S33005, a Novel Ligand at Both Serotonin and Norepinephrine Transporters: II. Behavioral Profile in Comparison with Venlafaxine, Reboxetine, Citalopram, and Clomipramine

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ABSTRACT

Reflecting its potent inhibition of serotonin (5-HT) reuptake (accompanying paper), S33005 blocked spontaneous tail-flicks induced by parachloroamphetamine in rats. This action was mimicked by the 5-HT reuptake inhibitor, citalopram, and the 5-HT/norepinephrine (NE) reuptake inhibitor, venlafaxine, whereas the preferential NE reuptake inhibitor, reboxetine, was inactive. Consistent with its less potent interaction with NE transporters, higher doses of S33005 attenuated induction of hypothermia by reserpine, an action mimicked by reboxetine and venlafaxine, whereas citalopram was ineffective. In mice, S33005 reduced immobility in forced-swim and tail-suspension procedures. It also inhibited marble-burying behavior and suppressed aggressive behavior between resident and intruder animals. In rats, S33005 generalized to a discriminative stimulus elicited by citalopram and attenuated hypnotic-sedative actions of the α2-adrenoceptor agonist, S18616. For these parameters, S33005 was a more potent agent (median, 1.2 mg/kg, s.c.) than venlafaxine, citalopram, reboxetine, or the tricyclic agent, clomipramine. Even at markedly higher doses (40.0–80.0 mg/kg, s.c.), S33005 little affected motor behavior. S33005 (10.0 mg/kg, s.c.) also increased responses in a learned helplessness paradigm in rats, whereas venlafaxine was ineffective. Finally, in a rat chronic mild-stress model, S33005 dose- (2.5–40.0 mg/kg) and time- (2–5 weeks) dependently enhanced sucrose consumption. Venlafaxine was likewise active in this procedure. In conclusion, in line with its inhibition of 5-HT and (less potently) NE reuptake, S33005 is active in a broad range of models suggestive of antidepressant activity. It exerts its actions more potently than venlafaxine and clomipramine, and its overall profile is distinct from those of citalopram and reboxetine.

The novel benzocyclobutane derivative, S33005, potently binds to native rat and cloned human 5-HT transporters (SERTs) and, less potently, to norepinephrine transporters (NETs), whereas it shows negligible affinity for dopamine (DA) transporters and all other (>50) sites examined (accompanying paper). Correspondingly, in freely moving rats, S33005 markedly elevates extracellular levels of 5-HT and NE in frontal cortex and other corticolimbic structures, while levels of DA are enhanced exclusively in the frontal cortex as compared with subcortical regions. S33005 can, thus, be distinguished from the selective 5-HT reuptake inhibitor (SSRI), citalopram (Pigott and Seay, 1999; Popik, 1999), and the preferential NE reuptake inhibitor, reboxetine (Riva et al., 1989; Versiani et al., 2000). Furthermore, while this dual influence of S33005 at SERTs and (less markedly) NETs resembles the structurally related venlafaxine, the latter shows considerably lower affinity for these sites (Muth et al., 1991; Schweizer et al., 1997; Béique et al., 1999). Moreover, although the interaction of S33005 with both SERTs and (less potently) NETs is also analogous to the tricyclic agent, clomipramine, S33005 lacks the latter’s affinity for α1-adrenoceptors (ARs) and histaminergic and muscarinic receptors, the blockade of which elicits cardiovascular and autonomic side effects (Frazer, 1997; Mir and Taylor, 1997).

The above-mentioned interaction of S33005 with SERTs and NETs, together with its facilitatory influence upon corticolimbic monoaminergic transmission in vivo, strongly suggests antidepressant properties, and the purpose of the complementary studies described herein was to characterize the behavioral profile of S33005 within this framework. In light of the above-specified similarities of S33005 to venlafaxine, one major, empirical approach was to systematically evalu-

ABBREVIATIONS: 5-HT, serotonin; AR, adrenoceptor; DA, dopamine; NE, norepinephrine; NET, norepinephrine transporter; PCA, parachloroamphetamine; S33005, (+)-(1-dimethylaminomethyl)-5-methoxybenzocyclobut-1-yl) cyclohexanol; S18616, (5)-spiro[(1-oxa-2-ami-no-3-aza-cyclocpent-2-ene)-4,2’-(1’,2’,3’,4’-tetrahydrodronaphthalene)]; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor; ANOVA, analysis of variance; CL, confidence limit.
ate the actions of S33005 in models responsive to venlafaxine. Moreover, we exploited several paradigms characterizing core symptoms of depression, responsive to diverse classes of antidepressant agent, and indicative of therapeutic efficacy in humans (Willner, 1991; Thiébot et al., 1992).

First, we examined the influence of S33005 upon induction of spontaneous tail-flicks by parachloroamphetamine (PCA), an amphetamine analog that enters serotonergic terminals via the SERT and thereby displaces 5-HT (Fuller et al., 1991; Bervoets et al., 1993). Furthermore, we determined its influence upon induction of hypothermia by the prodepressogenic agent, reserpine, a response reflecting NE depletion (Pawlowski and Nowak, 1987). Second, generalization studies were undertaken with rats recognizing a discriminative stimulus elicited by citalopram (Millan et al., 1999). Third, “despair”, considered a core symptom of depressive states, is mimicked by the forced-swim (Borsini and Meli, 1988; Rénergic and Lucki, 1998) and tail-suspension (Steru et al., 1987; Teste et al., 1993) paradigms in mice. Thus, actions of S33005 were examined in these models. Fourth, psychomotor retardation is similarly a cardinal symptom of depressive states (Caligiuri and Ellwanger, 2000). Although experimental models are not available, we determined the influence of S33005 upon sedative actions of the a2-AR agonist, S18616, in rats (Hayashi and Maze, 1993; Millan et al., 2000b). The learned helplessness paradigm in rats, a model incorporating elements both of despair and of psychomotor retardation (Martin et al., 1990; Thiébot et al., 1992), was also used. Fifth, depression is likewise characterized by melancholia, and the reduction of sucrose intake displayed by rats exposed to chronic mild stress reflects an anhedonic state (Papp et al., 1994; Willner, 1997). Thus, the modification of this behavior by S33005 was evaluated. Finally, impulsive symptoms are frequently comorbid with depressive states, and antidepressant agents are used for management of obsessive-compulsive disorders (Piccinelli et al., 1995; Blier and de Montigny, 1999; Pigott and Seay, 1999). Thus, we determined the influence of S33005 upon impulsive marble-burying behavior in mice, a model responsive to antidepressant agents (Njung’e and Handley, 1991; Sánchez and Meier, 1997). Monoamines are also implicated in aggressive behavior (Miczek et al., 1994; Edwards and Kravitz, 1997; Blanchard et al., 1998), and aggressive encounters in (isolated) mice are attenuated by several antidepressant agents, so actions of S33005 were also characterized in this model (White et al., 1991; Mitchell and Redfern, 1992; Sánchez and Meier, 1997).

