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Preclinical Profile of the Mixed 5-HT_{1A}/5-HT_{2A} Receptor Antagonist S 21357

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GRIEBEL, G., D. C. BLANCHARD, M.-C. RETTORI, B. GUARDIOLA-LEMAÎTRE AND R. J. BLANCHARD. *Preclinical profile of the mixed 5-HT_{1A}/5-HT_{2A} receptor antagonist S 21357.* PHARMACOL BIOCHEM BEHAV **54**(2) 509–516, 1996.—This study evaluated the pharmacological and behavioral effects of S 21357, a drug with high affinity for both 5-HT_{1A} and 5-HT_{2A} receptors. The drug behaved as antagonist at both 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors, as it prevented the inhibitory effect of lesopitron on the electrical discharge of the dorsal raphé nucleus (DRN) 5-HT neurons and the activity of forskolin-stimulated adenylate cyclase in hippocampal homogenates. In addition, S 21357 (4 and 128 mg/kg, PO) inhibited 5-HTP–induced head-twitch responses in mice, indicating that it possesses 5-HT_{2A} antagonistic properties. In a test battery designed to assess defensive behaviors of Swiss–Webster mice to the presence of, or situations associated with, a natural threat stimulus (i.e., rat), S 21357 (0.12–2 mg/kg, IP) reduced contextual defense reactions after the rat was removed, risk assessment activities when the subject was chased, and finally, defensive attack behavior. These behavioral changes are consistent with fear/anxiety reduction. Furthermore, the drug strongly reduced flight reactions in response to the approaching rat. This last finding, taken together with recent results with panic-modulating drugs, suggest that S 21357 may have potential efficacy against panic attack. Finally, our results suggest that compounds sharing high affinities for both 5-HT_{1A} and 5-HT_{2A} receptors may directly or synergistically increase the range of defensive behaviors affected.

S 21357 Mixed 5-HT_{1A}/5-HT_{2A} antagonist Serotonin 5-HT_{1A} receptor Antipredator defense Anxiety Flight Predator assessment Panic Head twitch

A substantial body of evidence points to an important role of the central 5-HT system in the pathophysiology of anxiety disorders. The strongest support for this comes from studies demonstrating that a variety of drugs modulating the release of 5-HT are either effective in treating anxiety disorders or that they induce or potentiate anxious responses. Among these, 5-HT_{1A} ligands and 5-HT_{2A} receptor antagonists have been of particular interest because clinical studies indicate that these drugs ameliorate anxiety disorders. In particular, treatment with 5-HT_{1A} receptor ligands, such as buspirone, gepirone, and ipsapirone, have been demonstrated to be effective in generalized anxiety disorder (GAD) [e.g., (7,11,16)] and buspirone also improved phobic anxiety [e.g., (35)]. These drugs behave as partial agonists having an agonist action at presynaptic (somatodendritic) receptors and a predominantly antagonist action with weak agonist activity at postsynaptic 5-HT_{1A} receptors (6). Furthermore, the nonselective 5-HT_{2A} receptor antagonist ritanserin was reported to be effective in several small studies of patients with GAD (2,8,12) and agoraphobia (27).

Several authors have recently suggested that pharmacological interactions of drugs combining both 5-HT_{1A} and 5-HT_{2A} properties may be of particular significance in the clinical management of anxiety, as they might increase therapeutic response (1,3,4,10,33,34). For instance, Millan and Brocco (33) demonstrated that the administration of S 14506 or S 14671, two drugs manifesting high efficacy and high potency at 5-HT_{1A} receptors and sharing similar affinities with 5-HT_{2A}

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binding sites, elicited anxiolytic-like effects superior to those of 5-HT_{1A} receptor ligands, 5-$HT_{2A/2C}$ receptor antagonists or benzodiazepines (BZPs).

In the present study, we report the preclinical pharmacology of a recently synthesized 5-HT receptor ligand, S 21357, which displays high affinity for both 5-HT_{2A} (K_i = 2 nM) and 5-HT_{1A} (K_i = 7 nM) receptors, and which shows moderate to low affinity for other 5-HT binding sites [for more details, see (39)]. First, we evaluated the effects of this compound in appropriate electrophysiological, biochemical, and behavioral tests to assess its potential antagonistic properties at the level of both somatodendritic and postsynaptic 5-HT_{1A} receptors as well as 5-HT_{2A} binding sites. Second, in a test battery designed to assess defensive behaviors of Swiss–Webster mice to the presence of, or situations associated with, a natural threat stimulus (i.e., rat), we investigated potential anxiolytic-like effects of S 21357.

