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Anxiogenic-like effect of serotonin$_{1B}$ receptor stimulation in the rat elevated plus-maze

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Abstract

Perturbations in serotonin [5-hydroxytryptamine (5-HT)] neurotransmission have been implicated in several psychiatric illnesses including depression and anxiety disorders. It is not yet clear, however, which of the 14 currently identified 5-HT receptor subtypes in the brain participate in the regulation of emotional states. This study investigates a role for the 5-HT$_{1B}$ receptor subtype in anxiety-related behaviors using the elevated plus-maze paradigm in rats. The selective 5-HT$_{1B}$ receptor agonist 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-*b*]pyridine (CP 94,253; 1–5.6 mg/kg) dose-dependently decreased the amount of exploration on the open arms of the plus-maze without altering overall locomotor activity. This 5-HT$_{1B}$ agonist-induced increase in anxiety-like behavior was dose-dependently reversed by coadministration of the selective 5-HT$_{1B/1D}$ receptor antagonist 2′-methyl-4′-(5-methyl[1,2,4]oxadiazol-3-yl)-biphenyl]-amide (GR 127,935). There was no significant effect of GR 127,935 administration alone on plus-maze behavior. These results indicate that 5-HT$_{1B}$ receptor activation increases anxiety-like behavioral responses as measured by the elevated plus-maze. Since 5-HT$_{1B}$ receptors modulate the activity of multiple neurotransmitter systems that have been implicated in anxiety disorders, these findings suggest that this receptor subtype may represent an important therapeutic target for the treatment of anxiety. © 2002 Published by Elsevier Science Inc.

Keywords: Plus-maze; 5-HT$_{1B}$; CP 94,253; GR 127,935; Anxiety; Serotonin

1. Introduction

Central serotonin [5-hydroxytryptamine (5-HT)] function in humans has been strongly implicated in depression (Owens and Nemeroff, 1994) and also plays an important role in aggression (Coccaro, 1989), impulsivity (Westenberg and den Boer, 1988; Coccaro and Kavoussi, 1996) and anxiety (Iversen, 1984). Although the distinct mechanisms involved in the etiology of anxiety are unclear, it has been hypothesized that increased 5-HT neurotransmission is associated with anxiogenesis, whereas decreased 5-HT function is associated with anxiolysis (Iversen, 1984; Briley et al., 1990). In support of this hypothesis, a number of clinical studies have reported increases in anxiety-like symptoms following the administration of direct 5-HT agonists (Murphy et al., 1989) and after the initial administration of selective 5-HT reuptake inhibitors (SSRIs; Westenberg and den Boer, 1988; Van Praag, 1988; Montgomery, 1991). Similarly, increases in anxiety-like behaviors in animal models have been reported following acute systemic administration of agents that increase serotonergic transmission, such as SSRIs (Griebel et al., 1994) and fenfluramine (File and Guardiola-Lemaitre, 1988; Graeff et al., 1998). Despite these observational trends, the role of 5-HT in anxiety is likely complex and may be dependent on 5-HT neurotransmission in multiple brain regions, the types of behavioral paradigms used to assess anxiety as well as the emotional and cognitive contexts of the tests (Handley et al., 1993).

5-HT neurotransmission is mediated by at least 14 different receptor subtypes (Hoyer et al., 1994; Barnes and Sharp, 1999). Thus, the effect of indirect 5-HT agonists on anxiety likely reflects a summation of activation at multiple receptor subtypes in different brain regions. Although it is not yet clear which 5-HT receptor subtypes mediate the anxiogenic-like effects of indirect 5-HT agonists, the 5-HT$_{1A}$ receptor has been the most extensively characterized in this regard (File et al., 1996). In addition, observa-

