

Inactivation of 5-HT_{2C} Receptors Potentiates Consequences of Serotonin Reuptake Blockade

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The enhancement of central serotonin system function underlies the therapeutic effects of selective serotonin reuptake inhibitors (SSRIs), which have become the most commonly used class of antidepressant agents. However, many individuals experience depressive episodes that are resistant to SSRI treatment. Homeostatic mechanisms that limit the extent to which SSRIs enhance serotonergic neurotransmission have been implicated in this phenomenon. Here, we report a novel strategy for enhancing the efficacy of SSRIs. Using *in vivo* microdialysis methods in rats, the nonselective 5-HT₂ receptor antagonist ketanserin was observed to produce a robust augmentation of citalopram-, fluoxetine-, and sertraline-induced elevations of hippocampal extracellular serotonin levels. Similar effects were also observed in cortex. The potentiation of SSRI-induced increases in hippocampal serotonin levels was reproduced by the 5-HT_{2C} receptor-selective antagonists SB 242084 and RS 102221, but not by the 5-HT_{2A} receptor-selective antagonist MDL 100907. Although 5-HT_{2C} receptor antagonists augmented the actions of SSRIs, they had no effect on extracellular serotonin levels or tail suspension responses when administered alone. These results were in strong accord with independent findings using a line of 5-HT_{2C} receptor-null mutant mice. Although this mutation did not affect baseline extracellular serotonin levels or tail suspension test (TST) behavior, it enhanced fluoxetine effects on serotonin levels and immobility in the TST. These findings reveal an unanticipated pharmacological action of 5-HT_{2C} receptors that warrants consideration in the development of novel strategies for the treatment of depression.

Neuropsychopharmacology (2004) 29, 1782–1789, advance online publication, 12 May 2004; doi:10.1038/sj.npp.1300474

Keywords: 5-HT_{2C}; receptor; antidepressant; microdialysis; serotonin; rat; mouse

INTRODUCTION

Major depressive disorder is among the most common and debilitating psychiatric conditions, with a lifetime prevalence of 20% for females and 10% for males (Judd, 1995). The high incidence and chronic recurrent course of unipolar depression has led the World Health Organization to predict that it will become the second-ranking global

cause of illness-related disability by 2020 (Murray and Lopez, 1997). Enhancement of central serotonin system function has been associated with the actions of a wide variety of antidepressant treatments, as indicated by the widespread use of selective serotonin reuptake inhibitors (SSRIs). The SSRIs, which are generally considered to be the first-line antidepressants of choice (Fava, 2000), block the plasma membrane serotonin reuptake transporter (SERT) and thus augment the availability of synaptic serotonin. However, this augmentation is blunted by SSRI-induced increases in serotonin levels in the vicinity of brainstem raphe nuclei serotonergic neurons. In response to elevated serotonin levels, somatodendritic 5-HT_{1A} autoreceptors are activated, reducing serotonergic neuronal firing rates and forebrain serotonin release (Artigas *et al*, 1996). The characteristic delay in the onset of antidepressant effects of SSRIs has been attributed to a gradual decrease in this inhibition, resulting from partial 5-HT_{1A} autoreceptor desensitization (Hervas *et al*, 2001).

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Received 23 September 2003; revised 2 March 2004; accepted 5 March 2004

Online publication: 29 March 2004 at <http://www.acnp.org/citations/Npp03290403435/default.pdf>

These findings led to the proposal that the coadministration of SSRIs with 5-HT_{1A} receptor antagonists could result in accelerated and possibly enhanced antidepressant responses (Artigas, 1993). This hypothesis was examined in clinical studies using the nonselective 5-HT_{1A} receptor antagonist pindolol. There was some indication that the onset of antidepressant responses was accelerated by the combined pharmacotherapy, although results were mixed (Artigas *et al*, 2001; Brousse *et al*, 2003). Since pindolol has partial 5-HT_{1A} receptor agonist activity, along with very high affinity at β -adrenoceptors, the receptor mechanisms through which pindolol influences antidepressant action have been called into question (Artigas *et al*, 2001; Cremers *et al*, 2001; Brousse *et al*, 2003). Thus, clinical tests of this antidepressant augmentation strategy await the availability of potent and selective 5-HT_{1A} receptor antagonist compounds.