Materials and Methods

Animals. Unless otherwise specified below, these studies used male Wistar rats of 200 to 250 g and NMRI mice of 22 to 25 g (Iffa-Credo, L’Arbresle, France) housed in sawdust-lined cages with unrestricted access to standard chow and water. There was a 12-h/12-h light/dark cycle with lights on at 7:30 AM. Laboratory temperature and humidity were 21 ± 0.5°C and 60 ± 5%, respectively. Animals were adapted to laboratory conditions for at least 1 week prior to testing. All animal use procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

PCA-Induced Spontaneous Tail-Flicks. Spontaneous tail-flicks were monitored in rats loosely restrained in horizontal cylinders (Bervoets et al., 1993). Following a 5-min adaptation, the number of spontaneous tail-flicks emitted over a 5-min period was counted. PCA was administered (10.0 mg/kg, s.c.) 30 min before observation, and S33005, venlafaxine, clomipramine, citalopram, reboxetine, or vehicle was administered 10 min before PCA. Data were analyzed by analysis of variance (ANOVA) followed by Dunnett’s test, and ID50 values plus 95% confidence limits (CL) were calculated.

Reserpine-Induced Hypothermia. Animals were male CD mice (Charles River, St Aubin-Les-Elbeuf, France), weighing 18 to 20 g, housed under laboratory conditions described above and placed into individual cages 1 day before testing. The test was performed essentially as described previously (Pawłowski and Nowak, 1987). On the test day, rectal temperature was measured (Thermometer Bat-12 Physitemp Instruments Inc., Clifton, NJ) before administration of reserpine (2.5 mg/kg, i.p.) or vehicle and again 3 h later just before the administration of drug or vehicle (s.c.). Two hours later, core temperature was again recorded. Data used for statistical analysis were the difference (°C) between the temperature recorded following the test drug and that 3 h following reserpine administration. Data were analyzed by ANOVA followed by Dunnett’s test.

Discriminative Stimulus Properties of Citalopram. As described previously (Millan et al., 1999), using a 2-lever, fixed-ratio 10-food-reinforced schedule, rats were trained to discriminate citalopram (2.5 mg/kg, i.p.) from saline administered in a quasi-random order. Daily sessions of 15 min were commenced 15 min after treatment. After the discrimination criterion (10 consecutive sessions of correct responding) had been obtained, generalization tests were performed on Wednesdays and Fridays for rats with correct responding on the 2 most recent training days. Thus, s.c. administration of S33005, venlafaxine, clomipramine, or citalopram itself was substituted for s.p. citalopram. On the other days, training sessions continued. Significance of generalization was evaluated by use of the Fisher’s exact probability test, and ED50 values (95% CL) were calculated. The influence of drugs upon response rates was analyzed by paired t tests.

Forced-Swim Test in Mice. The procedure was performed essentially as described by Redrobe et al. (1998). Briefly, male CD mice (Charles River) weighing 22 to 26 g were placed in individual glass cylinders (24 cm height × 12 cm diameter) containing 6 cm of water at 24 ± 0.5°C for 6 min. Duration (s) of immobility (defined as movement necessary to keep the head above water) was recorded during the last 4 min of the test. Mice were treated 30 min before the test with either drug or vehicle. Dose effects were analyzed using ANOVA followed by Dunnett’s test, and ID50 values (95% CL) were calculated.

Tail-Suspension Test in Mice. The procedure used was performed essentially as described by Steru et al. (1987) using an automated tail-suspension apparatus (Itematic-TST, Item-Labo, France). Each mouse was suspended by the tail using adhesive tape to a hook connected to a strain gauge. The duration of immobility (s) of each mouse was recorded during the 6-min test. Drug or vehicle was administered to the animals 30 min before testing. Dose effects were analyzed using ANOVA followed by Dunnett’s test, and ID50 values (95% CL) were calculated.

Inhibition of S18616-Induced Loss of Righting Reflex in Rats. The loss of righting reflex in rats was evaluated according to a scoring system described previously (Millan et al., 2000b). Briefly, rats were placed on their backs on a lab surface covered with paper wadding, and their ability to right themselves was assessed as follows. Score 0, normal, complete righting reflex; score 1, attempted righting reflex (turn of at least 90 degrees); score 2, attempted righting reflex (turn of less than 90 degrees); and score 3, total loss of righting reflex (no attempt to turn). S33005, venlafaxine, clomipramine, citalopram, reboxetine, or vehicle was administered 30 min prior to S18616, which was administered 30 min before the righting reflex was scored. All animals receiving S18616 (0.63 mg/kg, s.c.) displayed a score of 3; the number of rats displaying a score of 2 or less following drug treatment was calculated. Significance of inhibi-
tions was evaluated by use of the Fisher’s exact probability test, and ID$_{50}$ values (95% CL) were calculated.

