicle-treated animals (51%; \( P < 0.001 \) versus vehicle + vehicle) (Fig. 3C).

SB-269970 (1 mg/kg i.p.) and citalopram (1 mg/kg i.p.) did not significantly change locomotor activity compared with vehicle (\( P > 0.05 \) versus vehicle + vehicle; Fig. 4); in addition, coadministration of SB-269970 and citalopram also did not significantly change motoric function (Fig. 4). Likewise, coadministration of a higher dose of SB-269970 (10 mg/kg i.p.) with citalopram (1 mg/kg i.p.) did not significantly change motoric function (\( P > 0.05 \) versus vehicle + vehicle; data not shown).

Sleep-Wake States in Rats following the Coadministration of SB-269970 and Citalopram. To determine the sub efficacious dose of test reagents, a pilot study was conducted with two doses for each compound, citalopram (1 and 3 mg/kg) and SB-269970 (10 and 30 mg/kg). Results of this initial study identified the dose for citalopram (1 mg/kg) and SB-269970 (10 mg/kg) that elicited minimal effects on EEG sleep parameters in rats for the current study.

At the doses tested, citalopram and SB-269970 injected either alone or in combination did not influence the latency to the first episode of NREM sleep or the total time spent in NREM sleep during the 6-h recording period (data not shown). As shown in Fig. 5A, administration of citalopram induced a significant increase of REM sleep latency (\( P < 0.05 \)), whereas SB-269970 had no effect compared with vehicle treatment (Fig. 5A). The combination of citalopram with SB-269970 significantly delayed the onset of the first episode of REM sleep (~65 min) compared with citalopram alone (\( P < 0.05 \)). With regard to the time spent in REM sleep, a decrease was observed in rats treated with either citalopram (\( P < 0.01 \)) or SB-269970 (\( P < 0.05 \)) alone during the first 2 h after the treatment compared with vehicle-treated rats (Fig. 5B). In addition, the treatment with SB-269970 potentiated the decrease in REM sleep duration induced by citalopram during the first 2-h interval (\( P < 0.001 \) versus vehicle + vehicle) and the second 2-h interval (\( P < 0.001 \) versus vehicle + vehicle).
SB-269970 Enhances Citalopram Antidepressant-Like Effects

The present study was carried out to investigate the ability of a 5-HT7 receptor antagonist, SB-269970, to augment the effect of a selective serotonin reuptake inhibitor, citalopram, on 5-HT transmission, antidepressant-like behavior, and REM sleep suppression in rodents.

The pharmacological tools used in the present study, SB-269970 and citalopram, have been shown to be selective for 5-HT7 and the 5-HT transporter, respectively. In vitro, SB-269970 displays excellent selectivity (>100-fold) for 5-HT7, 5-HT3, 5-HT4, 5-HT6, 5-HT transporter, adrenergic α1, D1, and D2 receptors, and a panel of 50 receptors, transporters, enzymes, and ions channels (Hagan et al., 2000).

SB-269970 retains some affinity for the 5-HT5A receptor; however, the ratio 5-HT7/5-HT5A is close to 50-fold (Hagan et al., 2000). In vitro and in vivo antagonism properties of SB-269970 for the 5-HT7 receptor have been demonstrated (Hagan et al., 2000). Citalopram has excellent selectivity for the 5-HT transporter versus NE or DA transporters, other 5-HT, DA, adrenergic receptors, and a panel of 50 receptors, transporters, enzymes, and ions channels (Hyttel, 1994). Noteworthy, in vitro citalopram did not exhibit affinity for the 5-HT7 receptor (Shen et al., 1993).

Most of the currently available antidepressant drugs act through monoamines and in particular 5-HT. It has been demonstrated in the clinic that mood improvement in depressed patients who positively responded to treatment with citalopram-induced sleep fragmentation, resulting in a significant decrease in the number of microarousals ($P < 0.01$ versus vehicle + citalopram).