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tions from limited clinical and animal studies suggest that stimulation of 5-HT_{1B} receptors produces anxiogenic-like effects. For example, administration of *m*CPP, an agonist at 5-HT_{1B} and 5-HT_{2C} receptors and a partial agonist at the 5-HT_{2A} receptor, induced anxiety symptoms in healthy human volunteers (Murphy et al., 1989; Silverstone et al., 1994) and increased anxiety-like behaviors on the elevated plus-maze in rodents (Benjamin et al., 1990; Gibson et al., 1994). Although the anxiogenic effects of *m*CPP on plus-maze behavior has been ascribed to its activity at both 5-HT_{2C} (Gibson et al., 1994) and 5-HT_{1B} (Benjamin et al., 1990) receptors, the induction of increased anxiety-like behaviors by other nonselective 5-HT_{1B} agonists (RU 24969, TFMPP and CGS 12066B; Benjamin et al., 1990; Gibson et al., 1994; Critchley and Handley, 1987; Pellow et al., 1987; Critchley et al., 1992) provides additional support for an involvement of 5-HT_{1B} receptors. However, because each of these agents displays poor receptor selectivity and differing pharmacologic activity (e.g. partial 5-HT_{1B} agonist activity of CGS 12066B; Hoyer et al., 1994 for review), no firm conclusions regarding the effects of 5-HT_{1B} receptor manipulation on plus-maze behavior can be made from these studies. Furthermore, the specific action of putative 5-HT_{1B} agonists at 5-HT_{1B} receptors was not demonstrated pharmacologically due to the lack of specific 5-HT_{1B} receptor antagonists at the time.

The recent development of selective 5-HT_{1B} receptor agonists and antagonists makes it possible to test the effects produced by 5-HT_{1B} receptor manipulation on exploratory behavior by rats on the elevated plus-maze. This ethologically based animal model of anxiety, which exploits the rodents' natural avoidance of open spaces and height (Montgomery, 1955), has been extensively validated both behaviorally and pharmacologically (Pellow et al., 1985; Pellow and File, 1986). In the present study, the effects of the 5-HT_{1B} receptor agonist 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-*b*]pyridine (CP 94,253) on exploratory behavior in the elevated plus-maze were examined. To determine whether tonic activation of 5-HT_{1B} receptors by endogenous 5-HT contributes to the establishment of baseline anxiety states, we tested the effects of the selective 5-$HT_{1B/1D}$ receptor antagonist 2′-methyl-4′-(5-methyl[1,2,4]oxadiazol-3-yl)-biphenyl]-amide (GR 127,935) on plus-maze behavior. Lastly, the ability of GR 127,935 to alter the effects of CP 94,253 on plus-maze behavior was examined.

2. Materials and methods

2.1. Subjects

The subjects were 111 male Wistar rats (Charles River, Hollister, CA). Animals weighing 250–300 g upon arrival in the laboratory were group housed (two to three rats per cage) in a humidity- and temperature-controlled environment (21 °C) with a 12-h light/dark cycle (lights on at 06:00 h). Food and water were available ad libitum in the home cages. During the first week after arrival, animals were allowed to habituate to their new environment without handling. After the initial habituation period, all animals were handled briefly (3–5 min) for 7 consecutive days and were habituated to subcutaneous injections on the last 2 days prior to testing on the plus-maze. All handling and behavioral testing took place between 20:00 and 24:00 h during the dark phase of the rats' daily cycle. All procedures were in accordance with the National Institutes of Health's guidelines regarding the principles of animal care. The animal facilities and protocols were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute and in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.2. Drugs

CP 94,253 was generously provided by Pfizer (Groton, CT). GR 127,935 was generously provided by Glaxo Wellcome (Stevenage, UK). Both drugs were dissolved in sterile 0.9% saline with gentle heating. All drug injections were administered subcutaneously in a volume of 1 ml/kg.

2.3. Elevated plus-maze paradigm

2.3.1. Apparatus

The plus-maze apparatus was made of Plexiglas and consisted of four arms elevated 50 cm above the ground, with each arm (50 cm long, 10 cm wide) positioned 90° relative to the adjacent arms. The arms extended from a central platform with two of the arms enclosed by 40-cm high dark walls and the other two arms open. Testing was conducted in a quiet room in which the ventilation system provided approximately 65-dB background noise. The testing room was illuminated only by a dim light centered along one wall of the room, such that the open arms were illuminated from the side and not down the length of the arm. A light meter was used to adjust the lighting for all test sessions to provide approximately 1.5 lux of illumination on the open arms of the maze.

