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Antidepressant-Like Behavioral Effects in 5-Hydroxytryptamine$_{1A}$ and 5-Hydroxytryptamine$_{1B}$ Receptor Mutant Mice

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ABSTRACT

The development of serotonin receptor knockout mice has provided an opportunity to study antidepressant drug effects in animals with targeted genetic deletion of receptors involved in antidepressant responses. In the current study, the effects of two types of antidepressant drugs, the selective serotonin reuptake inhibitors fluoxetine and paroxetine and the selective norepinephrine reuptake inhibitor desipramine, were examined in 5-hydroxytryptamine (5-HT)$_{1A}$ and 5-HT$_{1B}$ receptor mutant mice using the tail suspension test (TST). Under baseline conditions, the immobility of 5-HT$_{1A}$ receptor mutant mice, but not 5-HT$_{1B}$ receptor mutant mice, was significantly lower than that of wild-type mice. The decreased baseline immobility in 5-HT$_{1A}$ receptor mutant mice was reversed by pretreatment with α-methyl-*para*-tyrosine, but not by *para*-chlorophenylalanine, suggesting mediation by enhanced catecholamine function. In wild-type mice, fluoxetine (10.0–20.0 mg/kg i.p.) and desipramine (5.0–20.0 mg/kg i.p.) both significantly decreased immobility in the TST. In 5-HT$_{1A}$ receptor mutant mice, desipramine (20.0 mg/kg i.p.) significantly decreased immobility, whereas fluoxetine (20.0 mg/kg i.p.) and paroxetine (20.0 mg/kg i.p.) had no effect. The immobility of 5-HT$_{1B}$ receptor mutant mice was decreased similarly by desipramine (5.0–20.0 mg/kg i.p.). However, the effect of low doses of fluoxetine were significantly augmented in the 5-HT$_{1B}$ receptor mutant mice (2.5–20.0 mg/kg i.p.) compared with wild-type mice. Administration of selective 5-HT receptor antagonists in wild-type mice partially reproduced the phenotypes of the mutant mice. These results suggest that 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors have different roles in the modulation of the response to antidepressant drugs in the TST.

Serotonin or 5-hydroxytryptamine (5-HT) has been strongly implicated in the etiology of depression and the mechanism of action of antidepressants (Maes and Meltzer, 1995). Selective serotonin reuptake inhibitors (SSRIs) are clinically effective antidepressants and their ability to increase serotonergic transmission is a critical component of their therapeutic antidepressant activity (Delgado et al., 1999). In contrast, increased catecholamine transmission appears to be more important for maintaining the clinical effects of antidepressants that enhance norepinephrine (NE) transmission. Although the 5-HT receptor subtypes that are responsible for the antidepressant actions of SSRIs are uncertain, pharmacological studies using rodent models of antidepressant-like behaviors have suggested important roles for individual 5-HT receptors by producing behavioral responses characteristic of conventional antidepressants using selective agonists or in blocking the activity of conventional antidepressants with antagonists (Lucki et al., 1994; Cryan and Lucki, 2000). Specifically, selective 5-HT$_{1A}$ receptor agonists produce responses in rodent behaviors, such as the forced swimming test (FST) and the tail suspension test (TST), that are similar to those produced by conventional antidepressants (Luscombe et al., 1993; Lucki et al., 1994; De Vry, 1995) and the effects of antidepressants are blocked by 5-HT$_{1A}$ receptor antagonists (Detke et al., 1995; Redrobe et al., 1996). Studies have also implicated the 5-HT$_{1B}$ receptor in antidepressant behavioral effects in the rat and mouse (Schlicker et al., 1992; O'Neill et al., 1996; Redrobe et al., 1996), although few compounds are available that substantially discriminate 5-HT$_{1B}$ receptors from other 5-HT receptors.

The recent development of 5-HT receptor knockout mice has permitted the study of behavioral effects and drug responses in animals that have genetic deletion of targeted

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ABBREVIATIONS: 5-HT, serotonin or 5-hydroxytryptamine; SSRI, selective serotonin reuptake inhibitor; NE, norepinephrine; FST, forced swimming test; TST, tail suspension test; PCPA, *para*-chlorophenylalanine; AMPT, α-methyl-*para*-tyrosine; DA, dopamine; DOPAC, 3,4-dihydrophenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; ANOVA, analysis of variance.

5-HT receptors. Behavioral studies using receptor knockout mice for the 5-HT_{1A} (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) and 5-HT_{1B} (Saudou et al., 1994) receptors have suggested that these receptor subtypes may play a role in affective disorders. The 5-HT_{1A} receptor knockout mice demonstrated behaviors consistent with an increase in anxiety (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) because they spent less time in the open arms of the elevated plus maze and the elevated zero maze, less time in the center of an open field, and less time exploring a novel object. These effects were independent of differences in the background strain. 5-HT_{1A} receptor knockout mice also demonstrated decreased baseline immobility in the FST and the TST without drugs given (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Although this behavior is similar to the expected response of wild-type mice if they were administered clinically effective antidepressants, the exact reason for this behavioral change is unknown. The 5-HT_{1B} receptor knockout mice showed a somewhat different, and in some cases opposite, behavioral profile than 5-HT_{1A} receptor mutants because they displayed decreases in measures of anxiety in the elevated plus maze and open field, as well as an increase in aggression in the resident intruder paradigm (Zhuang et al., 1999).

