Antidepressant-Like Behavioral Effects Mediated by 5-Hydroxytryptamine<sub>2C</sub> Receptors<sup>1</sup>

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ABSTRACT
The role of the 5-HT<sub>2C</sub> receptor in mediating active behaviors in the modified rat forced swim test was examined. Three novel selective 5-HT<sub>2C</sub> receptor agonists, WAY 161503 (0.1–3.0 mg/kg), RO 60-0175 (2–20 mg/kg), and RO 60-0332 (20 mg/kg), all decreased immobility and increased swimming, a pattern of behavior similar to that which occurs with the selective serotonin reuptake inhibitor fluoxetine (5–20 mg/kg). However, the prototypical but nonselective 5-HT<sub>2C</sub> receptor agonist m-chlorophenylpiperazine (1–10 mg/kg) increased immobility scores in the forced swim test. The selective 5-HT<sub>2C</sub> receptor antagonist SB 206533 was inactive when given alone (1–20 mg/kg). However, SB 206533 (20 mg/kg) blocked the antidepressant-like effects of both WAY 161503 (1 mg/kg) and fluoxetine (20 mg/kg). The typical antidepressant (noradrenergic α<sub>2</sub> and 5-HT<sub>2C</sub> receptor antagonist) mianserin reduced immobility and increased climbing at 30 mg/kg. At a behaviorally subactive dose (10 mg/kg), mianserin abolished the effects of WAY 161503 (1 mg/kg) on both swimming and immobility scores. Mianserin blocked the effects of fluoxetine (20 mg/kg) on swimming only; mianserin plus fluoxetine reduced immobility and induced a switch to climbing behavior, suggesting activation of noradrenergic transmission. These data exemplify the benefits of using the modified rat forced swim test, which is sensitive to serotoninergic compounds and distinguished behavioral changes associated with serotoninergic and noradrenergic effects. Taken together, the results strongly implicate a role for 5-HT<sub>2C</sub> receptors in the behavioral effects of antidepressant drugs.

The indoleamine serotonin (5-hydroxytryptamine, 5-HT) plays a major role in the pathogenesis and treatment of various psychiatric disorders including depression (Mael and Meltzer, 1995; Lucki, 1998). Selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed class of drugs for the treatment of clinical depression. SSRIs are believed to exert their antidepressant ability by blocking the reuptake of serotonin at synaptic terminals, resulting in an elevation of extracellular 5-HT concentrations in limbic regions of the brain that can act on various critical postsynaptic 5-HT receptors (Goodnick and Goldstein, 1998). At least 14 different subtypes of 5-HT receptors have been identified (Barnes and Sharp, 1999), and it is still unclear which of them are most relevant to the pathogenesis of depression and the mechanism of action of antidepressants (Cryan and Leonard, 2000). Whereas clinical and animal studies have strongly implicated 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, and 5-HT<sub>2A</sub> receptors as playing a critical role in the antidepressant response (Lucki et al., 1994; Barnes and Sharp, 1999; Cryan and Leonard, 2000), there has been less emphasis on other 5-HT receptor subtypes. This has been attributable, in part, to the unavailability of selective ligands.

The 5-HT<sub>2C</sub> receptor (formerly 5-HT<sub>1C</sub>) (Baxter et al., 1995) is a postsynaptically located, seven-transmembrane spanning receptor present in highest concentrations in the choroid plexus, but significant densities are also found in the subthalamic nucleus, hypothalamus, hippocampus, and amygdala (Mengod et al., 1990). The 5-HT<sub>2C</sub> receptor is believed to have an integral function in the control of many physiological and behavioral responses, including feeding, anxiety, temperature regulation, locomotion, sexual behavior, and the occurrence of seizures (Lucki et al., 1989; Bennett, 1993; Baxter et al., 1995). The first indication that this receptor may be relevant to antidepressant action occurred when it was realized that the atypical antidepressant mianserin has a high affinity for it (Pazos et al., 1984). Subsequently, a number of antidepressants, especially tricyclics, were shown to have high to moderate affinity for the 5-HT<sub>2C</sub> receptor (Jenck et al., 1993, 1994). Berendson and Broekkamp (1987, 1994) and others (Leander, 1987; Lucki et al., 1988) have shown that SSRIs may exert some of their