**Effect of SB-269970 on Citalopram Blood-Brain Barrier Penetration and Pharmacokinetics Parameters.** Citalopram plasma and brain concentrations were determined following s.c. dosing of citalopram alone (3 mg/kg) or coadministration of citalopram (3 mg/kg) with SB-269970 (10 mg/kg). SB-269970 did not affect citalopram plasma and brain concentration at all the time points tested ($P > 0.05$ versus vehicle + citalopram; Fig. 7, A and B). Pharmacokinetic and blood-brain barrier parameters are given in Table 2.

**Effect of Citalopram on SB-269970 Blood-Brain Barrier Penetration and Pharmacokinetics Parameters.** SB-269970 plasma and brain concentrations were also determined following s.c. dosing of SB-269970 alone (10 mg/kg) or coadministration of citalopram (3 mg/kg) with SB-269970 (10 mg/kg). Citalopram did not affect SB-269970 plasma and brain concentration at all the time points tested ($P > 0.05$ versus vehicle + SB-269970; plasma $C_{\text{max}}$ SB-269970 alone = 8.07 μM versus 7.1 μM for SB-269970 + citalopram; and brain $C_{\text{max}}$ SB-269970 alone = 0.37 μM versus 0.25 μM for SB-269970 + citalopram).

**Discussion**

The REM bout analysis following the s.c. coadministration of 1 mg/kg citalopram and 10 mg/kg SB-269970 in rats

**TABLE 1**

| Table 1 | REM bout analysis following the s.c. coadministration of 1 mg/kg citalopram and 10 mg/kg SB-269970 in rats
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<td>Values (means ± S.E.M.; n = 9) are calculated during the 6-h period following the treatment.</td>
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<tr>
<td></td>
<td>Vehicle + Vehicle</td>
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<tr>
<td>No. of REM bouts</td>
<td>22.4 ± 1.5</td>
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<tr>
<td>REM bout duration (min)</td>
<td>1.9 ± 0.1</td>
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* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Veh + Veh based on one-way ANOVA followed by Newman-Keuls post hoc test.
† $P < 0.001$ vs. Veh + citalopram based on one-way ANOVA followed by Newman-Keuls post hoc test.
‡ $P < 0.01$ vs. SB-269970 + Veh based on one-way ANOVA followed by Newman-Keuls post hoc test.

**Fig. 5.** REM sleep latencies (A) and REM sleep duration during the first 6 h (B) following the s.c. coadministration of 1 mg/kg citalopram and 10 mg/kg SB-269970 in rats ($n = 9$; means ± S.E.M.). *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ versus Veh + Veh; *, $P < 0.05$ versus Veh + citalopram based on one-way ANOVA followed by Newman-Keuls post hoc test (A) or two-way ANOVA followed by Bonferroni post hoc test (B).
various classes of antidepressants drugs could be rapidly impaired by 5-HT synthesis inhibition (Delgado et al., 1990). SSRIs are clinically effective antidepressants; however, most patients do not show signs of mood improvement until 2 to 3 weeks after the start of the treatment (Blier, 2003). About one third of these patients show only partial or no response to the treatment. Side effects are commonly reported during the chronic treatment, notably insomnia, somnolence, dizziness, akathisia, and long-term sexual dysfunction (decreased libido, delayed ejaculation) (Hamon and Bourgoin, 2006). Several strategies are in progress to improve the activity of the conventional antidepressant drugs (Blier, 2003; Adell et al., 2005). Additional blockade of aminergic autoreceptors (5-HT1A and 5-HT1B) or antagonism of certain postsynaptic receptors (5-HT2A and 5-HT2C) is among the proposed strategies (Adell et al., 2005).

The activation of 5-HT1A autoreceptors by 5-HT suppresses cell firing, whereas 5-HT1B receptor controls 5-HT synthesis and release at nerve terminals. The blockade of negative feedback mechanisms with 5-HT1A and/or 5-HT1B receptor antagonists potentiates the 5-HT increase produce by SSRIs. Clinical data with pindolol, a nonselective 5-HT1A antagonist, show that it hastens the effect of SSRIs in some studies, but not in others (Artigas et al., 2001). A similar negative feedback also occurs in noradrenergic neurons involving α2-adrenoceptor. Thus, α2-adrenoceptor antagonists have been used preclinically to potentiate the effect of SSRIs (Sanacora et al., 2004). Selective neurokinin 1 receptor antagonists such as GR205171 have also been used to augment the neurochemical and behavioral effect of SSRIs (Guiard et al., 2004). Coadministration of SSRI and a neurokinin 1 antagonist enhances 5-HT neurotransmission, presumably through a lower inhibitory feedback control of the serotoninergic neurons by 5-HT1A autoreceptors.

