A Paradoxical Locomotor Response in Serotonin 5-HT$_{2C}$ Receptor Mutant Mice

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Paradoxical behavioral responses to nonselective neuropsychiatric drugs are frequently encountered and poorly understood. We report that a single receptor gene mutation produces a paradoxical response to the nonspecific serotonin receptor agonist *m*-chlorophenylpiperazine (mCPP). Although this compound normally suppresses locomotion, it produces hyperactivity in mice bearing a targeted mutation of the 5-HT$_{2C}$ receptor gene. This effect was blocked by pretreatment with a 5-HT$_{1B}$ receptor antagonist, indicating that the behavioral consequences of mCPP-induced 5-HT$_{1B}$ receptor stimulation are unmasked in animals devoid of 5-HT$_{2C}$ receptor function. Furthermore, this paradoxical response to mCPP was reproduced in wild-type C57BL/6 mice by previous pharmacological blockade of 5-HT$_{2C}$ receptors, indicating that the mutant phenotype does not result from perturbations of brain development. These effects of 5-HT$_{1B}$ and 5-HT$_{2C}$ receptor antagonists likely reflected blockade of pharmacological actions of mCPP, because these compounds did not alter locomotor activity levels when administered alone. Thus, mCPP interacts with distinct 5-HT receptor targets that produce opposing effects on locomotor activity levels. A paradoxical behavioral response is produced by the genetic inactivation of the target that produces the prevailing effect of the drug in the wild-type animal. This genetically based paradoxical drug effect provides a model for considering the effects of genetic load on neurobehavioral responses to drugs.

Key words: serotonin; 5-HT$_{2C}$ receptor; mCPP; paradoxical; transgenic; locomotion

The brain serotonin [5-hydroxytryptamine (5-HT)] system modulates a diverse array of behavioral and physiological processes. Accordingly, defects of serotonergic function have been proposed to contribute to the manifestations of neuropsychiatric conditions such as depression, anxiety disorders, eating disorders, and migraine. The effects of serotonin are mediated by a heterogeneous family of at least 14 distinct 5-HT receptor subtypes. The contributions of particular subtypes to the actions of serotonin and nonselective agonists remain to be clarified, because the availability of subtype-selective agonist and antagonist compounds is limited. The application of molecular genetic approaches to this problem has led to the generation of mutant mouse strains with targeted disruptions of genes encoding particular 5-HT receptor subtypes. Such strains provide tools that complement pharmacological probes for the analysis of receptor function.

We have applied this approach to the 5-HT$_{2C}$ receptor subtype, which has been implicated in the serotonergic regulation of activity, feeding, and anxiety (Brennan et al., 1997). Animals bearing a targeted mutation of the 5-HT$_{2C}$ receptor gene display pleiotropic effects of the mutation, such as hyperphagia, altered spatial learning, and enhanced neuronal network excitability (Tecott et al., 1995; Nonogaki et al., 1998; Tecott et al., 1998). 5-HT$_{2C}$ receptor mutants were also used to determine the contribution of 5-HT$_{2C}$ receptors to the actions of dexfenfluramine, a compound producing nonselective 5-HT receptor activation by stimulating synaptic serotonin release. Mutants displayed reduced sensitivity to the anorectic effects of dexfenfluramine, implicating 5-HT$_{2C}$ receptors in this action of the drug (Vickers et al., 1999).

In an analogous manner, we sought to determine the extent to which 5-HT$_{2C}$ receptors contribute to the actions of the nonselective agonist *m*-chlorophenylpiperazine (mCPP). In clinical studies, responses to mCPP administration have been frequently used as indicators of central serotonin system function (Schwartz et al., 1997; Southwick et al., 1997; Hollander et al., 1998; Kaye et al., 1998; Broocks et al., 1999). In rodents, mCPP reduces locomotor activity (Kennett and Curzon, 1988; Lucki et al., 1989), suppresses feeding (Samanin et al., 1979; Kennett et al., 1987), and enhances anxiety-like behaviors (Kennett et al., 1989; Whitton and Curzon, 1990). Many of these effects are blocked by antagonists of the 5-HT$_{2C}$ receptor subtype, for which mCPP displays the highest affinity (pK_i, 7.7) (Hoyer, 1988). mCPP has

Received Dec. 22, 1999; revised Feb. 23, 2000; accepted Feb. 23, 2000.