The 5-HT_{2C} receptor is another 5-HT receptor subtype that warrants consideration in the development of novel treatment strategies for depression. Although a wide variety of antidepressants have high-affinity antagonist activity at 5-HT_{2C} receptors (Jenck *et al*, 1994; Palvimaki *et al*, 1996), the contributions of this activity to their therapeutic effects are poorly understood. Indirect activation of 5-HT_{2C} receptors by SSRI treatment may contribute to the transient anorectic and anxiogenic effects of these compounds (Halford and Blundell, 2000; Bagdy *et al*, 2001). However, the contribution of these receptors to the neurochemical and antidepressant behavioral effects of SSRIs remains unclear. Recent findings indicating that RNA editing of these receptors is regulated by SSRI treatment and altered in the prefrontal cortex of suicide victims highlights the importance of clarifying the contributions of these receptors to antidepressant action (Niswender *et al*, 2001; Gurevich *et al*, 2002a; Gurevich *et al*, 2002b).

Here, we report a novel interaction through which pharmacological blockade of 5-HT_{2C} receptors potentiates both the neurochemical and the antidepressant-like behavioral actions of SSRIs. We also report independently obtained data revealing that the effects of a 5-HT_{2C} receptor-null mutation on neurochemical and behavioral responses to SSRI administration are highly concordant with those of 5-HT_{2C} receptor antagonist treatment. These results suggest a potential treatment strategy that may counter SSRI-induced negative feedback of serotonergic neurotransmission and augment the antidepressant actions of these drugs.

MATERIALS AND METHODS

Animals

All animals were group-housed at 22°C on a 12-h light/dark cycle, with standard food and water *ad libitum*. Adult male albino rats of a Wistar-derived strain (285–320 g; Harlan, Zeist, Netherlands) were used. Effects of 5-HT_{2C} receptor antagonism in the mouse tail suspension test were determined using C57BL/6 mice (M & B A/S, Denmark). To examine the effects of genetic inactivation of 5-HT_{2C} receptors, male 8–12-week old mice hemizygous for a null mutation of the X-linked *htr2c* gene (backcrossed to a C57BL/6J background for at least 24 generations) and wild-

type littermates were used (Tecott *et al*, 1995). The line has been maintained through matings of heterozygous females with C57BL/6J males (Jackson Laboratory, Bar Harbor, ME). Experiments were conducted in accordance with the Declaration of Helsinki and the NIH Guide for the Care and Use of Laboratory Animals.

Drugs

For rat studies, citalopram hydrobromide, fluoxetine hydrochloride, irindalone, sertraline hydrochloride, olanzapine, RS 102221, and SB 242084 were obtained from Lundbeck A/S (Copenhagen, Denmark). Mepyramine, prazosin, and ketanserin were obtained from RBI (Natick, USA). MDL 100907 was synthesized at the University of Groningen. These drugs were dissolved in saline and injected subcutaneously in a volume of 1 ml/kg. Antagonists were administered immediately (within 15 s) prior to either SSRI or vehicle treatment. For mutant mouse studies, fluoxetine hydrochloride was obtained from Tocris (Ellisville, MO). Fluoxetine was dissolved in a sterile saline solution for i.p. injections. Control injections consisted of vehicle only.

Surgical Implantation of Microdialysis Probes

Rat microdialysis studies were performed at the University of Groningen, The Netherlands. For rats, microdialysis was performed using I-shaped probes, composed of polyacrylonitrile/sodium methyl sulfonate copolymer dialysis fiber (i.d. 220 μ m, o.d. 310 μ m, AN 69, Hospal, Italy). The exposed length of the membranes was 4 mm for both ventral hippocampus and prefrontal cortex. Prior to surgery, rats were anaesthetized using ketamine (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.), after premedication with midazolam (5 mg/kg s.c.). Lidocaine-HCl (10% (m/v)) was used for local anaesthesia. Rats were placed in a stereotaxic frame (Kopf, USA), and probes were implanted into the ventral hippocampus (L +4.8 mm, IA : +3.7 mm, V : -8.0 mm) and prefrontal cortex (L -0.9 mm, AP : +3.5 mm, V : -6.0 mm). After insertion, probes were secured with dental cement.

Microdialysis studies utilizing 5-HT_{2C} receptor mutant mice were performed at the University of California, San Francisco. For mice, concentric microdialysis probes were constructed using 23 gauge stainless steel and silica capillary tubing. The dialysis membrane (i.d. 240 μ m, o.d. 290 μ m, AN 69HF, Hospal, Italy) consisted of polyacrylonitrile/sodium methyl sulfonate copolymer with an average pore size of 29 Å. Dialysis probes had 2 mm of exposed membrane. Prior to surgery, mice were anesthetized using ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and then placed in a stereotaxic frame. Microdialysis probes were implanted into the right prefrontal cortex (PFC) at the following coordinates relative to bregma (AP +2 mm; L -0.5 mm; DV -4 mm; (Franklin and Paxinos, 1997)).