Some lines of evidence suggest that blockade of 5-HT2A receptors might enhance the therapeutic effectiveness of SSRIs. The selective blockade of 5-HT2A receptor by M100907 augments the antidepressant effect of fluoxetine in the DRL72s schedule (Marek et al., 2005). 5-HT2 receptors are involved in the feedback control of 5-HT neurons. 5-HT2 receptor agonists inhibit the firing of midbrain 5-HT neu-

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**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle + Citalopram</th>
<th>SB-269970 + Citalopram</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.26 ± 24</td>
<td>0.27 ± 35</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μM)</td>
<td>1.87 ± 6</td>
<td>1.39 ± 7</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{inf}}$ (h·μM)</td>
<td>2.57 ± 5</td>
<td>2.25 ± 7</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>0.74 ± 9</td>
<td>0.91 ± 11</td>
</tr>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.73 ± 8</td>
<td>1.00 ± 7</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μM)</td>
<td>2.99 ± 5</td>
<td>2.72 ± 4</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{inf}}$ (h·μM)</td>
<td>6.90 ± 5</td>
<td>7.47 ± 5</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>0.94 ± 11</td>
<td>0.80 ± 6</td>
</tr>
</tbody>
</table>

**Blood-brain barrier coefficient**

|                    | 0.47                  | 0.51                  |

---

**Fig. 6.** Number of microarousals after the coadministration of 1 mg/kg citalopram and 10 mg/kg SB-269970 in rats. Values (means ± S.E.M.; n = 9 animals) are calculated during the 6-h period after the treatment. *, P < 0.05 versus Veh + Veh; #, P < 0.01 versus Veh + citalopram; and ‡, P < 0.001 versus SB-269970 + Veh based on one-way ANOVA followed by Newman-Keuls post hoc test.

**Fig. 7.** Plasma (A) and brain (B) levels after s.c. administration of 3 mg/kg citalopram alone or coadministration of 3 mg/kg citalopram and 10 mg/kg SB-269970. Data represent the means ± S.E.M., n = 3. For both plasma and brain concentrations, $P > 0.05$ Veh + citalopram versus SB-269970 + citalopram based on paired t test. The corresponding pharmacokinetics and blood-brain barrier parameters are given in Table 2.
rons (Boothman et al., 2003). Recent data indicate that ketanserin or M100907 augments the effect of citalopram on extracellular 5-HT (Boothman et al., 2006). However, in the latter study, ketanserin or ritanserin did not augment the effect of fluoxetine on extracellular 5-HT. The authors suggested that the lack of augmentation of fluoxetine might reflect the intrinsic 5-HT₂ receptor antagonist properties of fluoxetine.

At this stage, it is unclear how 5-HT₇ blockade can facilitate 5-HT transmission, antidepressant-like behavior, and REM sleep suppression induced by citalopram in rodents. The presence of 5-HT₇ receptor mRNA and protein in 5-HT neurons suggests that this receptor may act as a 5-HT autoreceptor (To et al., 1995; Varnas et al., 2004). Recent data suggest that blockade of 5-HT₇ receptor might lead to an increased activity of 5-HT neurons (Faure et al., 2006). It has been suggested that 5-HT₇ antagonist might increase the firing rate of 5-HT neurons via an interaction with the glutamatergic system. However, others have found that blockade of 5-HT₇ receptors decreases 5-HT release in guinea pig dorsal raphe nucleus slices (Roberts et al., 2004) and in hamster suprachiasmatic nucleus (Glass et al., 2003), presumably by indirectly activating the GABAergic system.