**Learned Helplessness Test.** The procedure used was performed as previously described in detail (Martin et al., 1990; Millan et al., 1997). On day 1 of testing, “nonstressed” and “stressed” (“helpless”) rats were placed in a small chamber with a stainless steel gridfloor for 1 h. During this period, every minute, stressed rats were exposed to a 15-s inescapable shock (0.8 mA). On days 3, 4, and 5 of testing, both groups of rats were submitted to an avoidance task (sessions 1, 2, and 3, respectively) in a shuttle box. During the 15-min session, there were 30 stimuli (3 s) light-shock (3 s, 0.8 mA) trials, with a 24-s intertrial period. For each trial, the rat could terminate the shock by escaping into the other compartment. For each session, data were the number of escape failures (trials on which the rat received the shock by not crossing into the other compartment), intertrial crossings (transfer from one compartment to the other during the intertrial period), and exploratory crossings (locomotion during the 5-min shock-free period commencing the session). Imipramine, S33005, venlafaxine or vehicle (stressed groups), or vehicle (nonstressed group) was administered to rats as a single bolus (i.p.) on day 1, 6 h after exposure to shocks, and twice a day on days 2 to 5 (half the dose, 30 min before the avoidance session, and the other half 6 h later). For each avoidance session, differences between control (vehicle) stressed and nonstressed rats were evaluated by Student’s t test. Data obtained in stressed rats were analyzed by ANOVA followed by Dunnett’s test.

**Marble-Burying Behavior in Mice.** As described previously (Millan et al., 2000b), mice were individually placed in transparent polycarbonate cages (30 × 18 × 19 cm) containing a 5-cm layer of sawdust and 24 glass marbles (1.5 cm in diameter) evenly spaced against the wall of the cage. Thirty minutes later the animals were removed from the cages, and the number of marbles buried at least two-thirds into the sawdust was recorded. The mice were treated 30 min before the test with either S33005, venlafaxine, clomipramine, citalopram, reboxetine, or vehicle. Dose effects were analyzed with one-way ANOVA followed by Dunnett’s test, and the ID$_{50}$ values (95% CL) were calculated.

**Aggression in Preisolated Mice.** As described previously (Millan et al., 1997), pairs of mice were isolated in black cages for 1 month. On the test day, one mouse (intruder) was placed into the cage of the other (resident) and the total number and duration of fights determined. Both mice were treated 30 min before the test with either drug or vehicle. Data were analyzed by ANOVA followed by Dunnett’s test, and ID$_{50}$ values (95% CL) were calculated.

**Ataxia In Mice: Rotarod Test.** As described previously (Millan et al., 1997), the latency of mice to fall from an accelerating (4–40 rpm over 300 s) rotarod (Ugo Basile, Varese, Italy) was determined. There was a cut-off of 360 s. S33005, venlafaxine, clomipramine, citalopram, reboxetine, or vehicle was administered 30 min before the test. Dose effects were analyzed with ANOVA followed by Dunnett’s test.

**Spontaneous Locomotion In Rats.** As described previously (Millan et al., 2000b), locomotor behavior was evaluated in rats placed in transparent polycarbonate cages (45 × 30 × 20 cm) located in activity chambers (Lablinc System, Coulbourn, Lehigh Valley, PA). Locomotion was monitored over 12 min. Drugs were given 30 min prior to testing. A “movement” corresponded to the consecutive interruption of two infrared beams situated 24 cm apart and 4 cm above the cage floor. Data were analyzed by ANOVA followed by Dunnett’s test.

**Chronic Mild Stress-Induced Reduction in Sucrose Consumption.** This study used male Wistar rats weighing 220 to 250 g (Gorzowska, Warsaw, Poland) brought into the laboratory 2 months before the experiment. As described previously (Papp et al., 1994), animals were single-housed and initially trained to consume a 1% sucrose solution. Training consisted of 10 1-h baseline tests (twice weekly) in which sucrose was presented in the home cage following 14 h of food and water deprivation. Sucrose intake was measured by weighing bottles containing the sucrose solution before and at the end of the test. Subsequently, sucrose consumption was monitored under similar conditions at weekly intervals throughout the whole experiment. Animals were divided into two matched groups on the basis of their sucrose intakes in the final baseline test. One group was subjected to the chronic mild-stress procedure for 8 consecutive weeks. Each week of the stress regime consisted of two periods of food or water deprivation, two periods of 45 degree cage tilt, two periods of intermittent illumination (lights on and off every 2 h), two periods of soiled cage (250 ml of water in sawdust bedding), two periods of paired housing, two periods of low-intensity stroboscopic illumination (150 flashes/min), and two periods of no stress. All stressors were of 10 to 14 h in duration and were applied individually and continuously, day and night. Control animals were housed in separate rooms and had no contact with the stressed animals. On the basis of their sucrose intakes, following the initial 3 weeks of stress, both stressed and control animals were further divided into matched subgroups, and for the subsequent 5 weeks they received daily injections (i.p.) of S33005, venlafaxine, imipramine, or vehicle. The drugs were administered at 10:00 AM, and the weekly sucrose tests were carried out 24 h after the last drug injection. Data were analyzed by multiple ANOVA with three between-subjects factors (stress/control, drug treatment, and successive sucrose tests) followed by Fisher’s least-significant difference test.