In Vivo Microdialysis

All animals used in microdialysis studies were allowed to recover for at least 24 h following surgery. For rat studies, probes were perfused with artificial cerebrospinal fluid

containing (in mM): NaCl 147, KCl 3.0, CaCl₂ 1.2, and MgCl₂ 1.2, at a flow-rate of 1.5 µl/min (Harvard Apparatus pump, South Natick, MA, USA). Microdialysis samples were collected at 15 min intervals in HPLC vials containing 7.5 µl 0.02 M acetic acid for serotonin analysis (animals were injected at $t = 0$, data were not corrected for lag time). For mouse studies, probes were perfused with artificial cerebrospinal fluid containing (in mM): NaCl 145, KCl 2.7, MgCl₂ 1, CaCl₂ 1.2, Na₂HPO₄ 2, pH 7.40, at a flow rate of 1 µl/min. Microdialysis samples were collected at 20 min intervals. Fluoxetine was administered immediately before sample 6.

Determination of Dialysate Serotonin Levels

Serotonin concentrations were determined using HPLC coupled with electrochemical detection. For rat studies, 20 µl microdialysate fractions were injected via an autoinjector (CMA/200 refrigerated microsampler, CMA, Sweden) onto a 100 × 2.0 mm C18 Hypersil 3 µm column (Bester, Amstelveen, the Netherlands) and separated with a mobile phase consisting of 5 g/l di-ammoniumsulfate, 500 mg/l Na₂-EDTA, 50 mg/l heptane sulfonic acid, 4% methanol v/v, and 30 µl/l of triethylamine, pH 4.75 at a flow rate of 0.4 ml/min (Shimadzu LC-10 AD). 5-HT was detected amperometrically at a glassy carbon electrode at 500 mV vs Ag/AgCl (Antec Leyden, Leiden, Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µl sample (signal-to-noise ratio 3).

For mouse studies, the mobile phase consisted of a mixture of acetonitrile, phosphate buffer, and an ion-pairing agent (ESA, Chelmsford, MA). The mobile phase was delivered by a pump (model 582, Solvent Delivery Module; ESA) at a flow rate of 0.25 µl/min through an MD-150 narrowbore column (ESA). 5-HT was detected using a coulometric detector (Coulochem II; ESA) coupled to a single channel enhanced amperometric cell (Model 5041; ESA). The potential applied to the electrode was +175 mV; under these conditions, the limit of detection for 5-HT was approximately 2–3 fmol. Data were analyzed using a basic integrator (Model 3395; Hewlett-Packard).

For microdialysis data, basal 5-HT levels were defined as the average level of the first five samples. Differences between treatments were analyzed using a two-way ANOVA with repeated measurements, followed by Students–Newman–Keuls *post hoc* analysis. In separate analyses for evaluating the effects of treatments relative to baseline, one-way ANOVA for repeated measurements on ranks were used, followed by Dunnett's test. Level of significance was set at $p \leq 0.05$.

Tail Suspension Test (TST)

The mouse tail suspension test was designed as a screening assay for antidepressant compounds, and is sensitive to the major classes of antidepressants (Steru *et al*, 1985; Porsolt, 2000). TST studies employing the 5-HT_{2C} receptor antagonist SB 206553 were performed at Lundbeck A/S in Copenhagen, Denmark. TST studies utilizing 5-HT_{2C} receptor mutant mice were performed at the University of California, San Francisco. Animals were suspended by the tail using adhesive tape from either

a 1.2-cm diameter metal bar (5-HT_{2C} receptor mutant studies) or a 1 cm diameter hook (5-HT_{2C} receptor antagonist studies) elevated 30–35 cm. When suspended, rodents either make apparent escape attempt movements or adopt a characteristic immobile posture. The total immobility time was measured (manually for 5-HT_{2C} receptor mutant studies, and by automated strain gauge for 5-HT_{2C} receptor antagonist studies (Med Associates, St Albans, VT)) during the 6 min test period. The effects of treatments relative to baseline were analyzed using a one-way ANOVA, followed by Dunnett's test. Interactions between antagonist treatment and SSRI treatment, or between genotype and SSRI treatment, were analyzed by a two-way ANOVA.