METHOD

Drugs

The following drugs were used: S 21357 (trihydrochloride of 3-(-2-[-4-(-4-fluorobenzoyl)-piperidino]-ethyl)-6-4-[-4-(2-methoxyphenyl)1-piperazinyl]-1-nbutyl)benzothiazolin-2-one), lesopitron (E-4424, Labs Dr Esteve, Barcelona, Spain), forskolin (Calbiochem, Los Angeles, CA), 5-HTP (Sigma, St. Louis, MO), and cyproheptadine (Aldrich, St.-Quentin-Fallavier, France). [^{32}P]α-ATP (10–20 Ci/mmol) was from Amersham International, Ltd. (Buckinghamshire, UK).

Adenylate Cyclase Assays

Adult male Sprague–Dawley rats (250–350 g) were treated with reserpine (5 mg/kg IP) 24 h before killing so as to deplete endogenous 5-HT stores to levels producing no direct effect on 5-HT_{1A} receptor-coupled adenylate cyclase activity. Assays were performed on hippocampal membranes, which were incubated for 20 min at 30°C with forskolin (10 μM), [^{32}P]α-ATP (0.1 mM), lesopitron (3 μM) and S 21357 (10^{-9} to 10^{-5} M). Lesopitron was used because it binds to 5-HT_{1A} receptors with a relatively high affinity and behaves as full agonist at both pre- and postsynaptic 5-HT_{1A} receptors (23). The enzymatic activity was estimated from the conversion of [^{32}P]α-ATP into [^{32}P]cyclic AMP at the end of the incubation period [for more details, see (32)].

In Vitro Recording of the Electrical Activity of Dorsal Raphé Nucleus Serotonergic Neurons in Brain Slices

The experiment was performed using brain stem slices (0.3 mm thick), from young Sprague–Dawley rats (125–150 g). Slices were continuously perfused at 35°C, with an artificial cerebrospinal fluid (ACSF). Extracellular recordings were made with a single-barrel micropipette of 15 MΩ of impedance, filled with 2 M NaCl. When a cell was recorded, it was identified on line as being a serotonergic neuron when its electrical activity fulfilled the standard criteria (45). The electric signals were fed into a high-input impedance amplifier, an oscilloscope and an electronic ratemeter triggered by individual neuronal spikes. The integrated firing rate was computed and recorded graphically as consecutive 10 s samples. The effect of S 21357 (30 nM) was evaluated in the presence of 100 nM of lesopitron by comparing the mean discharge frequency during 2 min just before any treatment (baseline record), and for 2–5 min after their addition to the superfusing ACSF, when the resulting changes in firing frequency reached their maximal amplitude [for more details, see (32)].

Head-Twitch Behavior

Male Swiss mice (60–75 days old, Centre d'Elevage R. Janvier, France) were used in this experiment. They were housed by groups of five in polycarbonate cages in a room maintained under a 12 L : 12 D cycle. The number of head twitches was recorded during the time interval 10–20 min after subcutaneous (SC) administration of 5-HTP (400 mg/kg). S 21357 (4 and 128 mg/kg, per os (PO), n = 6) and cyproheptadine (16 mg/kg, intraperitoneal (IP), n = 6) were injected 20 min before 5-HTP administration. The data were evaluated by Student's t-test [for more details, see (37)].

Mouse Defense Test Battery

Subjects were 50 naive male Swiss–Webster mice obtained from Simonsen Laboratories (CA), 60–75 days old, and 2 male Long–Evans rats (400–500 g) bred in the laboratory. They were housed singly in polycarbonate cages in a room maintained under a 12 L : 12 D cycle. Mice were randomly assigned to following conditions: control group (n = 20) and drug treatment groups (0.12, 0.5, and 2 mg/kg; n = 10). The drug was administered IP 30 min before the experiment was carried out. Stimulus rats were deeply anaesthetized with pentobarbital (40 ml/kg, IP).

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 6.0 m in total length, consisting of two 2 m straight segments joined by two 0.4 m curved segments and separated by a median wall (2.0 × 0.30 × 0.06). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with videocameras mounted above the apparatus. Experiments were performed under red light between 1300 and 1700 h.