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); FST, forced swim test; SSRI, selective serotonin reuptake inhibitor; m-CPP, 1-(3-chlorophenyl)pyperazine 1:2; WAY 161503, 8,9-dichloro-2,3,4,4a-tetrahydro-1H,6H-pyrazino[1,2-a]quinoloxalin-5-one; RO 60-0175, (S)-2-(chloro-5-fluoro-indol-1-yi)-1-methylethylamine 1:1 C<sub>14</sub>H<sub>12</sub>O; RO 60-0332, (S)-2-(4,4,7-trimethyl-1,4-dihydro-indeno[1,2-b]pyrrol-1-methylethylamine 1:1 1:1 C<sub>25</sub>H<sub>26</sub>O; SB 206553, 5-methyl-1-(3-pyridylylcarbamoyl)-1,2,3,5-tetrahydropyrrole(2,3-f)indole).
behavioral effects in vivo by 5-HT
2C receptor activation. Indeed, a number of common behavioral effects are produced by both 5-HT
2C receptor agonists and SSRIs, including inhibition of escape from aversive periaqueductal gray stimulation, decreased defensive burying, induction of penile erections, hypophagia, and inhibition of muricide (Broekkamp and Be
rendson, 1992).

There have been relatively few studies investigating the role of 5-HT
2C receptors in animal behavior tests sensitive to antidepressants. The forced swim test (FST) as originally described by Porsolt et al. (1977) is the most widely used pharmacological model for assessing antidepressant activity (Weiss and Kilts, 1998). Rats develop immobility when they are placed in a cylinder of water without the opportunity for escape. The immobile behavior is thought to reflect either a failure to persist in escape-directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli (Lucki, 1997). A broad spectrum of antidepressant drugs selectively prevents the development of behavioral immobility in the FST (Borsini and Meli, 1988). However, a recent modification of the traditional FST demonstrated that specific behavioral components of active behaviors in the FST distinguished neurochemically distinct antidepressant drugs (Lucki, 1997). The modified FST differentiated swimming behavior, which was sensitive to SSRIs and 5-HT receptor agonists, and climbing behavior, which was sensitive to tricyclic antidepressants and drugs with selective effects on catecholamine transmission (Detke et al., 1995; Lucki, 1997). The distinctive active behaviors produced by pharmacologically selective antidepressants persisted with chronic treatment (Detke et al., 1997), and they were superimposable on combinations of serotonergic and catecholaminergic compounds (Reneric and Lucki, 1998). The increased swimming behavior produced by fluoxetine has been shown to be prevented by pretreatment with the tryptophan hydroxylase inhibitor parchlorophenylalanine but not the increased climbing produced by the norepinephrine reuptake inhibitor desipramine (Page et al., 1998). Although the modified rat FST is sensitive to the effects of serotonergic antidepressants, it is unclear which serotonergic receptors are responsible for the mediation of these antidepressant-sensitive behaviors. Therefore, the following series of studies were aimed at investigating the role of the 5-HT
2C receptor in mediating selective antidepressant-like effects in the modified rat FST.