2.3.2. Test procedure

To begin a test session, rats were placed individually in the central platform facing a closed arm and allowed to explore the maze for a 5-min test period (Pellow et al., 1985). Photobeam sensors located at the entry points to each arm were used to monitor the position of the rat in the maze. Two behavioral measures were recorded by computer for each rat: (1) duration of time spent on the various sections of the maze (open arms, closed arms and central platform) and (2) number of beam breaks at the entry points to the various compartments of the maze. The apparatus was wiped clean with a damp sponge and dried with paper towels between

tests. An adjacent antechamber served as a holding room where animals were kept before drug administration, during the interval between injection and testing and following maze testing.

2.3.2.1. Experiment 1: effects of the 5-HT$_{1B}$ receptor agonist CP 94,253 on plus-maze behavior. Separate groups of rats (n = 13/group) were tested with one of four doses of CP 94,253 (0, 1, 3 and 5.6 mg/kg). Injections of CP 94,253 were administered 25 min prior to testing on the plus-maze.

2.3.2.2. Experiment 2: effects of the 5-HT$_{1B/1D}$ receptor antagonist GR 127,935 on plus-maze behavior. Three doses of GR 127,935 (0, 3 and 10 mg/kg) were tested in separate groups of rats (n = 10/group). Injections of GR 127,935 were administered 40 min prior to testing on the plus-maze.

2.3.2.3. Experiment 3: effects of the 5-HT$_{1B/1D}$ receptor antagonist GR 127,935 on CP 94,253-induced changes in plus-maze behavior. Separate groups of rats (n = 9–10/group) were tested with one of three doses of GR 127,935 (0, 3 and 10 mg/kg) prior to an injection of CP 94,253 (3 mg/kg). GR 127,935 was administered 15 min prior to an injection of CP 94,253 and all animals were tested on the plus-maze 25 min after treatment with CP 94,253.

2.4. Data analysis

Drug-induced effects on anxiety on the elevated plus-maze are typically indicated by changes in the percentage of open-arm time and the percentage of open-arm entries. The number of closed-arm entries and total number of arm entries were analyzed as measures of general activity (Pellow et al., 1985). In all of the experiments described above, a between-subjects design was used to test the effects of different doses of agonist (CP 94,253), antagonist (GR 127,935) and agonist/antagonist interactions on the plus-maze. Thus, each animal received only one experimental treatment and was tested on the plus-maze for one trial. All data for the experiments examining the effects of CP 94,253 and GR 127,935 on plus-maze behavior were analyzed using analysis of variance (ANOVA) with drug dose as the between-subjects factor. The four dependent measures obtained from the plus-maze tests were (1) the amount of time spent on the open arms expressed as a percentage of the total time spent exploring both the open and closed arms, (2) the number of beam breaks at the entries to the open arms expressed as a percentage of the total number of beam breaks at both open- and closed-arm entries, (3) the number of beam breaks at the entry points to the closed arms and (4) the total number of beam breaks at the entries to both the open and closed arms of the maze. Significant differences between individual drug doses and saline treatment were subsequently determined by Fisher's Partial Least Squares Post Hoc Test.

3. Results

3.1. Experiment 1: effects of the 5-HT$_{1B}$ receptor agonist CP 94,253 on plus-maze behavior

The effects of pretreatment with CP 94,253 on exploratory behavior on the plus-maze are summarized in Fig. 1. CP 94,253 dose-dependently decreased the percentage of time spent exploring the open arms of the maze during a 5-min session [Fig. 1A; $F(3,49) = 5.18$, $P < .005$]. Relative to the control condition, both the 3- and 5.6-mg/kg doses of CP 94,253 produced significant reductions in the time spent on the open arms ($P < .05$ for each dose). The 5.6-mg/kg dose of CP 94,253 produced significantly greater effects than did either the 1- or 3-mg/kg doses ($P < .05$). Analyses of data characterizing the percentage of entries into the open arms of the maze [Fig. 1B; $F(3,49) = 3.02$, $P < .05$] yielded essentially the same results, but only the highest dose (5.6 mg/kg) was significantly different from that obtained with saline pretreatment ($P < .05$). Pretreatment with CP 94,253 did not alter the number of entries into the closed arms [Fig. 1C; $F(3,49) = 0.73$, NS] or the total number of open- and closed-arm entries [Fig. 1D; $F(3,49) = 0.29$, NS] relative to that obtained with saline pretreatment.