Our microdialysis data indicate that by itself SB-269970 does not increase cortical 5-HT release in rat. However, when coadministered with citalopram, SB-269970 significantly enhanced the effect of citalopram on 5-HT release. In theory, a high level of 5-HT at serotoninergic nerve terminals should lead to a higher degree of antidepressant effect by stimulating postsynaptic 5-HT receptors subtypes in brain regions involved in mood disorders. Interestingly, by itself SB-269970 did not increase cortical 5-HT release in rat cortex but reduced immobility time in the forced swim test/tail suspension test, increased REM sleep latency and suppressed REM sleep (Hagan et al., 2000; Guscott et al., 2005; Hedlund et al., 2005; Faure et al., 2006; Wesolowska et al., 2006a,b, 2007).

Our behavioral data indicate that blockade of 5-HT₇ receptors enhanced the antidepressant-like effect of citalopram. It has been demonstrated that citalopram decreased immobility of 5-HT₇ receptor knockout mice and wild-type controls in the tail suspension test and that this effect in 5-HT₇ knockout mice was additive to that of the genotype alone (Hedlund et al., 2005). This observation is consistent with our results showing that combining SB-269970 with ineffective dose of citalopram resulted in a significant decrease of immobility time in the tail suspension test. In a more recent article, Wesolowska et al. (2007) showed that blockade of 5-HT₇ receptor facilitates the anti-immobility effect of several antidepressants in mice. In the latter study, subefficacious doses of citalopram, imipramine, desipramine, and moclobemide administered with SB-269970 produced a significant antidepressant-like effect in the mouse forced swimming test without increasing spontaneous locomotor activity. Our data with citalopram and SB-269970 extend these findings using a different animal model of antidepressant-like activity. Furthermore, our study rules out a possible pharmacokinetic interaction between SB-269970 and citalopram and favors a pharmacodynamic interaction to explain the enhancement of citalopram effects. However, additional work is required to demonstrate that 5-HT₇ receptor blockade also augments the effect of tricyclic 5-HT/noradrenaline and monoamino oxidase-A inhibitors as well as other SSRIs on 5-HT transmission and REM sleep suppression.

Our EEG data are also consistent with the behavioral data. At the doses selected from a pilot experiment, 1 mg/kg citalopram and 10 mg/kg SB-269970 were expected to produce minimal sleep effects to reveal a potential enhancement of the combined treatment on sleep parameters, especially on REM sleep. At these low doses, citalopram treatment still induced a significant increase of REM sleep latency, whereas SB-269970 had no effect. Both citalopram and SB-269970 produced only a short-acting decrease in the time spent in REM sleep for 2 h. Accordingly, previous studies have shown that SB-269970 selectively reduced time spent in REM sleep and prolonged the latency for the first REM sleep episode at the dose of 30 mg/kg in rats (Hagan et al., 2000; Thomas et al., 2003). A number of animal studies have demonstrated the REM sleep suppressive effect of citalopram associated with minor changes in NREM sleep at doses up to 10 mg/kg in rats (Neckelmann et al., 1996; Ivarsson et al., 2005) and mice (Monaca et al., 2003; Hedlund et al., 2005). The combination of citalopram with SB-269970 significantly delayed the REM sleep latency compared with citalopram alone. In addition, the treatment with SB-269970 potentiated the decrease in REM sleep duration induced by citalopram. Interestingly, these results are in line with the observation that 5-HT₇ knockout mice exhibited a more pronounced reduction in REM sleep duration and a greater increase in REM latency after citalopram administration compared with wild-type mice (Hedlund et al., 2005).

Taken together, these data strongly suggest an involvement of 5-HT₇ receptors in the REM sleep effects induced by citalopram. At low dosage, SB-269970 also prevented the citalopram-induced sleep fragmentation, as evidenced by a significant decrease in the number of microarousals. Although no other animal study had previously reported an alteration in the frequency of wake episodes after citalopram treatment, polysomnographic studies in humans have consistently shown disruption of sleep continuity (with number of awakenings being increased) in addition to REM sleep suppression (Wilson et al., 2004). One of the hallmarks of depression is an alteration in the sleep-wake cycle, including a shortened REM sleep latency, an increase in REM sleep time and frequency during the first part of the night, and increased sleep fragmentation (Kupfer, 1984). Thus, as an additional benefit to the potential enhancement of the antidepressant effect, this combination may provide a novel therapy to alleviate sleep disturbances that are common in individuals with depression and that might be even worse with citalopram alone.

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References