**Drugs.** Actions of drugs in each of the specific behavioral paradigms used herein were evaluated concurrently. Full dose-response curves were generated for S33005 and other agents in all (acute) procedures. Drugs were dissolved in sterile water, plus a few drops of lactic acid if necessary, and pH-adjusted to as close to normality (>5.0) as possible. Drug salts and sources were as follows. S33005 [(−)-1-(1-dimethylaminomethyl-5-methoxybenzocyclobutan-1-yl)-cyclohexanone] HCl, S18616 [(S)-spiro[1-oxa-2-amino-3-azacyclopent-2-ene]-4,2’-[1’,2’,3’,4’-tetrahydroanaphthalene]] HCl, citalopram HCl, reboxetine methane sulfonate, and venlafaxine HCl were synthesized internally (A. Cordi, G. Lavielle and J.-L. Peglion). Clomipramine HCl, PCA HCl, and imipramine HCl were obtained from Sigma (Saint Quentin-Fallavier, France).

**Results**

**Reduction of Spontaneous Tail-Flicks Elicited by PCA.** PCA (10.0 mg/kg, s.c.) elicited a robust spontaneous tail-flick response in rats, corroborating our previous study (Bervoets et al., 1993) (Fig. 1; Table 1). The induction of

**Fig. 1.** Influence of S33005 as compared with reference antidepressants agents upon the induction of spontaneous tail-flicks by PCA (10.0 mg/kg, s.c.) in rats. Values are means ± S.E.M., n = 6 per value. ANOVA is as follows. S33005, F(5,27) = 5.2, P < 0.01; venlafaxine, F(4,31) = 4.0, P < 0.01; clomipramine, F(3,21) = 0.2, P > 0.05; and citalopram, F(4,19) = 3.5, P < 0.05. Asterisks indicate significance of differences to vehicle (VEH) values in Dunnett’s test following ANOVA. *P < 0.05.
spontaneous tail-flicks by PCA was potently and dose dependently inhibited by S33005. The action of S33005 was mimicked by venlafaxine and citalopram. Although clomipramine tended to decrease spontaneous tail-flicks, it did not attain statistical significance. Furthermore, reboxetine exerted no significant effect at a dose of 10.0 mg/kg, s.c. (not shown). S33005 and the other agents did not elicit spontaneous tail-flicks upon administration alone (not shown).

Inhibition of Reserpine-Induced Hypothermia.
Administration of reserpine (2.5 mg/kg, i.p.) elicited a pronounced reduction in core temperature of mice (Fig. 2; Table 1). This effect was significantly and dose dependently attenuated by S33005. Venlafaxine and (potently) reboxetine similarly attenuated the hypothermic properties of reserpine. In contrast, clomipramine was ineffective, and citalopram potentiated the action of reserpine:

<table>
<thead>
<tr>
<th>Drug</th>
<th>DDCIT, ID&lt;sub&gt;50&lt;/sub&gt;</th>
<th>FS, ID&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MB, ID&lt;sub&gt;50&lt;/sub&gt;</th>
<th>AGGN, ID&lt;sub&gt;50&lt;/sub&gt;</th>
<th>AGGD, ID&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S33005</td>
<td>0.1 (0.06–0.22)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1 (0.01–0.9)</td>
<td>6.8 (3.6–15.4)</td>
<td>3.5 (1.9–6.4)</td>
<td>1.2 (0.38–3.11)</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>0.7 (0.1–2.3)</td>
<td>2.3 (1.0–5.4)</td>
<td>11.7 (4.7–50.9)</td>
<td>2.7 (0.3–3.52)</td>
<td>5.1 (2.3–11.1)</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>6.6 (4.9–8.7)</td>
<td>3.6 (2.1–7.1)</td>
<td>9.6 (1.9–41.6)</td>
<td>3.0 (0.1–0.7)</td>
<td>6.6 (3.3–11.7)</td>
</tr>
<tr>
<td>Citalopram</td>
<td>0.3 (0.1–2.6)</td>
<td>0.16 (0.05–0.54)</td>
<td>NC</td>
<td>2.3 (1.3–4.3)</td>
<td>6.1 (3.0–19.6)</td>
</tr>
<tr>
<td>Reboxetine</td>
<td>&gt;10.0</td>
<td>NT</td>
<td>14.6 (10.4–18.4)</td>
<td>3.5 (1.4–8.9)</td>
<td>2.8 (1.3–9.2)</td>
</tr>
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</table>

Values in parentheses are 95% CI.

**Fig. 2.** Influence of S33005 as compared with reference antidepressants upon the induction of hypothermia by reserpine (2.5 mg/kg, i.p.) in mice. The data are presented as the percentage reversal of the hypothermic action of reserpine. Absolute basal core temperatures were, respectively, for the vehicle-treated group: 37.0 ± 0.1°C (basal), 32.7 ± 0.4°C (3 h following reserpine), and 30.6 ± 0.9°C (5 h following reserpine). Values illustrated are means ± S.E.M. ANOVA for repeated measures is as follows: S33005, F(4,35) = 5.4, P < 0.01; venlafaxine ( ), F(3,27) = 1.5, P > 0.05; clomipramine ( ), F(3,23) = 3.9, P < 0.05; reboxetine ( ), F(3,29) = 4.5, P < 0.01. Dunnett's test (following ANOVA) was used to test significant differences to vehicle (VEH) values. *P < 0.05.
Generalization of S33005 (●) as compared with reference antidepressant agents to a discriminative stimulus elicited by citalopram (2.5 mg/kg, i.p.) in rats. Values indicated are mean percentage of rats responding on the drug lever per dose. **n** = 4 to 6 per value. Asterisks indicate significance of difference to control values in the Fisher’s exact probability test. *P < 0.05. □, venlafaxine; ▽, clomipramine; ▲, citalopram.