RESULTS

Potentiation of SSRI-Induced Serotonin Release by Ketanserin

As part of an effort to probe the contribution of serotonin receptor subtypes to the regulation of brain serotonin systems and neurochemical responses to SSRIs, the effect of coadministration of the nonselective 5-HT₂ receptor antagonist ketanserin on SSRI-induced serotonin release was examined in rats using *in vivo* microdialysis. Microdialysis probes were placed in the ventral hippocampal formation, where baseline extracellular serotonin levels were 4.81 ± 0.38 fmol/sample ($n = 95$). Whereas the SSRI citalopram (3 mg/kg s.c.) produced 400–500% increases in serotonin levels relative to baseline ($X^2_{10} = 35.5$, $p < 0.0001$), ketanserin administration (40 µg/kg s.c.) alone produced no effect (Figure 1a). However, the effect of citalopram on extracellular serotonin was substantially augmented, in a dose-dependent manner, by coadministration with ketanserin. Whereas 0.4 µg/kg ketanserin was devoid of any effect, 4 and 40 µg/kg augmented the effect of citalopram, with the 40 µg/kg dose producing 1000% increases from baseline values ($F(1,106) = 15.82$, $p < 0.005$; Supplemental Figure 1). A parallel set of studies using another partially selective 5-HT₂ receptor antagonist, irindalone, yielded similar results (data not shown).

We then determined the extent to which the ability of ketanserin to potentiate citalopram-induced serotonin release generalizes to additional SSRIs. Whereas fluoxetine alone (4 mg/kg) increased serotonin levels to approximately 350% of the baseline value ($X^2_{10} = 34.3$, $p < 0.0005$), ketanserin coadministration (40 µg/kg s.c.) augmented this effect to produce a 600% increase relative to baseline values ($F(1,108) = 6.44$, $p < 0.05$) (Figure 1b). Sertraline (9 mg/kg, s.c.) also increased serotonin levels ($X^2_{10} = 33.0$, $p < 0.0005$) and ketanserin (40 µg/kg, s.c.) potentiated this effect ($F(1,87) = 4.19$, $p < 0.0005$) (Figure 1c). To determine whether the ability of ketanserin to potentiate citalopram-induced serotonin release generalizes to other forebrain regions implicated in the regulation of affect, the experiment was repeated with microdialysis probes located in the prefrontal cortex (PFC). In this region, baseline extracellular serotonin levels were 3.79 ± 0.37 fmol/sample (mean \pm SEM, $n = 19$). Citalopram administration (3 mg/kg s.c.) increased extracellular serotonin levels in the PFC ($X^2_{10} = 68.7$, $p < 0.0001$) and