Materials and Methods

Animals. A total of 458 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing from 175 to 200 g on arrival were used in these studies. The animals were housed in pairs in polycarbonate cages and maintained on a 12-h light/dark cycle (lights on at 7:00 AM) in a temperature-controlled (22°C) colony. The animals had free access to food and water. Animals were handled daily for at least 5 days before initiation of behavioral testing. Behavioral studies were carried out in the afternoon (12:00–6:00 PM) during the months of July through November. All experimental procedures were carried out in accordance with protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Rat Forced Swim Test. The modified rat forced swim test was conducted essentially as described by Detke et al. (1995). Briefly, rats were placed individually for 15 min in Pyrex cylinders (21 × 46 cm; Fisher Scientific, Pittsburgh, PA) that were filled with water to a depth of 30 cm. They were removed after 15 min, dried, and placed in their home cage. Twenty-four hours after their first exposure, the animals were replaced in the swim apparatus for 5 min, and the session was recorded using a video camera placed above the cylinder for subsequent analysis. Animals were randomly assigned to groups receiving various drug treatments or to a control group (0.9% saline). Injections were administered s.c. three times (1, 5, and 23.5 h) before the test session. The rater of the behavioral patterns was blind to the experimental conditions being scored. A time sampling technique was used whereby the predominant behavior in each 5-s period of the 300-s test was recorded. Climbing behavior consisted of upward-directed movements of the forepaws along the side of the swim chamber. Swimming behavior was defined as movement (usually horizontal) throughout the swim chamber, which also included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keep the rat’s head above the water.

The first study examined in a dose-response manner the effects of fluoxetine on active behaviors in the modified rat FST. The effects of three recently developed selective 5-HT
2C receptor agonists, WAY 161503, RO 60-0175, and RO 60-0332, were then investigated in the FST. These compounds are distinguished from conventional agonists, such as m-CPP and (±)-1-(2,5-dimethoxy-4-iodophenyl) 2-amino propane, by their greater selectivity for 5-HT
2C over 5-HT
2A receptors. WAY 161503 (Wyeth-Ayerst, Princeton, NJ) is a novel selective and potent 5-HT
2C receptor agonist (Rosenzweig-Lipson et al., 2000). In binding studies using [3H]KB-(±)-1-(2,5-dimethoxy-4-iodophenyl) 2-amino propane with human 5-HT
2C and 5-HT
2A receptors, WAY 161503 shows an approximate 3-fold selectivity for 5-HT
2C over 5-HT
2A receptors with an affinity of 3 nM. In functional studies using human receptors, WAY 161503 shows an approximate 2000-fold selectivity for 5-HT
2C over 5-HT
2A receptors with an EC
50 value of approximately 8 nM at the 5-HT
2C receptor in an agonist-stimulated inositol monophosphate formation assay (Rosenzweig-Lipson et al., 2000). RO 60-0175 and RO 60-0332 have been the most widely studied of the recently developed generation of selective 5-HT
2C receptor agonists (Moreau et al., 1996; Martin et al., 1998). Both compounds have at least greater than 25-fold affinity for the 5-HT
2C receptor over the 5-HT
2A receptor (RO 60-0175, 5-HT
2C p
K
i = 9.0 versus 5-HT
2A p
K
i = 7.5; RO 60-0332, 5-HT
2C p
K
i = 8.5 versus 5-HT
2A p
K
i = 7.0) (Martin et al., 1998). For comparison, we also investigated the effects of the prototypical, but nonselective, 5-HT
2C receptor agonist m-CPP on active behaviors in the FST.

The next set of studies focused on the effects of the potent selective 5-HT
2C receptor antagonist SB 206533 (Kennett et al., 1996) alone and in combination with fluoxetine and with WAY 161503 on active behaviors in the test. SB 206533 has a greater than 100-fold affinity for 5-HT
2C receptors over 5-HT
2A receptors (p
K
i ~ 7.9 versus 5.8) (Kennett et al., 1996), but it also has substantial affinity for the 5-HT
2B receptor (p
K
i = 8.9).

To complement these studies, we examined the effects of the antidepressant mianserin, which is a nonselective 5-HT
2C receptor antagonist, given alone and in combination with fluoxetine or WAY 161503, on active behaviors in the test. Mianserin has similar affinity for 5-HT
2A and 5-HT
2C receptors but has a lower affinity for the 5-HT
2B receptor (p
K
i = 8.0, 8.1, and 7.3, respectively) (Baxter et al., 1995).