Activity in the Learned Helplessness Procedure. As compared with control, nonstressed animals, those exposed to inescapable shock (helpless) manifested a significantly higher number of escape failures (Fig. 7). In analogy to imipramine (the internal, positive control), on each of the three avoidance sessions, at a dose of 10.0 mg/kg, i.p., S33005 showed a significant reduction in escape failures. In contrast, at a low dose of 0.63 as well as at doses of 2.5 and 40.0 mg/kg (not shown), S33005 did not exert a significant effect. Data for sessions 1 and 2 are shown in Fig. 7. For session 3, control nonstressed = 5.0 ± 2.0, vehicle stressed = 22.0 ± 3.0, imipramine stressed = 5.0 ± 2.0, S33005 (0.63) stressed = 21.0 ± 3.0, and S33005 (10.0) stressed = 11.0 ± 3.0. ANOVAs were performed across the entire dose range (all four doses). Session 7, *F*(4,55) = 4.7, *P* < 0.001; session 2, *F*(4,55) = 5.7, *P* < 0.001, and session 3, *F*(4,55) = 4.3, *P* < 0.001. As compared with control nonstressed animals, the helpless animals showed a significantly lower number of intertrial crossings. In analogy to imipramine, S33005 significantly increased intertrial crossings at the dose of 10.0 mg/kg, i.p., in each session, whereas the other doses did not elicit a significant effect. Data for sessions 1 and 2 are shown in Fig. 7. For session 3, control nonstressed = 24.0 ± 3.5, vehicle stressed = 3.0 ± 1.0, imipramine stressed = 13.0 ± 2.0, S33005 (0.63) stressed = 4.0 ± 1.0, and S33005 (10.0) stressed = 11.0 ± 3.0. ANOVAs performed across the entire dose range were as follows. Session 1, *F*(4,55) = 3.2, *P* < 0.01; session 2, *F*(4,55) = 3.7, *P* < 0.01; and session 3, *F*(4,55) = 3.1, *P* < 0.05. Imipramine significantly decreased exploratory crossings on session 1 (reflecting its influence on motor behavior), although it was without significant effect in the other sessions in which control levels of exploratory crossings were considerably lower. In contrast to imipramine, S33005 did not significantly modify exploratory crossings. Data for sessions 1 and 2 are shown in Fig. 7. For session 3, control nonstressed = 8.0 ± 2.0, vehicle stressed = 4.0 ± 2.0, imipramine stressed = 8.0 ± 1.0, S33005 (0.63) stressed = 8.0 ± 1.0, and S33005 (10.0) stressed = 8.0 ± 2.0. ANOVAs performed across the entire dose range were as follows. Session 1, *F*(4,55) = 1.2, *P* > 0.05; session 2, *F*(4,55) = 0.2, *P* > 0.05; and session 3, *F*(4,55) = 0.4, *P* > 0.05.

In contrast to S33005, venlafaxine did not significantly modify escape failures at any dose tested in sessions 1, 2, or 3. Data for session 3 as follows. Control nonstressed = 8 ± 2, vehicle stressed = 21 ± 2, venlafaxine (0.63 mg/kg, i.p.) = 18 ± 3, venlafaxine (2.5) = 18 ± 3, venlafaxine (10.0) = 21 ± 3, and venlafaxine (40.0) = 24 ± 3, *F*(4,47) = 0.7, *P* > 0.05. In comparison, imipramine = 5 ± 2, *P* < 0.05. Venlafaxine did not significantly modify intertrial crossings on any session (not shown). It also did not significantly affect exploratory crossings on sessions 2 and 3, although doses of 10.0 and 40.0 significantly reduced exploratory crossings on session 1; control nonstressed = 18 ± 1, vehicle stressed = 18 ± 1, venlafaxine (0.63) = 19 ± 1, venlafaxine (2.5) = 17 ± 1, venlafaxine (10.0) = 15 ± 1, and venlafaxine (40.0) = 13 ± 1, *F*(4,47) = 6.0, *P* < 0.001.

To summarize, the parameters of both escape failures and
intertrial crossings demonstrated that S33005 (10.0 mg/kg) was active in this procedure, whereas venlafaxine was ineffective. Furthermore, the actions of S33005 were selective inasmuch as it did not affect exploratory crossings.

**Influence upon Marble-Burying Behavior in Mice.** S33005 dose dependently reduced marble-burying behavior in mice, an action likewise seen with venlafaxine, clomipramine, citalopram, and reboxetine (Fig. 8; Table 1).

**Influence upon Aggressive Behavior in Mice.** In pairs of preisolated, unfamiliar mice, placement of the intruder mouse into the cage of the resident elicited aggressive behavior (Fig. 9; Table 1). Acute administration of S33005 dose dependently attenuated aggressive behavior as evaluated by the number and the duration of attacks. This action was reproduced less potently by venlafaxine, clomipramine, and citalopram. On the other hand, only high doses of reboxetine were effective.

**Induction of Ataxia in Mice.** In mice, administered up to a high dose (80.0 mg/kg, s.c.), S33005 did not markedly influence performance in the rotarod procedure (Fig. 10). Venlafaxine was also weakly active, while clomipramine, citalopram, and reboxetine elicited more pronounced effects in this procedure.

**Influence upon Spontaneous Locomotor Behavior in Rats.** S33005 did not significantly modify spontaneous locomotor behavior in rats (Fig. 10). Venlafaxine significantly decreased locomotor behavior, whereas the other agents did not significantly modify this parameter.

**Influence upon the Reduction of Sucrose Consumption Associated with Chronic Mild Stress.** Following exposure to chronic mild stress for 3 weeks, animals displayed a pronounced reduction in sucrose intake relative to control nonstressed rats (Fig. 11). Daily administration of S33005 was associated with a pronounced, dose- and time-dependent augmentation of sucrose consumption toward normal, control levels. Indeed, the highest dose of S33005 was significantly active after only 2 weeks of treatment. Venlafaxine also displayed a robust enhancement in sucrose consumption, although, at a low dose of 2.5, it was significantly active only after 5 weeks of administration. In comparison, at this dose, S33005 displayed significance at 3 weeks. S33005, imipramine, and venlafaxine did not significantly modify sucrose consumption in control nonstressed animals.