Studies of antidepressant-like behaviors using mice with genetic deletions targeted at 5-HT receptors may provide complementary information to pharmacological studies regarding the role of 5-HT receptor mechanisms in the actions of antidepressant drugs. Genetic techniques produce more selective and total deletion of targeted receptors than acute pharmacological tools. Although constitutive genetic deletions can produce physiological compensations for loss of function, the functional role of compensations in behavioral outcomes of knockout mice can often be evaluated using corresponding pharmacological antagonists. Targeted null mutations may produce aberrant behaviors resembling psychiatric disorders or induce variability in responses to psychiatric medications that may be related to human homologs of the murine-disrupted gene (Veenstra-VanderWeele et al., 2000).

The current studies were intended to clarify the roles of the 5-HT_{1A} and 5-HT_{1B} receptors in the actions of antidepressant drugs by studying mice with targeted deletion of the 5-HT_{1A} and 5-HT_{1B} receptor subtypes. The TST procedure induces behavioral immobility in mice by suspending them by the tail. Immobility in the TST is reduced by the administration of a wide range of antidepressant treatments, including tricyclic antidepressants, monoamine oxidase inhibitors, SSRIs, and atypical antidepressants (Steru et al., 1985, 1987; Porsolt et al., 1987; Perrault et al., 1992; O'Neill et al., 1996). The current studies examined the effects of antidepressant drugs on the immobility of 5-HT_{1A} and 5-HT_{1B} receptor mutant mice in the TST, in an attempt to better understand the 5-HT receptor mechanisms underlying antidepressant drug action.

Materials and Methods

Animals. Adult male wild-type, and homozygote 5-HT_{1A}, and 5-HT_{1B} receptor knockout mice, all generated on a 129/Sv background, were bred and housed in a colony at the University of Pennsylvania (Philadelphia, PA). Founders were obtained from established colonies derived originally on the 129/Sv strain (Saudou et al., 1994; Ramboz et al., 1998; Phillips et al., 1999) by Dr. René Hen, Columbia University (New York, NY). Mice were generated by breeding homozygote mutant or wild-type mice. Mice were housed in groups of three to four per cage for at least 2 weeks prior to study and were tested at 10 to 16 weeks of age. The animal room was maintained at a constant temperature (21 ± 1°C) and a 12-h light cycle (lights on at 7:00 AM). Food and water were freely available. All subjects were experimentally naïve and used only once.

Behavioral Procedure. The tail suspension test was a modified version of that validated for NMRI mice by Steru et al. (1985). Mice were transported a short distance from the holding facility to the testing room and left there undisturbed for at least 3 h. Subjects were randomly allocated to treatment conditions and tested in counterbalanced order. Thirty minutes after injection, mice were individually suspended by the tail to a horizontal ring-stand bar (distance from floor was 35 cm) using adhesive tape (distance from tip of tail was 2 cm). Typically, mice demonstrated several escape-oriented behaviors interspersed with temporally increasing bouts of immobility. A 6-min test session was videotaped. Videotapes were subsequently scored by a highly trained observer who was unaware of the treatment. The parameter recorded was the number of seconds spent immobile.

Drugs. All drugs were freshly prepared in a volume of 10 ml/kg just prior to use. Doses of desipramine hydrochloride, fluoxetine hydrochloride, paroxetine hydrochloride, *para*-chlorophenylalanine methyl ester (PCPA), α-methyl-*para*-tyrosine methyl ester (AMPT), WAY 100635 maleate, and GR 127935 hydrochloride were calculated as milligrams per kilogram of base. All drugs were dissolved in distilled water and administered via the intraperitoneal route. Control animals received physiological saline (0.9%).

Desipramine and fluoxetine were administered 30 min prior to behavioral testing. The selective 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg; Fletcher et al., 1996) and the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (0.056 mg/kg; Skingle et al., 1996) were administered immediately prior to fluoxetine or saline. In some experiments, an individual challenge dose of 20 mg/kg fluoxetine was selected based on the dose-response curves showing that it produced a near-maximal behavioral response but would be expected to maintain selectivity for 5-HT. Doses of the antagonists were selected on the basis of prior studies showing that they blocked the effects of corresponding agonists on 5-HT release (Knobelman et al., 2000).

PCPA (250 mg/kg) was administered twice daily for 3 days, with the last dose given 18 h prior to behavioral testing (Cesana et al., 1993). AMPT (200 mg/kg) was administered as a single dose 4 h prior to testing (Corrodi and Hanson, 1966).