Contextual defense test. This situation involves reactivity to stimuli associated with potential threat rather than the actual presence of an approaching predator. Subjects were placed into the runway for a 3-min familiarization period, in which line crossings, wall rears, wall climbs, and jump escapes were recorded (min 1 to 3). The same behavioral parameters were also recorded during an equivalent period following tests involving exposure to a predator (posttest) (min 12 to 14). Changes in the latter three (escape) measures during the post-predator period provide an index of contextual defense.

Reactions to the predator: (a) predator avoidance test (min 4 to 6). Immediately after the 3-min familiarization period, the hand-held stimulus rat was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. The experimenter stood adjacent to the runway while holding the anaesthetized rat. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. This was repeated five times.

(b) Chase/flight test (min 7 to 8). The hand-held rat is brought up to the subject at a speed of approximately 2.0 m/s. The time it took to chase the subject a distance of 15 m was recorded. Overall flight speed (m/s) and maximum flight speed (measured when the subject is running straight over a 1-m segment) were subsequently calculated from these mea-

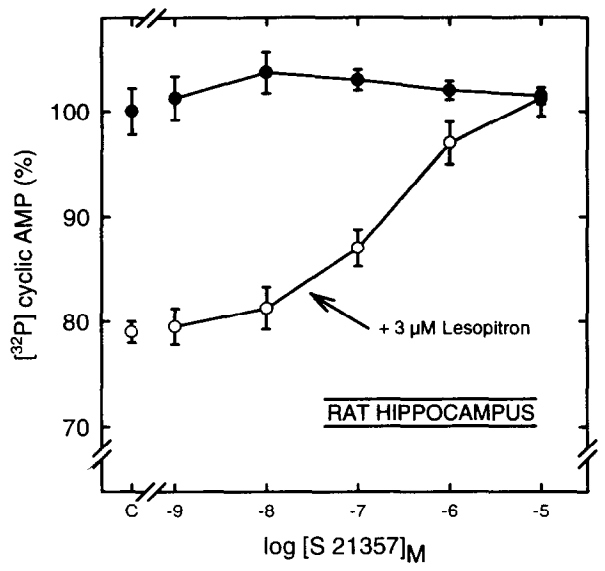

FIG. 1. Effects of S 21357 on the inhibition by lesopitron of forskolin-stimulated adenylate cyclase in rat hippocampal membranes. Adenylate cyclase activity is expressed as percentage [^{32}P]cyclic AMP synthesized in 20 min at 30°C. Assays were carried out with various concentrations of S 21357 in the presence (○) or the absence (●) of 3 μM lesopitron. Data represent mean ± SEM. C on absissa: assays without S 21357 or lesopitron.

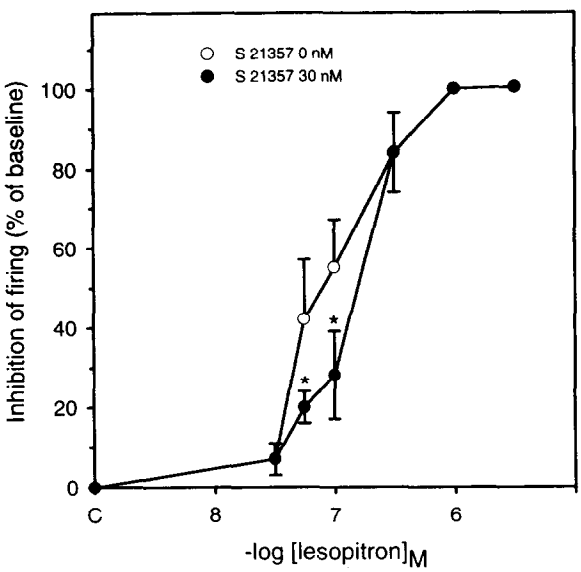

FIG. 3. Concentration-dependent inhibition by lesopitron of the firing of serotonergic neurons in the presence (●) or the absence (○) of 30 nM S 21357. Drugs were applied as shown in Fig. 2. The inhibition due to lesopitron is expressed as a percentage of the baseline firing rate. Data represent mean ± SEM. C on absissa: no lesopitron.

sures. In addition, the following parameters were recorded: number of stops (pause in movement), orientations (subject stops, then orientates the head toward the rat), and reversals (subject stops, then runs in the opposite direction).

(c) Straight alley (min 9 to 11). The runway was then converted to a straight alley by the closing two doors at both ends. Three approaches, 15 s each, respectively at 1.20, 0.80, and 0.40 m were made by a hand-held rat toward the subject in this inescapable runway. Measures taken included immobility time, closest distance between the subject, and the rat and the number of approaches/withdrawals (subject must move more than 0.2 m forward from the closed door, then return to it). Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites, vocalizations, upright postures and jump attacks by the subjects were noted. This was repeated three times.