This work was supported by grants from the National Alliance for Research on Schizophrenia and Depression (NARSAD) and the Bank of America Giannini Foundation to L.K.H. and the National Institute on Drug Abuse, the NARSAD, and the EJLB Foundation to L.H.T. We thank Preetpaul Bajwa for technical assistance with these studies, Noura Sall for mouse colony maintenance, and Nelson Freimer and Irwin Lucki for critical reading of this manuscript.

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This article is published in *The Journal of Neuroscience*, Rapid Communications Section, which publishes brief, peer-reviewed papers online, not in print. Rapid Communications are posted online approximately one month earlier than they would appear if printed. They are listed in the Table of Contents of the next open issue of JNeurosci. Cite this article as: JNeurosci, 2000, 20:RC71 (1–5). The publication date is the date of posting online at www.jneurosci.org.

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therefore been considered a pharmacological tool for evaluating 5-HT_{2C} receptor function (Curzon and Kennett, 1990).

In light of these studies, we anticipated that 5-HT_{2C} receptor mutant mice would exhibit reduced sensitivity to the behavioral effects of mCPP. However, attempts to determine the effects of this drug on anxiety-related behaviors were complicated by an unexpected hyperlocomotor response to mCPP in mutant mice; a finding antithetical to the suppression of activity produced in wild-type animals by this drug. In the present study, we describe this response and determine its underlying mechanism. Based on these findings, a model is proposed whereby paradoxical behavioral responses to nonselective psychoactive compounds are determined by genetic load.

MATERIALS AND METHODS

Subjects. 5-HT_{2C} receptor mutant mice were originally generated from a 129-derived embryonic stem cell line (Tecott et al., 1995) and have been back-crossed for 12 generations to a C57BL/6 genetic background. Wild-type C57BL/6 males were crossed with females heterozygous for the 5-HT_{2C} receptor mutation. A PCR-based genotyping strategy was used, as previously described (Brennan et al., 1997). Because the 5-HT_{2C} receptor gene is X-linked (Milatovich et al., 1992), 50% of the resulting males were hemizygous mutants, and 50% were wild types. This approach was chosen rather than the separate maintenance of mutant and wild-type strains to minimize interlitter variability attributable to potential differences in the maternal behavior of mutant and wild-type mothers. To further control for interlitter variability, wild-type littermate controls were used in studies of 5-HT_{2C} receptor mutant mice. Twenty 19- to 21-week-old drug-naïve 5-HT_{2C} receptor mutant and wild-type (n = 10 per genotype) mice were used. For studies using the 5-HT_{2C} receptor antagonist SB 206553, 16 drug-naïve C57BL/6 mice (Charles Rivers Laboratories, Wilmington, MA) of the same age were used. Animals were group-housed, two to six mice per cage, in standard polycarbonate mouse cages (29 × 18.5 × 13 cm). Animals were given *ad libitum* access to food and water and were maintained on a 12 hr light/dark cycle (lights on at 6 A.M.). Animals were tested a minimum of 2 weeks after transfer to the laboratory.

Apparatus. Horizontal activity and rearing were assessed with an automated Photobeam Activity System (version 70110; San Diego Instruments). This system consists of two metal rectangular frames surrounding a standard low-profile polycarbonate rat cage (48 × 27 × 13 cm). Horizontal locomotor activity was determined by the lower frame, consisting of a 4 × 8 array of infrared photobeams, spaced 4.4 cm apart on the *x*-axis and 5.5 cm apart on the *y*-axis, and elevated 2 cm. Frequency of rearing was identified with the upper frame through a set of eight infrared photobeams, spaced 2.5 cm apart and elevated 7 cm.