Neurochemical Procedure. Brain tissue samples were taken for determination of brain monoamine levels in mice treated with either PCPA or AMPT. Mice were sacrificed by decapitation 1 h after behavioral testing, corresponding to either 18 h after the last injection of PCPA or 5 h after the injection of AMPT. The whole brain was removed and separated from the cerebellum. Tissue samples were homogenized in 0.1 N perchloric acid with 100 μM EDTA (15 μl/mg of tissue) using a Tissuemizer (Tekmar, Cleveland, OH). Samples were centrifuged at 15,000 rpm (23,143*g*) for 15 min at 2–8°C. The supernatant was filtered through 0.45-μm nylon acrodisk syringe filters and divided for analysis of the monoamines, NE, dopamine (DA), and 5-HT as well as the metabolites 3,4-dihydrophenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA). Two separate high-pressure liquid chromatography systems were used for analysis. One consisted of an ESA solvent delivery system (ESA Inc., Chelmsford, MA) and a Velosep RP-18 column (100 × 3 mm, 3 μm; Varian Chromatography, Walnut Creek, CA). The mobile phase consisted of 60 mM sodium phosphate buffer (pH 4.2) with 100 μM EDTA, 1.5 mM sodium octyl-sulfate, 3.5% (v/v) methanol. The flow rate through the system was 700 μl/min. The detection system utilized an ESA 5200 electrochemical detector with three electrodes in series. The conditioning electrode was set at +270 mV. The applied

potential of the second electrode was set at −250 mV, and the compounds of interest (NE and DOPAC) were quantified at a third electrode, which was set at +270 mV. Peak heights were measured and compared with peak heights of standards at 10^{-8} M. The second high-pressure liquid chromatography system used to measure levels of 5-HT, DA, and 5-HIAA consisted of a PM80 solvent delivery system (Bioanalytical Systems, West Lafayette, IN), a 10-μl sample loop, and a sepstik microbore column (ODS 3 μm; 100 × 1 mm; Bioanalytical Systems, West Lafayette, IN). The mobile phase consisted of 90 mM NaAc, 35 mM citric acid, 0.34 mM EDTA, 1.2 mM octyl sulfate, and 9% (v/v) methanol adjusted to pH 4.2. The flow rate through the system was 110 μl/min and the detector was set at a potential of +0.60 V relative to a Ag/AgCl reference electrode. Peak heights were measured and compared with peak heights of standards at 10^{-8} M.

Statistical Analysis. Data were analyzed using one-way or two-way analysis of variance where appropriate. Planned comparisons between individual groups were conducted using the Student-Newman-Keuls procedure.

Results

Effects of PCPA and AMPT Pretreatment on Baseline Immobility in 5-HT$_{1A}$ Receptor Knockout Mice. In an attempt to understand the neurochemical mechanisms underlying the baseline differences in immobility between wild-type and 5-HT$_{1A}$ receptor mutants, animals were pretreated with either the tryptophan hydroxylase inhibitor PCPA or the tyrosine hydroxylase inhibitor AMPT. The neurochemical effects of these treatments are summarized in Table 1. PCPA reduced 5-HT levels by 70% in wild-type mice and 67% in 5-HT$_{1A}$ receptor knockout mice, without significant effects on DA or NE. AMPT reduced dopamine levels by 57% in wild-type mice and 56% in 5-HT$_{1A}$ receptor knockout mice. AMPT reduced NE levels by 53% in wild-type mice and by 42% in 5-HT$_{1A}$ receptor knockout mice. AMPT did not significantly affect levels of 5-HT.

The effects of monoamine depletions on TST performance are shown in Fig. 1. Although 5-HT$_{1A}$ receptor mutants were less immobile than wild-type mice [genotype: $F(1,29) = 12.79$, $p < 0.01$], depletion of 5-HT with PCPA had no effect on the baseline immobility of wild-type or 5-HT$_{1A}$ −/− mice [PCPA: $F(1,29) = 0.97$, $p = 0.37$; interaction: $F(1,29) = 0.08$,