Drugs. All drugs were freshly made before use and were injected s.c. in a volume of 2 ml/kg. Fluoxetine (Eli Lilly and Co., Indianapolis, IN), mianserin (Organon, Oss, The Netherlands), and WAY 161503 (Wyeth-Ayerst) were dissolved in deionized H
2O and sonicated mildly. m-CPP (Research Biochemicals, Natick, MA) dissolved readily in distilled H
2O. The selective 5-HT
2C receptor agonists RO 60-0175 (F. Hoffmann-La Roche, Basel, Switzerland), RO 60-0332 (F. Hoffmann-La Roche), and the 5-HT
2C receptor antagonist SB 206533 (Wyeth-Ayerst) were crushed using a pestle and mortar and moistened with 1 to 2 drops of Tween 80 (Sigma, St. Louis, MO). The resultant paste was suspended and brought up to volume with deionized H
2O.
ized H₂O. We have shown previously that the addition of small amounts of Tween 80, as used in these studies, does not have an impact on behavioral effects in the rat FST (Detke et al., 1995). For combination experiments, both drugs were made up in the same solution to eliminate any confounding stress effects of multiple injections. All drug doses were calculated as the base weight, except RO 60-0175 and RO 60-0332, which were calculated without correction.

**Statistical Analysis.** ANOVA was carried out in all studies. Any overall statistical differences were analyzed further using Fisher’s post hoc tests.

**Results**

As shown in Fig. 1, fluoxetine dose dependently decreased immobility [$F(3,36) = 5.21; P < .05$] while inducing a corresponding increase in swimming behavior [$F(3,36) = 8.70; P < .001$] without having any effect on climbing behavior [$F(3,36) = 0.27; P = .85$]. Similar effects were seen after the administration of the selective 5-HT₂C receptor agonists (Fig. 2). WAY 161503, RO 60-0175, and RO 60-0332 decreased immobility scores [$F(5,54) = 2.69, P < .05; F(3,36) = 4.45, P = .009$; and $F(3,36) = 3.49, P < .05$, respectively]. This was accompanied by an increase in swimming behavior for each agonist [$F(5,54) = 3.76, P < .01; F(3,36) = 5.31, P < .01$; and $F(3,36) = 3.04, P < .05$, respectively]. None of the three 5-HT₂C receptor agonists altered climbing behavior [$F(5,54) = 0.57, P = .57; F(3,36) = 0.35, P = .79$; and $F(3,36) = 0.78, P = .51$, respectively].

The effects of the nonselective 5-HT₂C receptor agonist m-CPP are shown in Fig. 3. m-CPP administration caused a dose-dependent increase in immobility [$F(3,36) = 3.50, P < .05$] and a corresponding decrease in climbing behavior [$F(3,36) = 7.57, P < .001$] without having any effect on swimming behavior [$F(3,36) = 0.07, P = .98$].

The 5-HT₂C receptor antagonist SB 2060533 did not alter immobility [$F(4,42) = 0.87, P = .49$], swimming [$F(4,42) = 0.94, P = .45$], or climbing [$F(4,42) = 0.46, P = .76$] behaviors in the rat forced swim test at all doses tested when given alone (Fig. 4A). When SB 206533 was administered in combination with either WAY 161503 (1 mg/kg) or fluoxetine (20 mg/kg), there was a significant overall effect of drug treatment on immobility [$F(5,73) = 7.85, P < .001$], on swimming [$F(5,73) = 14.66, P < .001$], but not on climbing [$F(5,73) = 0.33, P = .89$]. Post hoc analysis demonstrated that both WAY 161503 and fluoxetine decreased immobility and increased swimming behavior. These effects were completely
antagonized by combined treatment with the 5-HT_{2C} receptor antagonist SB 206533, which had no effect on any behavioral parameter when administered on its own (Fig. 4B).