(d) Ledge test (min 15). Subjects were placed on the median wall of the runway and their inability to remain on the ledge for 30 s was scored as an indication of potential myorelaxant activity.

Data were analyzed by a one-way analysis of variance (ANOVA) or the nonparametric Kruskal-Wallis ANOVA for some infrequently occurring or highly variable behaviors. Subsequent comparisons between treatment groups and control were carried out using Newman-Keuls procedures or the nonparametric Mann-Whitney *U*-test. In the contextual defense test, differences were evaluated by a combined repeated measures ANOVA followed by a Newman-Keuls posthoc comparison or by the Mann-Whitney *U*-test and Wilcoxon matched pair test if the behavior occurred infrequently. Data from the ledge test were analyzed by a χ^2 procedure.

RESULTS

Postsynaptic 5-HT$_{1A}$ Receptors: Influence on Lesopitron-Induced Inhibition of Adenylate Cyclase Activity in Hippocampal Homogenates

Figure 1 shows that S 21357 (10^{-8} to 10^{-5} M) prevented the inhibition of lesopitron-induced inhibition of adenylate cyclase activity in a concentration-dependent manner.

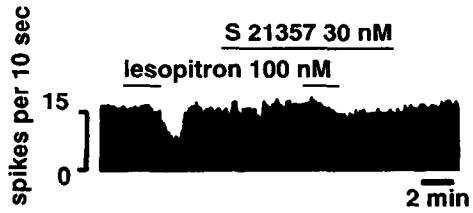

FIG. 2. Prevention by S 21357 of the inhibitory effect of lesopitron on the nerve impulse flow within serotonergic neurons in the dorsal raphe nucleus.

TABLE 1

EFFECT OF S 21357 AND CYPROHEPTADINE ON 5-HTP (400 mg/kg, SC)-INDUCED HEAD TWITCHES IN THE MOUSE

Treatment	Dose (mg/kg)	Head-Twitch Antagonism (%)	*n*
Cyproheptadine (IP)	16	100*	6
S 21357 (PO)	4	54*	6
	128	91*	6

*$p < 0.001$.

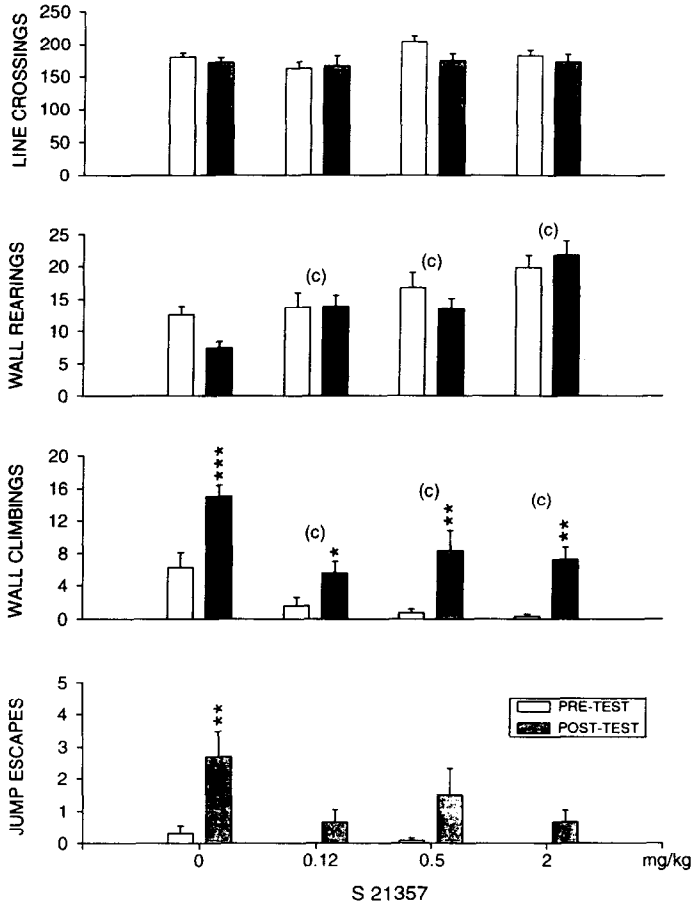

FIG. 4. Effects of S 21357 on the frequency of four response measures before (pre-test) and after (post-test) the exposure to the predator. Data are presented as means (SEM). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.0001$ (vs. pretest); (c) (vs. vehicle control).