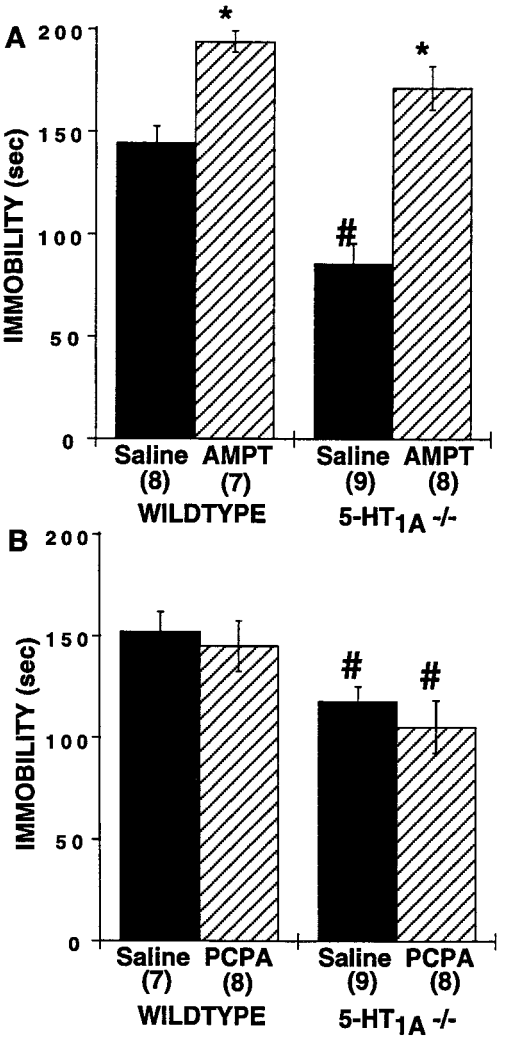

Fig. 1. Effects of pretreatment with AMPT (A) or PCPA (B) on the behavior of wild-type and 5-HT$_{1A}$ receptor knockout mice in the tail suspension test. n = 7–9 mice/group. *$p < 0.05$ versus same genotype saline control; #$p < 0.05$ versus wild-type mice treated with the same dose.

TABLE 1

Effects of PCPA (250 mg/kg b.i.d. for 3 days) or AMPT (250 mg/kg) pretreatment on 5-HT, 5-HIAA, NE, DA, and DOPAC levels in wild-type and 5-HT$_{1A}$ receptor mutant mice

Data are the mean value ± 1 S.E.M. expressed as picograms per milligram of tissue.

	Wildtype		5-HT$_{1A}$ Receptor Mutants	
	Saline	PCPA	Saline	PCPA
PCPA pretreatment				
n	7	8	8	8
5-HT[a,b]	1009.0 ± 27.7	300.6 ± 36.2*	1187.5 ± 57.7	394.7 ± 57.7*
5-HIAA[a,c]	880.3 ± 29.9	403.6 ± 19.2*	1109.8 ± 103.4	371.9 ± 37.2*
NE	101.3 ± 7.7	94.8 ± 2.2	104.3 ± 5.0	95.6 ± 1.5
DA[b]	1851.9 ± 185.4	1594.0 ± 37.8	2267.5 ± 285.4	2345.0 ± 293.4
DOPAC	25.3 ± 1.5	21.8 ± 1.1	20.8 ± 2.4	21.6 ± 1.0
AMPT pretreatment	Saline	AMPT	Saline	AMPT
n	8	7	9	8
5-HT	990.8 ± 31.4	979.4 ± 50.4	1047.0 ± 17.0	1055.5 ± 34.9
5-HIAA	752.8 ± 35.7	752.3 ± 42.5	883.4 ± 82.8	773.0 ± 25.8
NE[a]	92.1 ± 11.7	43.0 ± 4.5*	92.9 ± 5.8	54.3 ± 1.8*
DA[a]	2628.4 ± 214.8	1124.3 ± 56.5*	2587.9 ± 202.0	1136.1 ± 59.6*
DOPAC[a]	27.6 ± 1.0	5.7 ± 0.6*	26.1 ± 1.6	4.9 ± 0.4*

*$p < 0.05$ vs. corresponding genotype saline control.
ANOVA results: [a]Treatment, $p < 0.001$; [b]Genotype, $p < 0.05$; [c]Treatment × genotype interaction, $p < 0.05$.

$p = 0.82$]. In contrast, the depletion of catecholamines with AMPT reversed the reduced baseline immobility of 5-HT_{1A} receptor knockout mice [genotype: $F(1,28) = 20.34, p < 0.001$; AMPT: $F(1,28) = 56.72$, $p < 0.001$; interaction: $F(1,28) = 4.02$; $p = 0.05$]. Although AMPT pretreatment produced a significant increase in the immobility of wild-type mice (34% increase), the increase in immobility in 5-HT_{1A} −/− mice was much larger (100% increase).

Effects of Antidepressants on Immobility in 5-HT_{1A} Receptor Knockout Mice. The effects of desipramine, fluoxetine, and paroxetine (each tested at 20.0 mg/kg) on TST immobility in wild-type and 5-HT_{1A} receptor knockout mice are shown in Fig. 2. ANOVA revealed a significant genotype × treatment interaction [$F(3,74) = 4.15$, $p < 0.01$]. Planned comparisons using Newman-Keuls tests indicated that the immobility of 5-HT_{1A} receptor knockout mice was significantly lower than that of wild-type mice at baseline ($p < 0.05$). Test doses of the SSRIs fluoxetine and paroxetine failed to reduce immobility values in 5-HT_{1A} receptor mutants even though these treatments were effective in reducing immobility by 30 and 32% in wild-type mice, respectively. The highest dose of desipramine that was effective in the 5-HT_{1B} receptor knockout mice (see below), however, significantly reduced immobility ($p < 0.05$) in the 5-HT_{1A} receptor knockout mice (55%, $p < 0.05$). All three of these treatments produced a significant decrease in immobility in concurrent wild-type controls ($p < 0.05$).