As shown in Fig. 5A, the 5-HT_{2C} receptor antagonist/atypical antidepressant mianserin altered immobility \( F(3,38) = 3.93, \ P < .05 \) and climbing behavior \( F(3,38) = 7.71, \ P < .001 \) without having any effect on swimming behavior \( F(3,38) = 0.58, \ P = .63 \). Post hoc analysis revealed that only the highest dose of mianserin (30 mg/kg) reduced immobility and increased climbing behavior, whereas the other doses did not affect any behavioral parameters. The effects of mianserin, at a behaviorally inactive dose (10 mg/kg), given in combination with either WAY 161503 (1 mg/kg) or fluoxetine (20 mg/kg), were then examined and the results are shown in Fig. 5B. ANOVA demonstrated that there was a significant alteration of immobility \( F(5,49) = 4.75, \ P = .001 \), swimming \( F(5,49) = 18.94, \ P < .001 \), and climbing behavior \( F(5,49) = 2.85, \ P < .05 \). Post hoc analysis confirmed that both fluoxetine and WAY 161503 reduced immobility and increased swimming behavior without altering climbing scores. Mianserin significantly abolished the effects of WAY 161503 when given in combination. In addition, mianserin counteracted the fluoxetine-induced increase in swimming behavior. However, mianserin caused an increase in climbing behavior when combined with fluoxetine and therefore did not block fluoxetine’s effect on immobility. Mianserin at the dose used had no effect of its own on any of the behavioral parameters.

Discussion

The precise specification of the neural mechanisms underlying the effects of antidepressant drugs can identify novel candidates involved in the pathophysiology of depression and possible new targets for therapeutic intervention. In this study, we provided direct evidence for a role of the 5-HT_{2C} receptor in mediating the antidepressant-like behavioral effects of both the novel 5-HT_{2C} receptor agonist WAY 161503 and those of the SSRI and established antidepressant drug fluoxetine. Three selective 5-HT_{2C} receptor agonists, WAY 161503, RO 60-0175, and RO 60-0332, were demonstrated to have antidepressant potential in the modified rat FST paradigm. Although fluoxetine and the three novel 5-HT_{2C} receptor agonists produced qualitatively similar responses in the modified rat FST, the effects of fluoxetine could be attributable either to its direct action at the 5-HT_{2C} receptor or to an indirect consequence of 5-HT transporter blockade. The latter seems more likely because prior depletion of 5-HT prevents the antidepressant behavioral response of fluoxetine (Page et al., 1999). This initial demonstration of common effects of all three novel 5-HT_{2C} receptor agonists with the SSRI fluoxetine in the rat FST paradigm suggested that compounds with similar actions may have clinical antidepressant ability. Both RO 60-0175 and RO 60-0332 have been shown previously to be active in other animal models of depression (Moreau et al., 1996; Martin et al., 1998), but the specificity of their behavioral effects for the 5-HT_{2C} Receptor was not investigated using antagonism studies. The fact that...
SB 206533 antagonized the antidepressant-like behavioral effects of both WAY 161503 and fluoxetine suggests that these effects were mediated by the activation of 5-HT<sub>2C</sub> receptors. Although SB 206533 is also a 5-HT<sub>2B</sub> receptor antagonist, the functional relevance of this effect is not known. Previous behavioral studies have shown that SB 206533 is effective at blocking 5-HT<sub>2C</sub> receptor-mediated behaviors induced by m-CPP (Kennett et al. 1996) and more selective 5-HT<sub>2C</sub> receptor agonists such as RO 60-0175 (Sukoff and Goldstein, 1998), fluoxetine treatment can impact noradrenergic neurotransmission (Li et al., 1996; Gobert et al., 1997). With the combined administration of mianserin and fluoxetine, at a dose of mianserin that is subactive in our behavioral paradigm but that increases discharge of the noradrenergic nucleus locus ceruleus (Curtis and Valentino, 1998), and the expression of both behavioral components may predict more effective antidepressant drug treatments (Lucki, 1997). Because the affinity of fluoxetine for the 5-HT transporter is only an order of magnitude greater than that for the norepinephrine transporter (Goodnick and Goldstein, 1998), fluoxetine treatment can impact noradrenergic neurotransmission (Li et al., 1996; Gobert et al., 1997). With the combined administration of mianserin and fluoxetine, at a dose of mianserin that is subactive in our behavioral paradigm but that increases discharge of the noradrenergic nucleus locus ceruleus (Curtis and Valentino, 1991), a synergistic effect on climbing behavior was seen. It is tempting to speculate that the addition of α<sub>2</sub> receptor block-
ade by mianserin to the inhibition of 5-HT reuptake blockade by fluoxetine might enhance effects on catecholamine and serotonergic transmission through a variety of potential mechanisms (Gobert et al., 1997). Because the pattern of behavior produced by mianserin and fluoxetine combinations manifested in an increase in climbing behavior, a response mediated by catecholamines, mianserin may have augmented any effects of fluoxetine on catecholamine transmission past the threshold where they would influence behavior. These data from the mianserin/fluoxetine combination study are but one example of the utility of specifying active behaviors in the FST because otherwise, mianserin’s blockade of fluoxetine’s antidepressant-like effects, i.e., swimming behavior, could not be shown. The combination of mianserin with fluoxetine may lead to augmentation of their clinical effects (Maes et al., 1999).