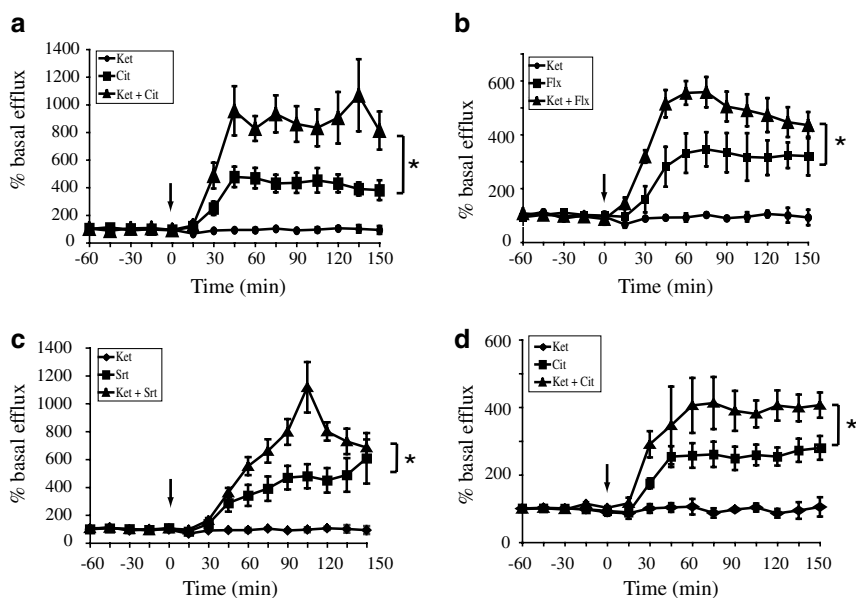


Figure 1 Augmentation of SSRI-induced serotonin release in hippocampus (a–c) and prefrontal cortex (d) of freely moving rats by coadministration of the 5-HT₂ receptor antagonist ketanserin. All drugs were administered at time 0. (a) Citalopram administration (3 mg/kg s.c.) significantly increased extracellular serotonin levels in the ventral hippocampus ($X^2_{10} = 35.5$, $p < 0.0001$, $n = 7$). Ketanserin (40 μ g/kg s.c.) did not alter hippocampal serotonin levels when administered alone ($n = 4$). However, when coadministered with citalopram, it augmented the serotonin-releasing effects of the SSRI ($F(1,106) = 15.82$, $**p < 0.005$, $n = 4$). (b) Fluoxetine administration (4 mg/kg s.c.) significantly increased hippocampal extracellular serotonin levels ($X^2_{10} = 34.3$, $p < 0.0005$, $n = 5$). Ketanserin (40 μ g/kg s.c.) augmented the serotonin-releasing effects of fluoxetine ($F(1,108) = 6.44$, $*p < 0.05$, $n = 5$). (c) Sertraline administration (9 mg/kg s.c.) significantly increased hippocampal extracellular serotonin levels ($X^2_{10} = 33.0$, $p < 0.0005$, $n = 4$). Ketanserin (40 μ g/kg s.c.) augmented the serotonin-releasing effects of sertraline ($F(1,87) = 4.19$, $***p < 0.0005$, $n = 4$). (d) In the PFC, citalopram administration (3 mg/kg s.c.) significantly increased extracellular serotonin levels ($X^2_{10} = 68.7$, $p < 0.0001$, $n = 10$). As observed in the hippocampus, ketanserin (40 μ g/kg s.c.) alone produced no effect ($n = 7$), but it significantly augmented the effect of citalopram (3 mg/kg s.c.) ($F(1,163) = 6.27$, $*p < 0.05$, $n = 4$).

ketanserin potentiated this effect ($F(1,163) = 6.27$, $p < 0.05$) (Figure 1d).

Identification of Receptor Subtype Mediating Potentiation of SSRI-Induced Serotonin Release

Ketanserin interacts with high affinity at 5-HT₂ receptors and at several non-5-HT receptor subtypes (Roth et al, 2000). To determine the extent to which these receptors contribute to the ability of ketanserin to potentiate SSRI-induced serotonin release, antagonists of these receptors were coadministered with citalopram.

The α_1 -adrenoceptor antagonist prazosin (0.4 mg/kg s.c.) significantly decreased 5-HT levels when administered alone ($X^2_{10} = 19.9$, $p < 0.05$), but did not alter the effect of citalopram (Figure 2a). The histamine H₁-receptor antagonist mepyramine (0.3 mg/kg s.c.) did not alter 5-HT levels when administered alone, nor did it modify the effect of citalopram (Figure 2b). Similarly, the selective 5-HT_{2A} receptor antagonist MDL 100,907 (0.4 mg/kg s.c.) had no effect on 5-HT levels when administered alone or in combination with citalopram (Figure 2c). In contrast, two selective 5-HT_{2C} receptor antagonists, SB 242084 and RS 102221, potentiated citalopram-induced serotonin release. Although these agents did not produce significant alterations of extracellular serotonin levels when given alone (0.4 and 0.6 mg/kg s.c., respectively), they substantially augmented the serotonergic response to citalopram, as indicated by 900% increases of extracellular serotonin levels from

baseline values (SB 242084: $F(1,117) = 9.19$, $p < 0.05$; RS 102221: $F(1,120) = 12.14$, $p < 0.005$; Figures 3a, b).

Effect of *htr2c* Gene Inactivation on Neurochemical Responses to Fluoxetine

To examine the contributions of 5-HT_{2C} receptors to the actions of SSRIs, we also used a mouse molecular genetic approach. An *in vivo* microdialysis study was performed to examine the effect of fluoxetine (20 mg/kg, i.p.) on extracellular serotonin levels in the PFC of 5-HT_{2C} receptor-null mutant mice (Figure 4). No phenotypic effects on basal extracellular serotonin levels were observed (5.8 ± 0.7 and 5.6 ± 0.6 fmol/sample (mean \pm SEM) in the PFC of mutant and wild-type mice, respectively). By contrast, the mutation significantly augmented the effect of fluoxetine on extracellular serotonin levels in the PFC ($F(1,18) = 6.018$, $p < 0.05$).