Effects of Antidepressants on TST Immobility in 5-HT_{1B} Receptor Knockout Mice. There were no significant differences in immobility between wild-type and 5-HT_{1B} receptor knockout mice at baseline in the TST. The effects of fluoxetine (1.25–20.0 mg/kg) on immobility in wild-type and 5-HT_{1B} receptor knockout mice in the TST are shown in Fig. 3. Two-way ANOVA revealed a significant genotype × treatment interaction on immobility [$F(5,131) = 2.36$, $p < 0.05$]. 5-HT_{1B} −/− mice demonstrated an increase in sensitivity to the behavioral effects of fluoxetine. Immobility was reduced significantly by fluoxetine in wild-type (10.0–20.0 mg/kg) and by a wider range of fluoxetine doses (2.5–20.0 mg/kg) in 5-HT_{1A} −/− mice relative to their respective saline controls ($p < 0.05$). Furthermore, at the 2.5-mg/kg dose of fluoxetine, 5-HT_{1B} receptor knockout mice displayed an enhanced anti-immobility response compared with wild-type mice ($p < 0.01$).

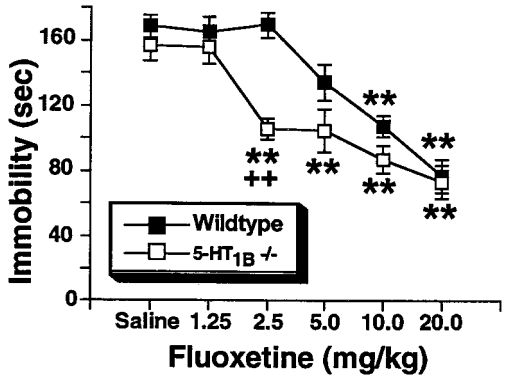

Fig. 3. Effects of fluoxetine (1.25–20.0 mg/kg) on the behavior of wild-type and 5-HT_{1B} receptor knockout mice in the tail suspension test. For both genotypes, $n = 9$ to 22 mice for each dose. $^{**}p < 0.01$ versus same genotype saline control; $^{++}p < 0.01$ versus wild-type mice treated with the same dose.

The effects of desipramine (2.5–20.0 mg/kg) on immobility in wild-type and 5-HT_{1B} receptor knockout mice in the TST are shown in Fig. 4. Two-way ANOVA indicated a significant main effect of treatment [$F(4,98) = 29.37$, $p < 0.001$]. Desipramine significantly reduced immobility in both wild-type (5.0–20.0 mg/kg, $p < 0.05$) and 5-HT_{1B} receptor knockout mice (5.0–20.0 mg/kg; $p < 0.05$). There was no main effect of genotype [$F(1,98) = 2.65$, N.S.] and no genotype × treatment interaction [$F(4,98) = 1.28$, N.S.].

Pretreatment with 5-HT Receptor Antagonists and Effect of Fluoxetine in Wild-Type Mice. The effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg) administered immediately prior to fluoxetine in wild-type mice is shown in Fig. 5. Two-way ANOVA indicated a significant pretreatment × treatment interaction [$F(2,42) = 5.62$, $p < 0.01$]. Planned comparisons using Newman-Keuls tests indicated that the anti-immobility effect of 20.0 mg/kg fluoxetine was completely blocked by pretreatment with WAY 100635 ($p < 0.05$).

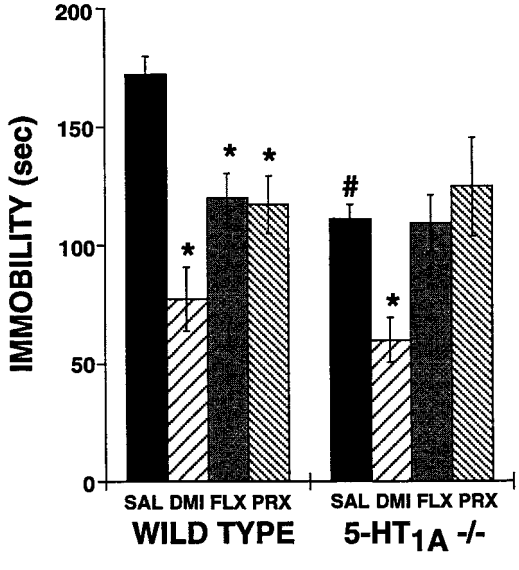

Fig. 2. Effects of desipramine (20.0 mg/kg), fluoxetine (20.0 mg/kg), and paroxetine (20.0 mg/kg) on the behavior of wild-type and 5-HT_{1A} receptor knockout mice in the tail suspension test. $^{*}p < 0.05$ versus same genotype saline control; $^{\#}p < 0.05$ versus wild-type mice treated with the same dose.