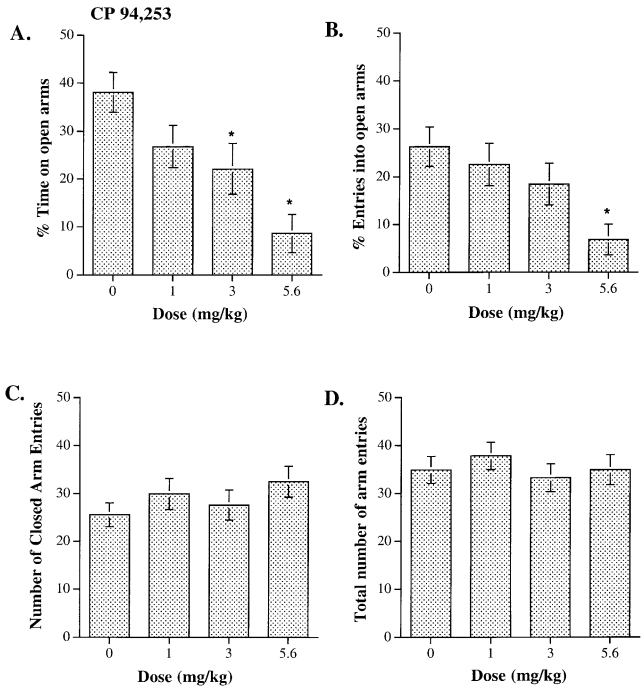

Fig. 1. Effects of CP 94,253 pretreatment on exploratory behavior on the elevated plus-maze (n = 13/dose). CP 94,253 dose-dependently decreased the percentage of time spent on the open arms of the plus-maze (A) and decreased the percentage of entries into the open arms (B). Pretreatment with the 5-HT$_{1B}$ agonist did not affect the number of closed-arm entries (C) or the total number of open- and closed-arm entries (D). Asterisks denote significant differences between drug doses and saline controls ($P < .05$).

3.2. Experiment 2: effects of the 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 on plus-maze behavior

Pretreatment with the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 (3 and 10 mg/kg) did not alter the percentage of time spent exploring the open arms of the plus maze [Fig. 2A; $F(2,27)=0.05$, NS] or the percentage of open-arm entries [Fig. 2B; $F(2,27)=0.3$, NS] relative to saline-pretreated controls. Pretreatment with GR 127,935 also did not alter the number of entries into the closed arms [Fig. 2C; $F(2,27)=0.04$, NS] or the total number of open- and closed-arm entries [Fig. 2D; $F(2,27)=0.47$, NS].

3.3. Experiment 3: effects of the 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 on CP 94,253-induced changes in plus-maze behavior

Pretreatment with CP 94,253 (3 mg/kg) and GR 127,935 (0, 3 and 10 mg/kg) significantly altered exploration of the open arms of the plus-maze relative to saline-treated controls [$F(3,38)=5.85$, $P<.005$ and $F(3,38)=4.39$, $P<.01$, respectively]. Post hoc analyses indicated that animals pretreated with CP 94,253 (3 mg/kg) and saline (0-mg/kg GR 127,935) spent significantly less time on ($P<.05$) and made fewer entries into ($P<.05$) the open arms of the plus-maze relative to saline-treated controls. CP 94,253-induced decreases in the percentage of time spent on the open arms of the plus-maze were reversed by pretreatment with GR 127,935 (Fig. 3A) at both the 3- and 10-mg/kg doses ($P<.05$ for each dose). Similar results were obtained for analyses of data characterizing the percentage of entries into the open arms of the maze (Fig. 3B; $P<.05$ for both doses). On both measures, pretreatment with GR 127,935 (3 and 10 mg/kg) and CP 94,253 (3 mg/kg) resulted in levels of open-arm exploration equivalent to those obtained after saline treatment alone.

The number of closed-arm entries was significantly altered following pretreatment with GR 127,935 and CP 94,253 relative to saline-treated controls [$F(3,38)=2.90$, $P<.05$]. Post hoc analyses indicated that treatment with CP 94,253 and saline increased the number of closed-arm entries relative to saline-treated controls ($P<.05$), whereas no significant differences were observed in animals pretreated with either CP 94,253 or either 3- or 10-mg/kg GR 127,935 (Fig. 3C). No significant differences between the treatment groups were detected, however, in the total number of open- and closed-arm entries [$F(3,38)=0.98$, NS].