Procedure. Approximately 3 min before each assay, animals were removed from their home cage and placed in a clean holding cage. Animals were quickly transferred to the center of the testing chamber, and horizontal activity and rearing were monitored in 5 min intervals for 3 hr. Animals were exposed to the apparatus in two trials before drug treatment to determine basal activity levels and to acclimate animals to the testing environment. During each 3 hr session, drug treatments were administered after 2 hr of habituation to the chamber, and drug pretreatments were injected 30 min before this. Experiments were conducted using a within-subjects design with a 3 d interval between drug trials. Testing was counterbalanced by genotype and treatment condition and conducted during the light cycle. All holding and testing cages were autoclaved between subjects. For dose–response studies with GR 127935 and mCPP, 10 5-HT_{2C} mutant and 10 wild-type mice were treated with three doses of GR 127935 (0.3, 1.5, and 7.5 mg/kg) and mCPP (2.5, 5.0, and 10.0 mg/kg) and vehicle. To determine the effect of GR 127935 pretreatment on mCPP responses in mutant and wild-type animals, four treatment conditions were used: (1) vehicle pretreatment followed by vehicle, (2) GR 127935 pretreatment (7.5 mg/kg) followed by vehicle, (3) vehicle pretreatment followed by mCPP (2.5 mg/kg), and (4) GR 127935 (7.5 mg/kg) pretreatment followed by mCPP (2.5 mg/kg). To determine the effect of SB 206553 pretreatment on mCPP responses in C57BL/6 mice, 16 animals were pretreated with vehicle or three doses of SB 206553 (1.0, 2.5, and 5.0 mg/kg) before treatment with vehicle or 2.5 mg/kg mCPP. Experimenters were blind to the genotypes of the mice and to the drugs administered in pharmacological experiments

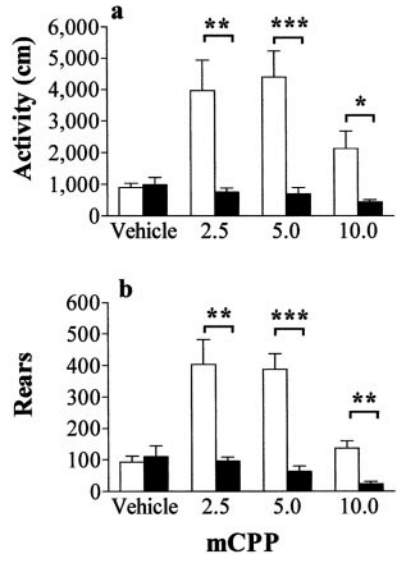

Figure 1. Effect of mCPP on activity. *White bars* indicate 5-HT_{2C} receptor mutant mice (n = 10), and *black bars* represent wild-type mice (n = 10). *a,* Horizontal activity; *b*, rears after mCPP treatment (vehicle or 2.5, 5.0, or 10.0 mg/kg, i.p.). Values are expressed as mean + 1 SEM. Significant differences by genotype, $^{***}p \leq 0.001$; $^{**}p \leq 0.01$; $^{*}p \leq 0.05$.

Drugs. mCPP (Sigma, St. Louis, MO) was dissolved in 0.9% sterile saline; the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (courtesy of Glaxo Group Research Ltd.) was dissolved in distilled water; and the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 (Research Biochemicals International, Natick, MA) was dissolved in 0.32% Tween 80 (ICN Biomedicals, Costa Mesa, CA). Appropriate solvents were used for vehicle comparisons, and all injections were made intraperitoneally in a volume of 10 μl/gm of body weight.

Data analysis. The effects of drug treatment and genotype on horizontal activity and rearing were analyzed using repeated measures ANOVA, followed by Tukey's honestly significant difference *post hoc* tests. For all analyses, significance was assigned at the $p \leq 0.05$ level.