Presynaptic 5-HT$_{1A}$ Receptors: Prevention by S 21357 of Lesopitron-Induced Inhibition of Serotonergic Neurons in the Dorsal Raphé Nucleus (DRN)

Figure 2 shows that superfusion of the brain slice with ACSF containing lesopitron produced an inhibition of the firing of 5-HT neurons. S 21357 (30 nM) significantly reduced the inhibitory effect of lesopitron on cell firing. Further characterization of the interaction between the two compounds indicated that in the presence of 30 nM S 21357, the concentration curve for the inhibition by lesopitron of the nerve impulse flow within these neurons was shifted to the right. However, high concentration of lesopitron still induced complete blockade of the electrical activity of serotonergic neurons as expected from competitive interaction between the two drugs (Fig. 3).

Evaluation of 5-HT$_{2A}$ Receptor-Mediated Behavior: Effect on 5-HTP-Induced Head-Twitch Behavior

Cyproheptadine (16 mg/kg) completely abolished 5-HTP induced head-twitch responses in mice, while S 21357 antagonized 54% of them at the low dose (4 mg/kg) and 91% at 128 mg/kg (Table 1).

Evaluation of the Anxiolytic-Like Activity: The Mouse Defense Test Battery

Contextual defense: locomotor activity before and after exposure to the predator (fig. 4). Drug effect: comparisons (ANOVA) of S 21357 treatment and saline control group failed to show a reliable main effect for frequency of line crossings, $F(3, 52) = 1.94$, and jump escapes, $F(3, 52) = 7.52$, but indicated that the drug had a significant overall effect on wall rearing, $F(3, 52) = 13.2$, $p < 0.001$, and wall climbing [Kruskal-Wallis ANOVA: $H(3, 56) = 19.4$, $p < 0.002$]. Newman-Keuls comparisons indicated a reliable increase in the number of wall rearings at each dose level, and Mann-Whitney test showed that the frequency of wall climbings were significantly decreased, again at each dose level used.

Pre/postpredator exposure differences: predator exposure produced a reliable effect on wall climbing (Wilcoxon pair test: $p < 0.0000001$) and on jump escape (Wilcoxon pair test: $p < 0.00007$), but failed to alter the number of line crossings, $F(1, 52) = 2.62$, and the frequency of wall rearings, $F(1, 52) = 1.75$.

Dose-related pre- vs. posttest comparisons: 4 × 2 (dose × pre/posttest) ANOVA failed to reveal a reliable interaction for

line crossing, $F(3, 52) = 0.94$, or wall rearing, $F(3, 52) = 1.89$. Friedman ANOVA indicated reliable effects on wall climbing, $N(1, 56) = 38.7$, $p < 0.00001$, and jump escape, $N(1, 56) = 20.2$, $p < 0.00001$, and subsequent analyses (Wilcoxon pair test) showed a posttest increase in these two measures, which occured at all doses with respect to wall climbing and only in saline-treated group for the number of jump escapes.

Reactions to the predator. Predator avoidance test (Fig. 5): ANOVA revealed that both behavioral measures were significantly affected by drug treatment [number of avoidances: $H(3, 56) = 9.18$, $p < 0.03$; avoidance distance: $F(3, 50) = 7.42$, $p < 0.0003$]. Post hoc comparison showed that the drug reduced the prey-predator distance at all doses and decreased the number of avoidances at 0.12 and 0.5 mg/kg.

Flight/predator orientation test (Table 2): ANOVA failed to reveal a reliable drug effect on overall flight speed, $F(3, 51) = 0.61$, maximum flight speed, $F(3, 51) = 1.68$, arrests in movement, $F(3, 51) = 0.94$, and orientation to the predator [Kruskal-Wallis: $H(3, 55) = 2.08$] but indicated a reliable main effect on the frequency of reversals [Kruskal-Wallis: $H(3, 55) = 15.1$, $p < 0.002$]. This latter behavior was significantly inhibited by the drug at all doses.

Predator approach: straight alley (Table 3): ANOVA failed to reveal any reliable main effects of treatment with S 21357 [closest distance between animals: $F(3, 52) = 0.18$; immobility time: $F(3, 52) = 1.66$; number of approaches/withdrawals: $H(3, 56) = 1.26$].