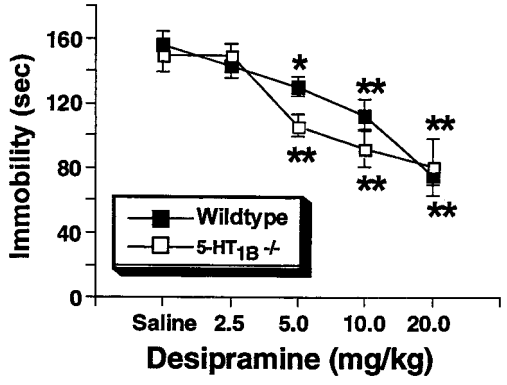

Fig. 4. Effects of desipramine (2.5–20.0 mg/kg) on the behavior of wild-type 5-HT_{1B} receptor knockout mice in the tail suspension test. For both genotypes: saline and 10.0 mg/kg, $n = 13$; all other doses, $n = 10$. $^{*}p < 0.05$; $^{**}p < 0.01$ versus same genotype saline control.

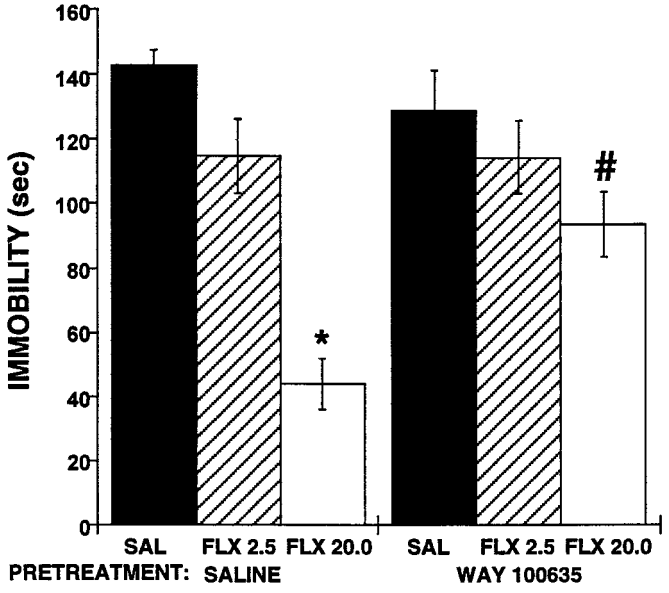

Fig. 5. Effects of WAY 100635 (0.1 mg/kg) pretreatment on the effect of fluoxetine (2.5 and 20.0 mg/kg) on the behavior of wild-type mice in the tail suspension test. $n = 8$ mice/group. $^{*}p < 0.05$ versus saline control with same pretreatment; $^{\#}p < 0.05$ versus corresponding dose with different pretreatment.

The effects of the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (0.056 mg/kg) administered immediately prior to fluoxetine in wild-type mice is shown in Fig. 6. There was a significant main effect of fluoxetine treatment [$F(2,56) = 61.07$, $p < 0.001$]. Planned comparisons using Newman-Keuls tests indicated that pretreatment with GR 127935 significantly enhanced the anti-immobility effect of 2.5 mg/kg fluoxetine ($p < 0.05$).

Discussion

The current study provides evidence that 5-HT_{1A} and 5-HT_{1B} receptors have different roles in the modulation of the response to antidepressant drugs in the TST. The absence of 5-HT_{1A} receptors was associated with a decrease in immobility under baseline conditions, while genetic deletion of 5-HT_{1B} receptors had no effect on baseline immobility. Also, there was no effect of the SSRIs fluoxetine and paroxetine in 5-HT_{1A} receptor mutant mice at the doses tested, whereas 5-HT_{1B} receptor knockout mice demonstrated increased sensitivity to fluoxetine. Despite the differing baselines, the effects of the selective NE reuptake inhibitor desipramine were similar in 5-HT_{1A} and 5-HT_{1B} receptor knockout mice, revealing distinctions between antidepressants with different pharmacological mechanisms. Although 5-HT_{1A} and 5-HT_{1B} receptors are both located postsynaptically in limbic regions and known to regulate the release of 5-HT, the current results suggest that they modulate the antidepressant-like effects of the SSRI fluoxetine in different ways.