RESULTS

A marked difference in response to mCPP was observed by genotype for both horizontal activity [treatment, $F_{(3,54)} = 6.30$; $p < 0.001$; genotype, $F_{(1,18)} = 20.31$; $p < 0.001$; treatment × genotype, $F_{(3,54)} = 7.38$; $p < 0.001$ (Fig. 1*a*)] and rearing [treatment, $F_{(3,54)} = 12.83$; $p < 0.001$; genotype, $F_{(1,18)} = 30.93$; $p < 0.001$; treatment × genotype, $F_{(3,54)} = 11.67$; $p < 0.001$ (Fig. 1*b*)]. *Post hoc* comparisons of the interactions showed that all doses of mCPP were associated with hyperactivity and enhanced rearing in the mutant but not wild-type mice. Analysis of variance revealed no significant order of injection × drug response interactions in these studies.

To determine whether mCPP-induced hyperactivity in mutant mice was dependent on 5-HT_{1B} receptor stimulation, the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 was used (Skingle et al., 1993; Starkey and Skingle, 1994). When administered alone (at 0.3, 1.5, or 7.5 mg/kg), this compound did not significantly alter levels of activity or rearing (Fig. 2*a*). However, pretreatment with 7.5 mg/kg GR 127935 markedly altered the responses of mutant mice to mCPP. Repeated measures ANOVA of both horizontal activity and rearing revealed a significant interaction between treatment condition and genotype [main effects of treatment and genotype, NS; horizontal activity treatment × genotype interaction, $F_{(3,54)} = 5.92$; $p < 0.01$ (Fig. 2*b*); rearing treatment × genotype interaction, $F_{(3,54)} = 5.76$; $p < 0.01$ (Fig. 2*c*)]. *Post hoc* comparisons showed dramatic hyperactivity and en-

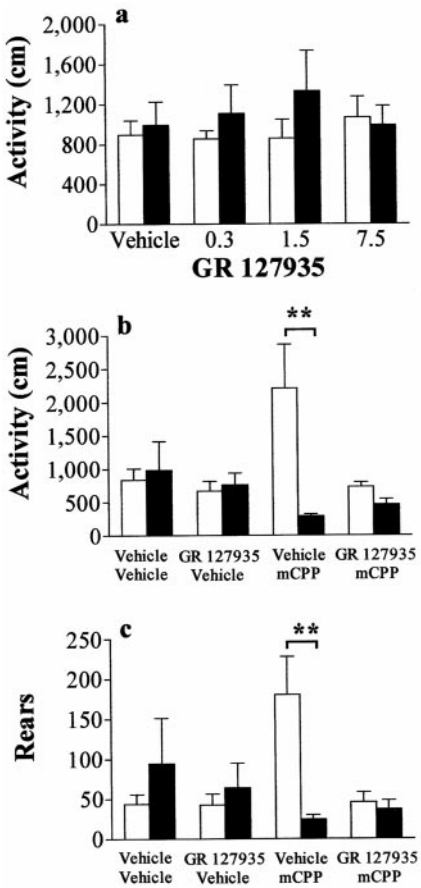

Figure 2. Effect of GR 127935 and mCPP on activity. *White bars* indicate 5-HT$_{2C}$ receptor mutant mice, and *black bars* represent wild-type mice. *a*, Horizontal activity after GR 127935 treatment (vehicle or 0.3, 1.5, or 7.5 mg/kg, i.p.; $n = 10$ per genotype). *b*, Horizontal activity; *c*, rears after either vehicle or GR 127935 (7.5 mg/kg, i.p.) pretreatment and vehicle or mCPP (2.5 mg/kg, i.p.) treatment ($n = 10$ per genotype). Values are expressed as mean + 1 SEM. Significant differences by genotype, $^{**}p \leq 0.01$.