To summarize, S33005 elicited a dose- and time-dependent increase in sucrose intake in stressed rats. This action was specific inasmuch as S33005 did not significantly modify sucrose consumption in nonstressed animals. Venlafaxine behaved similarly to S33005 in this model, although, at a dose of 2.5 mg/kg, it was active only at week 5 as compared with week 3 for S33005.

**Discussion**

**Blockade of PCA-Induced Spontaneous Tail-Flicks and Reserpine-Induced Hypothermia.** Induction of spontaneous tail-flicks by PCA is prevented by interfering with its access to serotonergic neurones via SERTs (Bervoets et al., 1993). Correspondingly, in line with its ability to block depletion of cerebral pools of 5-HT by PCA (accompanying paper), almost identical doses of S33005 (ID50 values, 0.12 versus 0.18 mg/kg, s.c.) potently blocked induction of spontaneous tail-flicks by PCA. Citalopram, venlafaxine, and clomipramine (weakly) were similarly active, in distinction to reboxetine, which shows low affinity for SERTs. These data underpin the importance of SERTs to the functional profile of S33005. Following entry into neurones via NETs, the monoamine depletor, reserpine, elicits a diminution in core temperature (Pawlowski and Nowak, 1987; Popik, 1999). Accordingly, reboxetine potently blocked reserpine-induced hypothermia (Wong et al., 2000), an action mimicked by S33005 and venlafaxine (Muth et al., 1991). Reflecting their less potent interactions with NETs versus SERTs (accompanying paper), S33005 and venlafaxine attenuated reserpine-hypothermia only at doses markedly higher than those inhibiting PCA-induced spontaneous tail-flicks. Furthermore, inasmuch as citalopram potentiated induction of hypothermia by reserpine (see Results), the NET-mediated inhibition

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**Fig. 6.** Influence of S33005 as compared with reference antidepressant agents upon the induction of loss of righting reflex (LRR) by S18616 in rats. Values are percentage of rats displaying a score of less than or equal to 2 (see Materials and Methods), n > 6 per value. Asterisks indicate significance of differences to vehicle values in the Fisher’s exact probability test. *P < 0.05.

**Fig. 5.** Influence of S33005 as compared with reference antidepressant agents upon the duration of immobility in the tail-suspension test in mice. Values are means ± S.E.M., n > 6 per value. ANOVA is as follows. S33005, F(4,43) = 8.4, P < 0.01; venlafaxine, F(4,73) = 6.3, P < 0.01; clomipramine, F(3,29) = 4.4, P < 0.05; citalopram, F(5,62) = 7.3, P < 0.01; and reboxetine, F(5,70) = 7.0, P < 0.01. Asterisks indicate significance of differences to vehicle (VEH) values in Dunnett’s test following ANOVA. *P < 0.05.
of reserpine by S33005 and venlafaxine may be counteracted by their interaction with SERTs. This factor may also contribute to the failure of clomipramine to reduce reserpine-induced hypothermia, although its antagonist properties at α2-ARs are also relevant in this regard (Pawlowski and Nowak, 1987).

Fig. 7. Activity of S33005 as compared with imipramine in a learned helplessness paradigm in rats. IMI, imipramine; NLH, nonlearned helpless (nonstressed, control group); LH, learned helpless (stressed) groups. Values are means ± S.E.M., n = 12 per value. Open asterisks indicate significance of difference between LH and NLH values. For ANOVA of S33005 data, see Results. Closed asterisks indicate significance of differences of imipramine to vehicle (LH) values, and of S33005 to vehicle (LH) values in Dunnett’s test. *P < 0.05.

Fig. 8. Influence of S33005 as compared with reference antidepressant agents upon marble-burying behavior in mice. Values are means ± S.E.M., n = 5 per value. ANOVA is as follows. S33005, F(4,28) = 14.0, P < 0.001; venlafaxine, F(5,34) = 17.2, P < 0.01; clomipramine, F(3,21) = 55.5, P < 0.001; citalopram, F(4,34) = 6.6, P < 0.001; and reboxetine, F(4,25) = 10.9, P < 0.001. Asterisks indicate significance of drug to vehicle (VEH) values in Dunnett’s test following ANOVA. *P < 0.05.
Generalization to a Citalopram Discriminative Stimulus. Citalopram elicits a discriminative stimulus to which other SSRIs generalize, whereas antidepressants devoid of activity at SERTs are ineffective (Millan et al., 1999; Dekeyne et al., 2001). Clomipramine similarly generalized to citalopram, indicating that its actions at SERTs can be identified among its numerous other receptorial interactions (accompanying paper). Inasmuch as venlafaxine also generalized, its actions at SERTs are likewise recognized, and S33005, which displays more pronounced affinity for SERTs than venlafaxine, generalized over a lower dose range. These observations probably reflect interoceptive properties mediated centrally, although not necessarily antidepressant properties per se (Millan et al., 1999).

**Forced-Swim and Tail-Suspension Tests.** The forced-swim model consistently reveals activity of tricyclics and NE reuptake inhibitors, while the more variable actions of SSRIs may be a function of individual behaviors evaluated (Borsini Fig. 9. Influence of S33005 as compared with reference antidepressant agents upon aggressive behavior of isolated mice. Upper panels, number of attacks; lower panels, duration of attacks. Values are means ± S.E.M., n > 6 per value. ANOVA is as follows. Number of attacks: S33005, F(5,40) = 7.2, P < 0.01; venlafaxine, F(3,26) = 3.4, P < 0.05; clomipramine, F(3,24) = 7.8, P < 0.01; citalopram, F(4,27) = 5.8, P < 0.05; and reboxetine, F(3,32) = 11.2, P < 0.01. Duration of attacks: S33005, F(5,40) = 6.1, P < 0.01; venlafaxine, F(3,26) = 3.6, P < 0.05; clomipramine, F(3,24) = 8.4, P < 0.01; citalopram, F(4,27) = 6.0, P < 0.05; and reboxetine, F(3,32) = 7.2, P < 0.01. Asterisks indicate significance of differences to vehicle (VEH) values in Dunnett’s test following ANOVA. *P < 0.05.