Forced contact with the predator (Table 4): ANOVA indicated a reliable effect for frequency of biting to the rat [Kruskal-Wallis: $H(3, 56) = 13$, $p < 0.005$], but not for the occurence of upright posture [Kruskal-Wallis: $H(3, 56) = 4.2$], frequency of vocalizations [Kruskal-Wallis: $H(3, 56) = 7.47$] or jump attacks toward the predator [Kruskal-Wallis: $H(3, 56) = 5.73$]. Subsequent Mann-Whitney U-tests revealed significant reductions in biting at all doses tested.

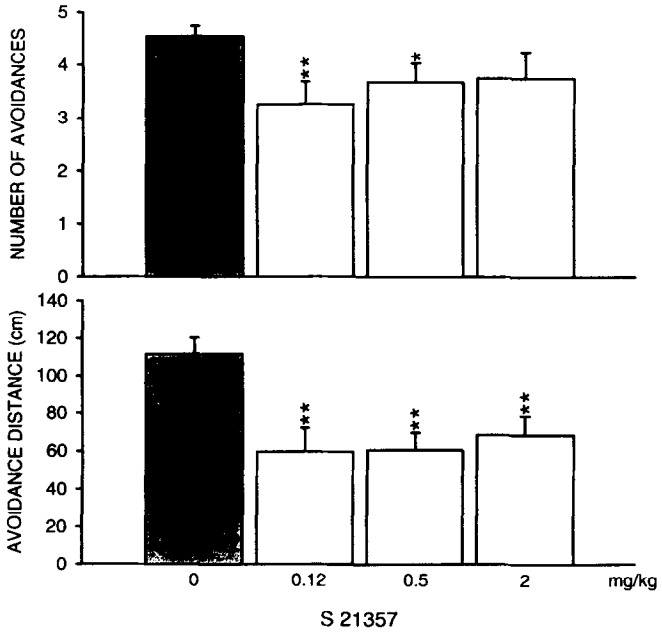

FIG. 5. Runway measures of avoidance to an approaching predator for mice administered S 21357. Data represent mean ± SEM. *$p < 0.05$ and **$p < 0.01$ (vs. vehicle control).

Ledge test. S 21357 did not affect the behavior of mice in this test situation. In fact, only one fall was observed at 0.12 mg/kg.

DISCUSSION

Pharmacological Characterization

The present findings clearly showed that S 21357 possess 5-HT_{1A} antagonistic properties. In the rat hippocampus, postsynaptic 5-HT_{1A} receptors are negatively coupled to adenylate cyclase (25), and the effects of 5-HT_{1A} receptor ligands on the forskolin-stimulated enzyme are of value for assessing their potential agonist/antagonist properties. For instance, the 5-HT_{1A} receptor agonist 8-OH-DPAT, S 20499, and lesopitron inhibit forskolin-stimulated adenylate cyclase activity in rat hippocampal membranes, and this effect can be counteracted by nonselective 5-HT_{1A} receptor antagonists (32). In the present study, S 21357 prevented the inhibition due to lesopitron suggesting that both drugs interact at the same sites (i.e., postsynaptic 5-HT_{1A} receptors).

In order to demonstrate that S 21357 displays antagonistic properties at somatodendritic 5-HT_{1A} receptors, an electrophysiological investigation was performed in vitro using brain stem slices (24). Previous studies have confirmed that such investigations allow the clear-cut identification of agonist/antagonists acting at 5-HT_{1A} autoreceptors within the rat dorsal raphé nucleus (30,31). The present data with S 21357 showed that the drug efficiently antagonized the effect of lesopitron on somatodendritic 5-HT_{1A} autoreceptors. Thus, the reduction in the firing rate due to 100 nM lesopitron no longer occurred in the presence of 30 nM S 21357. Furthermore, the concentration curve for lesopitron-inducing inhibition was shifted to the right when the superfusing ACSF was supplemented with 30 nM S 21357, indicating a competitive interaction between the two drugs at the same receptors.

S 21357 has fourfold selectivity for the 5-HT_{2A} (K_i = 2 nM) over the 5-HT_{1A} (K_i = 7 nM) receptor and has even greater selectivity over all other 5-HT receptor tested (39). The present data showed that it potently antagonized 5-HTP-induced head-twitch behavior, a putative model of central 5-HT_{2A} receptor function (47). Together, these findings suggest that S 21357 also possesses 5-HT_{2A} antagonistic properties.

Behavioral Characterization

These MDTB results show that S 21357 produce complex patterns of behavior change that differ from those seen with other 5-HT receptor ligands (21,22). These differences may most parsimoniously be attributed to the differential selectivity of these ligands for 5-HT binding sites.