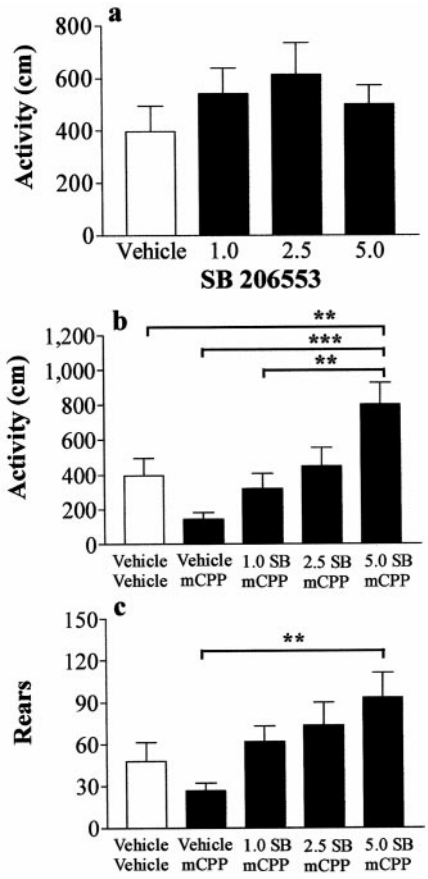

Figure 3. Effect of SB 206553 and mCPP on activity in C57BL/6 mice. *White bars* indicate vehicle treatment, and *black bars* represent drug treatment. *a*, Horizontal activity after SB 206553 treatment (vehicle or 1.0, 2.5, or 5.0 mg/kg, i.p.; $n = 16$). *b*, Horizontal activity; *c*, rears after either vehicle or SB 206553 (1.0, 2.5, or 5.0 mg/kg, i.p.) pretreatment and vehicle or mCPP (2.5 mg/kg, i.p.) treatment ($n = 16$). Values are expressed as mean + 1 SEM. Significant differences, $^{***}p \leq 0.001$; $^{**}p \leq 0.01$.

hanced rearing in response to 2.5 mg/kg mCPP in mutant but not wild-type mice. However, after GR 127935 pretreatment, a complete abolition of mCPP-induced hyperactivity was observed in mutant mice.

To determine whether mCPP-induced hyperactivity resulted from developmental compensations in 5-HT$_{2C}$ receptor mutants, we tested whether 5-HT$_{2C}$ receptor antagonist pretreatment would alter mCPP responses of C57BL/6 mice in a manner that mimicked the mutant phenotype. Although the 5-HT$_{2C/2B}$ receptor antagonist SB 206553 (Kennett et al., 1996) (1.0, 2.5, and 5.0 mg/kg) did not alter horizontal activity or rearing when administered alone (Fig. 3*a*), it produced a substantial increase in activity when combined with mCPP. When administered alone, the dose of mCPP used (2.5 mg/kg) produced a significant reduction in locomotor activity relative to a vehicle control [dependent *t* test, $t_{(13)} = 2.57$; $p < 0.05$ (Fig. 3*b*)]. However, SB 206553 pretreatment combined with mCPP administration produced a significant effect on horizontal activity [$F_{(4,60)} = 9.18$; $p < 0.001$ (Fig. 3*b*)], such that the highest dose of SB 206553 administered before mCPP produced marked hyperactivity compared with mCPP and vehicle alone. A significant treatment effect was also found for rearing [$F_{(4,60)} = 4.71$; $p < 0.01$ (Fig. 3*c*)]. *Post hoc* analysis revealed that pretreatment with the highest dose of SB 206553 before mCPP administration was associated with significantly greater rearing than mCPP alone.

DISCUSSION

These results reveal a mechanism through which a single receptor gene lesion can predispose animals to a completely paradoxical behavioral response to a nonselective drug. The locomotor-suppressing effects of mCPP have been well established and attributed to its 5-HT$_{2C}$ receptor agonist activity (Kennett and Curzon, 1988; Lucki et al., 1989). Based on such studies, it was expected that 5-HT$_{2C}$ receptor mutant mice would exhibit decreased mCPP-induced suppression of locomotor activity. We were therefore surprised to observe a robust enhancement of locomotor activity in the mutants.