Fig. 10. Influence of S33005 as compared with reference antidepressant agents upon motor behavior in mice and rats. Upper panels, induction of ataxia in the rotarod test in mice; lower panels, influence upon spontaneous locomotor behavior in rats. Values are means ± S.E.M., n ≥ 5 per value. ANOVA is as follows. Rotarod: S33005, F(3,27) = 3.9, P < 0.05; venlafaxine, F(4,38) = 3.3, P < 0.05; clomipramine, F(3,25) = 5.4, P < 0.01; citalopram, F(3,21) = 7.9, P < 0.01; and reboxetine, F(4,31) = 8.6, P < 0.001. Spontaneous locomotion: S33005, F(5,34) = 0.6, P > 0.05; venlafaxine, F(5,39) = 4.5, P < 0.01; clomipramine, F(4,31) = 0.4, P > 0.05; citalopram, F(4,30) = 0.3, P > 0.05; and reboxetine, F(4,25) = 1.2, P > 0.05. Asterisks indicate significance of drug to vehicle (VEH) values in Dunnett’s test following ANOVA. *P < 0.05.
and Meli, 1988; Renéric and Lucki, 1998; Popik, 1999). Herein, using the parameter of immobility, clomipramine and citalopram were effective, and the activity of reboxetine extends previous observations in rats (Connor et al., 1999; Cryan and Lucki, 1999; Wong et al., 2000). Actions of venlafaxine in the forced-swim model in rats are variable (Renéric and Lucki, 1998; West and Weiss, 1998; Connor et al., 2000), but its robust effects in mice (Redrobe et al., 1998) were confirmed herein. Accordingly, S33005 also expressed marked activity in this forced-swim model. The tail-suspension test is likewise responsive to tricyclics, NE reuptake inhibitors and, more consistently than the forced-swim paradigm, to SSRIs (Steru et al., 1987; Teste et al., 1993). Indeed, in corroboration of previous studies, citalopram (Steru et al., 1987), reboxetine (Wong et al., 2000), and clomipramine (Teste et al., 1993) all decreased immobility. Similarly, both S33005 and venlafaxine displayed robust activity, although in contrast to the forced-swim procedure, S33005 was more potent than venlafaxine.

S18616-Induced Loss of Righting Reflex. Hypnotic-sedative actions of the α₂-AR agonist, clonidine, which reflect suppression of adrenergic transmission via activation of α₂-AR autoreceptors (Hayashi and Maze, 1993; Millan et al., 2000b), are inhibited by antidepressants (Von Voigtländer et al., 1978). Accordingly, the loss of righting reflex elicited by the efficacious α₂-AR agonist, S18616 (Millan et al., 2000b), was potently inhibited by reboxetine. This action may involve elevation of extracellular levels of NE and activation of postsynaptic α₁-ARs facilitatory to motor activity (Hayashi and Maze, 1993). Nevertheless, citalopram was also active, indicating that serotonergic mechanisms are also pertinent. Thus, the mechanistic basis of the potent activity of S33005 (and venlafaxine) requires elucidation. The weak effects of clomipramine probably reflect its antagonist properties at α₁-ARs, blockade of which potentiates sedative properties of α₂-AR agonists (Hayashi and Maze, 1993; Millan et al., 2000b).

Learned Helplessness Paradigm. Although venlafaxine was ineffective, S33005 markedly suppressed escape failures in a learned helplessness paradigm at a dose of 10.0 mg/kg. In fact, the dose-response curve of S33005 was biphasic, in analogy to SSRIs (Martin et al., 1990), suggesting that serotonergic mechanisms may be involved in its actions. However, adrenergic mechanisms should not be discounted since the NE reuptake inhibitor, desipramine, is also active in this procedure (actions of reboxetine remain to be characterized). Interestingly, in analogy to SSRIs (Martin et al., 1990; Millan et al., 1997), S33005 increased intertrial intervals, a finding indicating a “disinhibitory” influence upon behavior (Martin et al., 1990). However, this does not represent a motor stimulant effect per se inasmuch as exploratory locomotion was not affected by S33005. Furthermore, S33005 enhanced neither locomotor behavior in rats nor mesolimbic dopamine release (accompanying paper). Thus, these observations are consistent with the view that antidepressant actions in the learned helplessness model reflect an influence not only upon “resignation”, but also upon “psychomotor retardation” (Willner, 1991; Thiebot et al., 1992).

Marble Burying. SSRIs such as citalopram (Fig. 8) suppress marble burying without disrupting general behavior (Njung’e and Handley, 1991; Sánchez and Meier, 1997). Although it remains to be established whether blockade of marble-burying behavior in mice is genuinely predictive of clinically relevant, anti-impulsive properties, this action is of interest in view of the increasing utility of SSRIs in the treatment of obsessive-compulsive disorders (Piccinelli et al., 1995; Blier and de Montigny, 1999; Pigott and Seay, 1999). The tricyclic, clomipramine, is likewise a well established therapeutic option for this disease (Piccinelli et al., 1995), and it potently suppressed marble-burying behavior. Clinical data concerning a potential influence of the NE reuptake inhibitor, reboxetine, upon impulsive disorders are not currently available, but adrenergic mechanisms were recently implicated in their control (Evenden, 1999). The potent inhibition of marble-burying behavior by reboxetine is, thus, of interest. Indeed, it is unclear whether the potent actions of S33005 (and venlafaxine) reflect serotonergic and/or adrenergic mechanisms.