Myorelaxation and sedation measures. In previous studies using the MDTB (19,22), drug effects on measures of sedation and/or myorelaxation (wall rearings, line crossings, maximum flight speed, and falls from the wall) were very similar, with a good relationship between those drug doses at which wall rearings, line crossings, and flight speed decreased, and falls increased. In the present study, evidence for sedation and/or myorelaxation was minimal, with a general lack of effect of S 21357 on these responses. Only wall rearings were significantly changed during both pre- and both tests, but they were increased.

Effects preceding and following predator exposure: contextual defense. The present findings confirm earlier observations (18,19,22) of behavior change in mice, after presentation and removal of a predator. These changes included an increase in wall climbing and jump escape against the wall in the post-

TABLE 2

EFFECTS OF S 21357 ON BEHAVIORAL RESPONSES OF MICE CHASED BY A PREDATOR

	Overall Speed (m/s)	Maximum Speed (m/s)	Frequency of Stops	Frequency of Orientations	Frequency of Reversals
0	0.58 ± 0.05	1.11 ± 0.04	10.15 ± 0.99	4.05 ± 0.69	2.90 ± 0.52
0.12 mg/kg	0.54 ± 0.07	1.08 ± 0.06	8.25 ± 0.83	3.33 ± 0.54	1.33 ± 0.59*
0.5	0.60 ± 0.05	1.00 ± 0.04	8.17 ± 1.30	2.75 ± 0.55	0.50 ± 0.36†
2	0.68 ± 0.11	1.15 ± 0.04	8.18 ± 1.31	3.00 ± 1.04	0.91 ± 0.41‡

Data represent mean ± SEM.
*$p < 0.05$, †$p < 0.0001$, ‡$p < 0.01$ (vs. vehicle control).

predator period, compared to an equivalent period prior to the introduction of the predator.

S 21357 counteracted the potentiation of jump escapes during the postpredator period, and reduced the postpredator frequency of wall climbing, relative to saline controls, while significantly increasing the frequency of wall rearing during the pre- and postrat period. This pattern of effects suggests that the drugs induced a shift from a more intense defensive response (jump escapes and wall climbings) to a less intense one (wall rearings). In fact, given that S 21357 increased prerat wall rearing at least as much as postrat wall rearing, there was no evidence of a selective drug-associated increase in contextual defense, even for the wall rearing measure. These findings thus indicate that S 21357 consistently reduce contextual fear/anxiety, countering the postpredator potentiation of escape attempts. The data are very similar to previous findings with the recently synthesized 5-HT_{1A} receptor antagonist S 21187 in this test (22). In addition, the present data also resemble those we obtained with 8-OH-DPAT and gepirone (21). However, unlike S 21357, the latter also strongly decreased spontaneous horizontal and vertical activities, suggesting that some components of the mouse 5-HT syndrome might be involved in its reduction of contextual defensive behaviors (21).

Drug effects during exposure to the predator. S 21357 attenuated flight responses as it reduced the prey-predator distance at which flight occurred, at all doses tested and the avoidance frequency at 0.12 and 0.5 mg/kg. Several authors have identified flight/avoidance reactions of rodents as panic-like (17,26) and, therefore, suggested that an experimental model of flight responses may have face validity as model of panic attacks (PA). This view is further supported by clinical observations indicating that panic disorder patients often report intense desire to flee or escape from the place the PA is occurring (13). In addition, electrical stimulation of the hypothalamic-periaqueductal gray (PAG) fight-flight system in man elicits symptoms and autonomic changes that closely resemble panic (41). The extensive pharmacological evaluation of the MDTB has clearly demonstrated that panic-modulating agents specifically affect animals' flight responses with panicogenic treatment increasing flight and panicolytic drug challenge decreasing it (18,19,21). In this context, the present results suggest that S 21357 may have some efficacy in reducing PA.