Previous studies of the influence of 5-HT$_{2C}$ receptor antagonists on the locomotor effect of mCPP have yielded inconsistent results. After pretreatment with such antagonists, mCPP has been observed to produce decreases (Bonhaus et al., 1997), increases (Gleason and Shannon, 1998), or no change (Kennett and Curzon, 1988; Lucki et al., 1989; Kennett et al., 1994) in locomotor activity, depending on the particular antagonist used. This may reflect the limited specificity of both the antagonists and mCPP, which displays nearly equivalent high affinities for the 5-HT$_{2B}$,

5-HT$_{2C}$, and 5-HT$_3$ receptor subtypes and significant affinity for the 5-HT$_{1B}$ receptor subtype (Hoyer, 1988; Hamik and Peroutka, 1989; Baxter et al., 1995). We hypothesized that mCPP-induced hyperactivity in animals lacking 5-HT$_{2C}$ receptors reflected an unmasking of the actions of this compound at other 5-HT receptors. The 5-HT$_{1B}$ receptor was the leading candidate for this effect because of its high-affinity interactions with mCPP and because of the known hyperlocomotor effects of 5-HT$_{1B}$ receptor stimulation (Oberlander et al., 1987; Hoyer, 1988; Saudou et al., 1994; O'Neill et al., 1996). The blockade of mCPP-induced hyperactivity in mutants by pretreatment with the 5-HT$_{1B/1D}$ receptor antagonist GR 127935 supported this hypothesis.

The interpretation of these results was complicated, however, by the constitutive nature of the 5-HT$_{2C}$ receptor mutation. It remained possible that this anomalous drug response resulted from developmental compensation in animals that lacked 5-HT$_{2C}$ receptors throughout development. To determine whether mCPP-induced hyperactivity required developmental perturbations in 5-HT$_{1B}$ signaling and/or in other neural systems, we used a pharmacological approach in normal adult mice. Pretreatment of C57BL/6 mice with the relatively selective 5-HT$_{2C/2B}$ receptor antagonist SB 206553 converted the mCPP response from activity reduction to an enhancement of activity, resembling the mutant phenotype. Thus, the mutant phenotype predicted this behavioral consequence of 5-HT$_{2C}$ receptor blockade in the wild-type animal.

These findings provide a simple model for explaining the complex effects of mCPP on locomotor activity. Previous studies and our results indicate that the stimulation of brain 5-HT$_{2C}$ and 5-HT$_{1B}$ serotonin receptors produces opposite effects on locomotor activity levels. When both receptor subtypes are activated by mCPP, the locomotor suppression produced by 5-HT$_{2C}$ receptor stimulation predominates. However, when this component of the mCPP response is eliminated by either genetic means or by antagonist pretreatment, then the 5-HT$_{1B}$ receptor-stimulating properties of the drug are unopposed, leading to paradoxical hyperactivity.

Thus, the paradoxical response of 5-HT$_{2C}$ receptor mutants to mCPP provides an example of the complexity of the mechanisms through which nonselective serotonergic agonists modulate behavior. This model may be generalized for considering the genetic basis of paradoxical behavioral responses to nonselective drugs. When a compound alters the function of multiple gene products with opposing influences on behavior, then mutations or allelic variants of such genes may lead to individual differences in responses and to paradoxical effects.

In humans, paradoxical behavioral responses to nonselective drugs are frequently encountered. For example, mCPP administration produced paradoxical effects in a study of alcoholics, leading to enhanced anger and anxiety in some and to euphoria in others (George et al., 1997). In addition, serotonin reuptake-blocking antidepressants (e.g., fluoxetine and fluvoxamine) that nonspecifically enhance serotonin receptor activation frequently produce agitation in some individuals and sedation in others (Beasley et al., 1991; Freeman, 1991). Both environmental and genetic factors interact to influence individual variability in responses to drugs. Heritable differences in genes encoding drug-metabolizing enzymes have long been known to influence drug responses; the impact of variability in neural genes is a more recent focus of attention (Lin and Poland, 1995). mCPP-induced hyperactivity in 5-HT$_{2C}$ receptor mutant mice provides a useful model for considering the effects of neural genes on neurobehavioral responses to drugs.

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