Aggressive Behavior. Serotonergic mechanisms modulate a further mode of impulsive behavior, aggression, and clinical studies suggest anti-aggressive actions of SSRIs (Miczek et al., 1994; Edwards and Kravitz, 1997). Corre-
spondingly, SSRIs reduce isolation-induced aggression (White et al., 1991; Sánchez and Meier, 1997), and a marked inhibition of agonistic behavior was observed herein with citalopram. In comparison, acute administration of NE reuptake inhibitors only weakly reduces aggressive behavior (White et al., 1991; Mitchell and Redfern, 1992; Matsumoto et al., 1995). Indeed, reboxetine blocked aggressive behavior only at high doses compromising performance in the rotated test. This provides an interesting distinction to the marble-burying paradigm in which reboxetine was highly active. Tricyclics, such as clomipramine, suppress aggression in isolated mice (White et al., 1991), an observation reproduced herein. Venlafaxine was likewise effective, in line with Mitchell and Redfern (1992) and, similarly, S33005 was potently active in this model. Like other antidepressants, S33005 displays anxiogenic activity upon acute administration (A. Dekyne, unpublished observations), and benzodiazepines are ineffective in this model at nonsedative doses (White et al., 1991; Miczek et al., 1994). Thus, potential anxiolytic properties do not underlie this action of S33005. However, the relationship between serotonergic activity, aggressive behavior, and depressive states is not completely understood (Mitchell and Redfern, 1992; Cases et al., 1995).

**Restoration of Suppressed Sucrose Consumption Associated with Chronic Mild Stress.** Repetitive exposure to mild stressors reduced consumption of sucrose, an effect dose dependently reversed by S33005 (and venlafaxine), concerning both magnitude and onset of action. Like tricyclics and SSRIs (Willner, 1997), S33005 may, then, increase the incentive value of reward. As such, these data are relevant to its potential utility in the treatment of the melancholic component of depressive states. Anhedonia associated with chronic mild stress involves a perturbation of activity at mesolimbic D2 receptors (Papp et al., 1994; Willner, 1997). Thus, although S33005 does not increase mesolimbic DA release (accompanying paper), a potential role of (postsynaptic) dopaminergic mechanisms in its anti-anhedonic actions justifies investigation.

**General Discussion.** The present studies raise several general comments. First, the present and accompanying papers constitute an extensive and homogeneous body of neurochemical and behavioral data characterizing actions of several prototypical antidepressants: citalopram (SSRI), reboxetine (NE reuptake inhibitor), venlafaxine (5-HT/NE reuptake inhibitor), and clomipramine (tricyclic). As such, they constitute an optimal framework for interpretation of the functional profile of S33005. In this regard, S33005 clearly resembles venlafaxine and clomipramine. However, in line with its more pronounced activity at native rat and cloned human SERTs and NETs (accompanying paper), S33005 was more potent than the former in the behavioral models used herein, and it lacks the latter’s action at other receptor types. The contrasting functional profile of S33005 in comparison with citalopram and reboxetine should also be emphasized.

Second, this study used a broad range of paradigms, including models of cardinal symptoms of depression. The activity of S33005 in all procedures mimics venlafaxine, which is distinguished by marked clinical efficacy and, possibly, rapid onset of action (see Introduction). Whether S33005 shows such a favorable therapeutic profile necessitates clinical verification.

Third, as outlined under the Introduction, and in line with other investigations, these studies exploited many paradigms in which antidepressant agents (notably, venlafaxine) exert their actions upon acute administration. While the robust activity of S33005 in all of these procedures strongly suggests antidepressant properties, procedures using single administration obviously do not mimic the clinical requirement for repeated administration and cannot provide insights into the delay of onset of action. In this regard, the learned helplessness model (5 days) and, in particular, the chronic mild stress (5 weeks) paradigm, both of which demonstrated sustained actions of S33005 upon recurrent administration, are of pertinence. The marked activity of long-term (2 weeks) administration of S33005 in a model of olfactory bulbectomy-induced hyperactivity should also be mentioned in this regard (C. MacSweeny, unpublished observations).

Furthermore, the chronic mild stress protocol suggests that, at a low dose, S33005 acts relatively rapidly, an assertion underpinned by studies of diverse biochemical markers (accompanying paper and T. Sharp, unpublished observations). Nevertheless, additional studies of chronic administration of S33005 are necessary to further characterize its long-term actions.

Fourth, inasmuch as several models predictive of antidepressive activity are responsive to agents acting at SERTs or NETs, an intriguing question concerns their relative contribution to actions of S33005. This may depend upon the active dose range. Indeed, venlafaxine may act principally via serotonergic mechanisms at low doses, yet recruit adrenergic mechanisms at higher doses (Redrobe et al., 1998; Réneric and Lucki, 1998; Porter et al., 1999; Béique et al., 2000; Harvey et al., 2000). The role of individual receptor types also requires clarification since there is a complex and divergent body of data implicating diverse serotonergic and adrenergic receptors in the mediation of antidepressant properties (see Millan et al., 2000a).

Finally, at doses expressing behavioral and neurochemical (accompanying paper) actions indicative of antidepressant properties, S33005 did not perturb motor performance. This separation was more marked than for other agents, indicating that S33005 possesses a substantial therapeutic window to generalized disruption of behavior.

**Conclusion.** In line with its actions at SERTs and (less potently) NETs, S33005 displays marked activity in diverse models indicative of antidepressant properties. S33005 can be distinguished from citalopram and reboxetine, and it closely resembles the 5-HT/NE reuptake inhibitor, venlafaxine. However (see also accompanying paper), S33005 is more potent, displays a broader pattern of antidepressant activity, may possess a more rapid onset of action, and displays a particularly marked therapeutic window. Furthermore, S33005 is more potent than clomipramine, and it lacks the latter’s interaction with receptors other than 5-HT/NE transporters. Together with the accompanying paper, this extensive receptorial, neurochemical, electrophysiological, and behavioral characterization suggests that S33005 should be a potent and effective antidepressant, a hypothesis requiring clinical evaluation.
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References


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