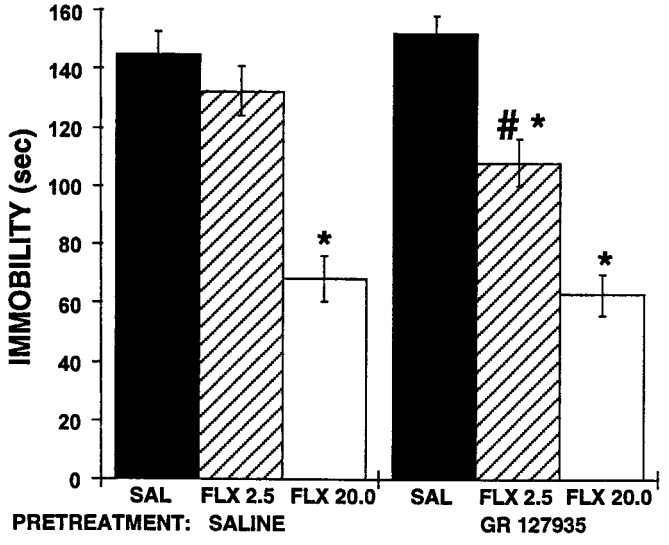

Fig. 6. Effects of GR 127935 (0.056 mg/kg) pretreatment on the effect of fluoxetine (2.5 and 20.0 mg/kg) on the behavior of wild-type mice. $n = 10$ to 11 mice/group. $^{*}p < 0.05$ versus saline control with same pretreatment; $^{\#}p < 0.05$ versus corresponding dose with different pretreatment.

5-HT receptor mutant mice showed substantial differences in their baseline responses to tests of antidepressant activity. 5-HT_{1A} receptor mutant showed a decrease of baseline immobility values when tested in the TST, similar to a previous report (Heisler et al., 1998). 5-HT_{1B} receptor mutants did not show similar effects. 5-HT_{1A} receptor mutant mice have also been reported to show reduction of immobility in the FST (Parks et al., 1998; Ramboz et al., 1998). Because the TST is a behavioral test for antidepressant activity, the existence of these behavioral differences in 5-HT_{1A} receptor mutant mice at baseline may result from pre-existing neurochemical changes that simulate the effects of antidepressants. For example, antidepressant-like responses of 5-HT_{1A} receptor mutants could reflect a disinhibition of serotonergic neuronal activity resulting from the absence of 5-HT_{1A} autoreceptors. This hypothesis was tested using the tryptophan hydroxylase inhibitor PCPA but the depletion of serotonin failed to reverse the decreased immobility of the 5-HT_{1A} receptor mutant mice. Although PCPA produced only a 67% depletion of forebrain 5-HT, this was sufficient to prevent the effects of fluoxetine in the mouse FST and TST (Cesana et al., 1993; O'Leary et al., 2001). The lack of a behavioral response to PCPA pretreatment is quite significant because it suggests that the TST behavior is not caused by the absence of presynaptic 5-HT_{1A} receptors. Parsons et al. (2001) reported increases in basal and stress-induced extracellular levels of 5-HT in 5-HT_{1A} receptor knockout mice. However, we found no such change in basal levels testing a larger group of 5-HT_{1A} receptor knockout mice from the same background (129 mice) as used in this behavioral study (Knobelman et al., 2001). Although differences in age and background strain of the mice may explain these experimental differences, assessing the role of 5-HT transmission in mediating baseline behavioral differences in 5-HT_{1A} receptor knockout mice has taken on added importance in view of these reports. Alternatively, antidepressant-like responses of 5-HT_{1A} receptor mutants resulting from the absence of 5-HT_{1A} receptors could involve altered regulation of NE or DA transmission. This hypothesis was tested by the depletion of catecholamines using the tyrosine hydroxylase inhibitor AMPT. Although the immobility of wild-type mice was increased by AMPT pretreatment, the significantly larger proportional increase in the immobility of the 5-HT_{1A} receptor knockout mice suggested selective vulnerability for the effects of catecholamine depletion. In the absence of other evidence, however, AMPT may have had a greater effect in the 5-HT_{1A} receptor knock-

out mice because of differences in initial baseline. Nevertheless, these data suggest that genetic deletion of the 5-HT_{1A} receptor may activate compensatory mechanisms during development, leading to an enhancement of catecholaminergic function. Future studies could address the specific physiological mechanism of that compensation.

5-HT receptor mutant mice also showed substantial differences in their behavioral responses to antidepressant drugs. The SSRIs fluoxetine and paroxetine failed to decrease immobility in 5-HT_{1A} receptor mutant mice at a test dose that was active in wild-type and 5-HT_{1B} receptor mutant mice. Although starting from a lower baseline, 5-HT_{1A} receptor mutant mice still demonstrated an antidepressant-like response to the selective NE reuptake inhibitor desipramine, thus distinguishing antidepressants with different pharmacological effects. These data suggest that the presence of 5-HT_{1A} receptors may be critical for the expression of the antidepressant-like behavioral responses of SSRIs in the TST.