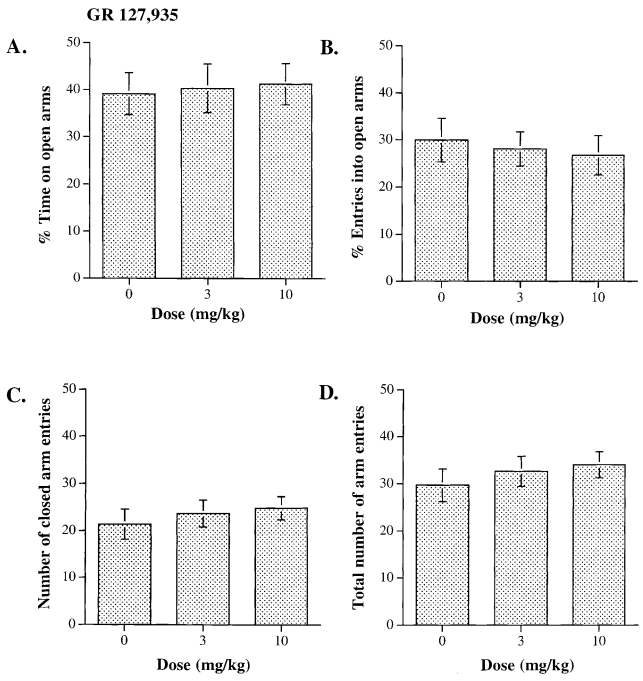

Fig. 2. Effects of pretreatment with the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 on plus-maze behavior ($n=10$/dose). Pretreatment with GR 127,935 had no effect on the percentage of time spent exploring the open arms of the plus-maze (A) or the percentage of entries into the open arms (B). The number of closed-arm entries (C) and the total number of open- and closed-arm entries (D) were not affected by pretreatment with GR 127,935.

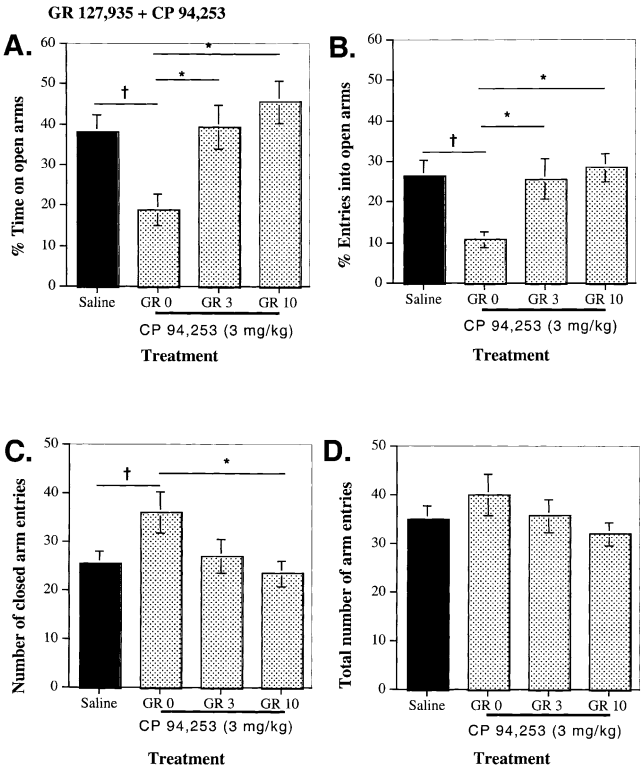

Fig. 3. Effects of GR 127,935 on CP 94,253-induced changes in exploratory behavior on the plus-maze ($n=9-10$/treatment). Pretreatment with GR 127,935 blocked CP 94,253-induced reductions in the percentage of time spent on the open arms (A) and the percentage of open-arm entries (B). Pretreatment with CP 94,253 increased the number of closed-arm entries (C) but did not affect the total number of open- and closed-arm entries (D). Daggers denote significant differences between saline-treated controls and saline/CP 94,253-pretreated animals. Asterisks denote significant differences between GR 127,935/CP 94,235 pretreatment and saline/CP 94,253 pretreatment ($P<.05$).