Most challenge studies that have examined functional responses associated with 5-HT2C Receptor activation have relied on the use of m-CPP as a 5-HT2C receptor agonist. However, the lack of effect of m-CPP in the rat FST, even though it has substantial affinity for 5-HT2C receptors, indicates that its behavioral effects may be complicated by effects at many other receptors with which it interacts (Murphy et al., 1991). The inactivity of m-CPP in the FST could be attributable to behavioral suppression caused by its potent inhibitory effects on general locomotion (Lucki et al., 1989). Nevertheless, as with many antidepressants, RO 60-0175, RO 60-0332, and WAY 161503 at WAY 161503 at behaviorally active doses in the FST have been shown to produce similar hypomotility effects that do not obscure their antidepressant-like effects (Martin et al., 1998; S. Rosenzweig-Lipson, personal communication). Another possibility is that the effects of m-CPP on immobility may be mediated through its actions at the 5-HT1B receptor. Previous studies have shown that activation of 5-HT1B receptors can block the anti-immobility effects of desipramine in the rat FST (Cervo et al., 1989; but see Schlicker et al., 1992). It is of interest that the antidepressant nefazodone, which has m-CPP as one of its primary metabolites, is inactive in this test (J. F. Cryan and I. Lucki, unpublished observations). This effect may also be attributable to functional antagonism by m-CPP through its actions at the 5-HT1B receptor. It is unclear whether there are other functional receptors that might be produced by newly selective 5-HT2C receptor agonists that would differ from those produced by m-CPP.

Several 5-HT receptor subtypes, alone or interdependent with the 5-HT2C receptor, are likely to play a role in the mediation of antidepressant effects. Other 5-HT receptors such as 5-HT1A, 5-HT1B, and 5-HT2A receptors have been suggested to mediate antidepressant responses and play a role in the responses produced by SSRIs (Lucki et al., 1994; Berendson, 1995). Although the 5-HT2C receptor antagonist SB 2060533 completely blocked behavioral responses to fluoxetine, the FST paradigm used was not designed to reveal a residual role for other 5-HT receptors in the effects of fluoxetine. Nevertheless, results with novel and selective 5-HT2C receptor agonists present new evidence that the 5-HT2C receptor may indeed be a novel target for the development of antidepressants and perhaps of drugs effective in other psychiatric disorders involving 5-HT (Lucki, 1998).

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