In contrast, 5-HT_{1B} receptor mutant mice demonstrated a dramatically enhanced response to low doses of fluoxetine produced by an apparent leftward shift in the dose-response curve for the TST. 5-HT_{1B} receptor deletion did not appreciably alter the antidepressant behavioral response to desipramine, a selective NE reuptake inhibitor. Because 5-HT_{1B} autoreceptors ordinarily restrain 5-HT release, the increased response to fluoxetine may be related to the loss of inhibition of 5-HT transmission in areas critical for the expression of antidepressant-like behaviors. Microdialysis studies have shown that in 5-HT_{1B} receptor mutant mice, a low 2.5-mg/kg dose of fluoxetine produced an augmented increase of 5-HT in the ventral hippocampus, a dose that was behaviorally inactive in wild-type mice (Knobelman et al., 2001). Thus, 5-HT_{1B} receptor mutant mice may potentiate antidepressant-like behaviors to SSRIs because they are important in regulating extracellular 5-HT in regions, like the hippocampus, that are critical for their expression (Knobelman et al., 2001; Malagie et al., 2001). However, 5-HT_{1B} receptors also mediate the release of other neurotransmitters, such as DA and acetylcholine (Consolo et al., 1996; Ase et al., 2000; Shippenberg et al., 2000), that are potential substrates for enhancing the behavioral response to fluoxetine.

To evaluate whether developmental compensation could be an important factor mediating altered behavioral responses of the mutant mice to antidepressant drugs, studies examined whether similar effects could be produced in wild-type mice pretreated with pharmacological antagonists. Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY 100635 blocked the behavioral effects of fluoxetine, just as they were blocked in 5-HT_{1A} receptor mutants. However, the decreased baseline immobility in 5-HT_{1A} receptor mutant mice was not mimicked by administration of WAY 100635 alone. Although the duration of treatment with the antagonist was brief, developmental compensation may account for the dramatic differences between the effects of the 5-HT_{1A} receptor antagonist and 5-HT_{1A} receptor mutant mice on baseline TST performance. Pretreatment with the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935 enhanced the effect of low doses of fluoxetine, although the magnitude of the enhancement was not as large as that measured in 5-HT_{1B} receptor knockout mice. Interestingly, pretreatment of wild-type mice with GR 127935 also augments the increase of 5-HT produced by SSRIs (Knobelman et al., 2001; Malagie et al., 2001). Thus, pharmacological antagonists reproduced a substantial component of the altered response to fluoxetine in both 5-HT_{1A} and 5-HT_{1B} receptor mutant mice. The limited response to GR 127935 may be due to its poor selectivity, partial efficacy at 5-HT_{1B} receptors, or the need to block receptors for longer periods of time. Newer compounds may be available that can discriminate the effects of 5-HT_{1B} and 5-HT_{1D} receptors (Price et al., 1997).

The results of the present study disagree with the view of others that 5-HT_{1B} receptors are essential for the expression of antidepressant behavioral responses. In a previous study, pretreatment of mice with higher doses of GR 127935 (>10 mg/kg) were shown to block the effects of paroxetine in the TST (O'Neill et al., 1996). Because of its shortcomings as a selective pharmacological antagonist, additional studies are needed to evaluate components of pharmacological selectivity of GR 127935, particularly the role of 5-HT_{1D} receptors (De Vries et al., 1997). Another study reported that 5-HT_{1B} receptor knockout mice fail to demonstrate antidepressant-like responses to fluoxetine in the FST (Trillat et al., 1998). Differences between the TST and FST or testing procedures could mediate these divergent results. However, in a recent strain survey study, 129 mice were among the mouse strains that failed to show antidepressant-like responses to SSRIs in the FST (Lucki et al., 2001). In our experience, SSRIs produced hind limb rigidity in 129 mice when they were placed in the water and increased rather than decreased immobility time in the FST. In the Trillat et al. (1998) study, wild-type 129 mice demonstrated immobility for nearly the entire 4-min testing period, except for a 5 to 10% reduction from baseline produced by SSRIs. The unusually long immobility period and small magnitude of response to antidepressants in this FST procedure may not be sufficiently robust and representative for distinguishing neural mechanisms typically associated with antidepressant responses.

In conclusion, these studies demonstrated that mutations of 5-HT_{1A} and 5-HT_{1B} receptors produce different, almost opposite, effects in modulating the behavioral effects of antidepressants, just as they have been shown to produce opposite effects on other behaviors (Zhuang et al., 1999). Specifically, these data contend that the function of 5-HT_{1A} receptors (likely postsynaptic) may be necessary for the expression of fluoxetine's behavioral effects, whereas those effects were enhanced by genetic deletion of the 5-HT_{1B} receptor. The functional consequences of 5-HT receptor mutations may depend on the pharmacological selectivity of the antidepressants because they were unnecessary for the behavioral activity of the selective NE reuptake inhibitor desipramine. These data provide important information concerning the potential role for genetic regulation of 5-HT receptors in clinical depression and antidepressant response (Veenstra-VanderWeele et al., 2000). Future studies with genetic mutant mice will be able to delineate specific roles for presynaptic and postsynaptic receptors with regionally selective genetic deletions and address the role of developmental compensation with conditional mutations.

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