4. Discussion

In the present study, the selective 5-HT_{1B} receptor agonist CP 94,253 dose-dependently decreased exploration of the open arms in the elevated plus-maze. Compared to vehicle-treated controls, animals treated with CP 94,253 spent significantly less time exploring the open arms of the maze and made fewer entries into the open arms. None of the doses of CP 94,253 significantly altered the number of entries into the closed arms or the total number of entries, suggesting that the drug did not significantly affect locomotor activity. Pretreatment with the selective 5-HT_{1B} receptor antagonist GR 127,935 blocked the effects of CP 94,253 on open-arm exploration of the plus-maze but did not significantly alter open-arm exploration or locomotor activity when administered alone.

The reduction in open-arm exploration produced by CP 94,253 suggests that activation of 5-HT_{1B} receptors has anxiogenic-like effects, which resemble those observed after administration of inverse agonists at the benzodiazepine receptor (Pellow and File, 1986). CP 94,253 has approximately 45-fold selectivity for 5-HT_{1B} receptors over 5-HT_{1A} receptors and binds with very low affinity at other 5-HT receptor subtypes (Koe et al., 1992). Although CP 94,253 shows a much higher selectivity for 5-HT_{1B} receptors than previously investigated compounds, it may be postulated that the anxiogenic-like effects of CP 94,253 observed in the present study are due in part to the activation of 5-HT_{1A} receptors. In this regard, two lines of evidence argue against an involvement of 5-HT_{1A} receptors in the effects of CP 94,253. First, systemic administration of 5-HT_{1A} receptor agonists in rats produce equivocal results on plus-maze exploration (Handley, 1991; Handley and McBlane, 1993), which may be due to opposing effects of 5-HT_{1A} receptor activation in different brain regions. Local infusion of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the dorsal or medial raphe nuclei produces anxiolytic-like effects on the plus-maze (File et al., 1996; Hogg et al., 1994), presumably by stimulating 5-HT_{1A} autoreceptors located on 5-HT-producing cell bodies and decreasing cell firing rate. By contrast, local infusion of 8-OH-DPAT into the dorsal hippocampus activates 5-HT_{1A} receptors on postsynaptic targets and produces anxiogenic-like effects (File et al., 1996). Second, the presently observed behavioral effects of CP 94,253 were dose-dependently blocked by GR 127,935, a compound that is nearly 1000-fold selective for 5-HT_{1B} vs. 5-HT_{1A} receptors (Skingle et al., 1993). Taken together, these findings indicate a selective involvement of 5-HT_{1B}, and not 5-HT_{1A} receptors, in the anxiogenic-like profile of CP 94,253 on the elevated plus-maze.

As recently reviewed by Dawson and Tricklebank (1995), the assessment of anxiolytic- or anxiogenic-like effects on the rodent elevated plus-maze can be confounded by drug-induced changes in locomotor activity. It is thus important to note that the anxiogenic-like effects of CP 94,253 were not accompanied by altered locomotor activity on the plus-maze at any of the doses tested. The lack of CP 94,253-induced motor effects is consistent with recent observations from our laboratory (Parsons et al., unpublished observations) and is in contrast to the motor perturbations produced by other putative 5-HT_{1B} receptor agonists such as RU 24969 (Geyer, 1996), TFMPP and *m*CPP (Lucki et al., 1989) previously investigated on the elevated plus-maze (Benjamin et al., 1990; Gibson et al., 1994; Critchley and Handley, 1987; Pellow et al., 1987; Critchley et al., 1992). Thus, the presently observed decrease in open-arm exploration produced by CP 94,253 cannot be attributed to a generalized alteration in motor activity and likely reflects a specific anxiogenic-like effect of this drug.