This effect on flight might be attributable to the 5-HT_{1A} antagonist component of the drug. Preclinical as well as human studies provide consistent evidence of a lack of efficacy of 5-HT_{1A} receptor agonists in flight/panic reactions (28,29, 38,40,42–44). This view is further supported by recent data from the MDTB showing that the 5-HT_{1A} receptor full agonist 8-OH-DPAT failed to affect flight responses. Moreover, clinical studies revealed that panic attacks may even be exacerbated by 5-HT_{1A} receptor agonists (9,15,36,46). Based on these latter findings, several authors have recently suggested that blockade of the 5-HT_{1A} receptor might prevent panic reactions (14). The flight-reducing action recently observed in the MDTB with the novel 5-HT_{1A} receptor antagonist S 21187 supports this view (22). An alternative explanation of the flight-reducing action of S 21357 might be that the effect is mediated through action at the 5-HT_{2A} receptor. This view is supported by a recent finding in the MDTB, that the preferential 5-HT_{2A} receptor antagonist pirenperone reduced flight responses (21). In addition, in Graeff's (17) paradigm in which the activation of the rat dorsal periaqueductal gray (DPAG) leads to behavioral manifestations identified as panic-like, the 5-HT_{2A} receptor blockers ketanserin and pirenperone dose dependently increased the aversive threshold of DPAG stimulation (28,29).

When chased by a predator, mice treated with S 21357 tended to display less predator assessment activities. A similar behavioral profile was obtained with the administration of

TABLE 3

EFFECTS OF S 21357 IN THE STRAIGHT ALLEY ON BEHAVIORAL REACTIONS TO A PREDATOR THAT REMAINS AT CONSTANT DISTANCE FROM THE SUBJECT

	Frequency of Approaches/Withdrawals	Closest Distance Between Animals (cm)	Immobility Time
0	4.00 ± 0.40	87.40 ± 11.28	3.90 ± 0.91
0.12 mg/kg	3.50 ± 0.44	99.67 ± 13.52	7.41 ± 2.03
0.5	3.83 ± 0.58	93.33 ± 15.15	7.91 ± 2.75
2	4.00 ± 0.46	95.50 ± 10.79	4.16 ± 0.82

Data represent mean ± SEM.

TABLE 4

MEAN FREQUENCY OF BITING, DEFENSIVE THREAT VOCALIZATION, UPRIGHT POSTURE, AND JUMP ATTACKS TO FORCED CONTACT WITH A DEEPLY ANESTHETIZED RAT FOR SUBJECTS UNDER VARYING DOSES OF S 21357

	Vocalization	Upright Posture	Jump Attack Toward Rat	Biting To Rat
0	3.00 ± 0.00	2.55 ± 0.14	0.10 ± 0.24	2.90 ± 0.10
0.12 mg/kg	2.67 ± 0.26	1.83 ± 0.37	0.37 ± 0.23	1.83 ± 0.37*
0.5	3.00 ± 0.00	2.08 ± 0.23	0.30 ± 0.30	2.00 ± 0.30†
2	3.00 ± 0.00	2.00 ± 0.30	0.30 ± 0.27	2.17 ± 0.30†

Data represent mean ± SEM.
*$p < 0.0001$ and †$p < 0.01$ (vs. vehicle control).

chlordiazepoxide and alprazolam (19,20). Because these data, in particular, agree with the rat response of reduced risk assessment with administration of classic BZPs in a situation in which controls show such assessment, we have interpreted changes in predator assessment as an especially important index of effect on anxiety (5). Thus, the present results provide some evidence of anxiolytic-like effects for S 21357. It is intriguing that, while both 8-OH-DPAT, gepirone, and S 21187 did reduce some predator assessment behaviors, they had no reliable effect on this particular threat assessment pattern (21). This is consonant with a view that the combination of 5-HT_{1A}/5-HT_{2A} properties within a single molecule may yield drugs superior to either 5-HT_{1A} receptor ligands or 5-HT_{2A} receptor antagonists as concerns their therapeutic potential.

Finally, S 21357 had a very clear impact on defensive biting, reliably reducing it at all dose levels. These results are consonant with previous findings of reduced biting for a variety of typical and atypical anxiolytic drugs including BZPs (5,20) and selective 5-HT_{1A} or 5-HT_{2A} agents (21).

In summary, the present study provides evidence of anxiolytic-like effects in the MDTB, for the mixed 5-HT_{1A}/5-HT_{2A} antagonist S 21357. The drug reduced contextual defense responses, predator assessment activities and defensive attacks without affecting motor responses. Also, in light of previous results with specific panic-modulating compounds, the flight-reducing action of the drug suggests that S 21357 represents a possible panicolytic agent. Finally, our results suggest that the combination of 5-HT_{1A} and 5-HT_{2A} properties may, through either direct and independent effects or synergistically, expand the range of defensive behaviors affected by 5-HT_{1A} receptor ligands or 5-HT_{2A} receptor antagonists. Insofar as target symptoms of psychopathology reflect a variety of different defensive behaviors, this feature might be of particular significance in the clinical management of such disorders.

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