Although the neuronal substrates that underlie the anxiogenic-like effects of 5-HT_{1B} receptor agonists are not known, several hypotheses can be proposed based on the neuroanatomical localization of the receptor and the neurophysiological consequences of 5-HT_{1B} receptor activation. The distribution of 5-HT_{1B} receptors in the brain is heterogeneous, and regions that have been implicated in the modulation of anxiety states, such as the hippocampus and periaqueductal gray, express high levels of 5-HT_{1B} receptors (Bruinvels et al., 1993). 5-HT_{1B} receptors are predominantly located on axon terminals (Boschert et al., 1994), and their activation inhibits the release of multiple neurotransmitters in different brain regions through a G-protein-coupled inhibition of adenylate cyclase (Bouhelal et al., 1988). For example, activation of 5-HT_{1B} receptors has been shown to inhibit acetylcholine release in both the hippocampus (Maura and Raiteri, 1986) and cortex (Feuerstein et al., 1996). Findings from a recent study indicate that activation of both nicotinic and muscarinic acetylcholine receptors produces anxiolytic-like effects on the elevated plus-maze (File et al., 1998). Furthermore, administration of both nicotinic and muscarinic receptor antagonists produces anxiogenic-like effects (File et al., 1998), which suggests that tonic activation of these receptors plays a role in establishing baseline anxiety states. Thus, 5-HT_{1B} receptors may modulate anxiety states through an inhibitory control of acetylcholine release. The inhibitory modulation of GABA neurotransmission by 5-HT_{1B} receptors (Johnson et al., 1992; Parsons et al., 1999; Stanford and Lacey, 1996) represents another potential mechanism for regulating anxiety behaviors. Manipulations of GABAergic transmission in the periaqueductal gray have been shown to affect exploration on the plus-maze (Russo et al., 1993), and 5-HT_{1B} receptors in this region have been implicated in the modulation of anxiety states (Audi et al., 1991). It is unlikely that the anxiogenic-like effects of CP 94,253 are related to 5-HT_{1B}-mediated modulation of 5-HT neurotransmission. An inhibition of 5-HT release by terminal 5-HT_{1B} autoreceptors (Trillat et al., 1997) is not consistent with the notion that postsynaptic 5-HT receptor activation

in terminal regions is associated with increased anxiety (File et al., 1996).

Consistent with the present observation that 5-HT_{1B} receptor stimulation produces enhanced anxiety-like behavior, mutant mice that lack functional 5-HT_{1B} receptors have recently been found to display lower levels of anxiety-like behavior in several different paradigms. For example, 5-HT_{1B} receptor knockout mice display greater exploration in the center portion of an open field apparatus than do wild-type controls (Zhuang et al., 1999). Furthermore, 5-HT_{1B} knockout pups vocalize less in the isolation-induced vocalization paradigm than do wild-type pups (Brunner et al., 1999). Together, these findings suggest that 5-HT_{1B} knockout mice show reduced anxiety-like behaviors relative to wild-type control mice. However, 5-HT_{1B} knockout mice do not differ from wild-types with regard to the entries into and time spent in the open arms of the elevated plus-maze (Brunner et al., 1999). Together with the present observation that 5-HT_{1B} receptor blockade in rats by GR 127,935 does not alter plus-maze behavior (in the absence of 5-HT_{1B} agonist administration), these findings suggest that tonic activation of 5-HT_{1B} receptors does not contribute to baseline behavior elicited on the elevated plus-maze. In this regard, it should be noted that the expression of anxiety-like behaviors is highly sensitive to "background" test conditions (Hogg, 1996; Rodgers, 1997), which in turn may determine endogenous transmitter release and receptor activation. In the present study, low ambient illumination levels and extensive pretest animal handling were employed to minimize baseline anxiety "tone" as indicated by the relatively high level of open-arm exploration in the saline-treated control animals. Under such conditions, it may be difficult to detect anxiolytic-like effects of test drugs, as has been previously shown for classic benzodiazepine anxiolytics (Hogg, 1996). Thus, although the present results do not point to an anxiolytic profile for 5-HT_{1B} receptor antagonists, firm conclusions in this regard must await further study.

The present observations indicate that 5-HT_{1B} receptor stimulation increases anxiety-like behavior in rats tested on the elevated plus-maze. Although various animal models of anxiety probe different aspects of emotional response (e.g. proximal vs. distal threats; conditioned vs. unconditioned stressors; see Rodgers, 1997 for review), these findings indicate value in further investigations into the possible role played by central 5-HT_{1B} receptors in the etiology of anxiety disorders.

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