Effect of *htr2c* Gene Inactivation and 5-HT_{2C} Receptor Antagonist treatment on Tail Suspension Responses

If inactivation of 5-HT_{2C} receptors potentiates antidepressant-like behavioral responses to SSRIs, then we would expect the effects of fluoxetine to be augmented in 5-HT_{2C} receptor mutant mice during tail suspension testing. Saline-treated mutant and wild-type mice displayed similar immobility times. Fluoxetine treatment (20 mg/kg i.p., 40 min prior to testing) significantly enhanced immobility, revealed by two-way ANOVA as a significant overall effect

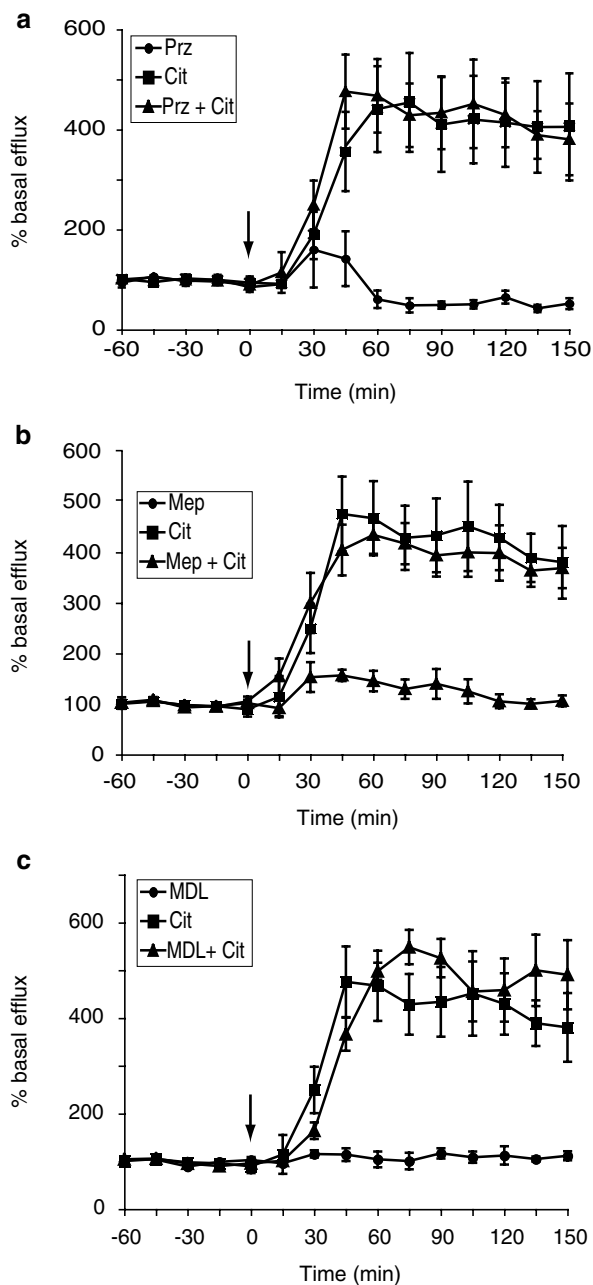


Figure 2 SSRI-induced hippocampal serotonin release in freely moving rats is not potentiated by coadministration of an α_1 -adrenoceptor antagonist, a histamine receptor I antagonist, or a 5-HT_{2A} receptor antagonist. All drugs were administered at time 0. (a) Administration of the α_1 -adrenoceptor antagonist prazosin (0.4 mg/kg s.c.) alone significantly reduced 5-HT extracellular levels ($\chi^2_{10} = 19.9$, $p < 0.05$, $n = 4$). However, prazosin did not alter serotonin release induced by citalopram (3 mg/kg s.c.; coadministration $n = 8$, citalopram alone $n = 4$). (b) Administration of the H1 receptor antagonist mepyramine (0.3 mg/kg s.c.) did not alter extracellular serotonin levels when administered alone ($n = 5$) or in combination with citalopram (3 mg/kg s.c.; coadministration $n = 7$, citalopram alone $n = 7$). (c) Administration of the selective 5-HT_{2A} receptor antagonist MDL 100,907 (0.4 mg/kg s.c.) did not alter extracellular serotonin levels when administered alone ($n = 5$) or in combination with citalopram (3 mg/kg s.c.; coadministration $n = 5$, citalopram alone $n = 7$).

of drug treatment ($F(1,27) = 139$, $p < 0.001$). This effect of fluoxetine was potentiated in 5-HT_{2C} receptor mutant mice, as indicated by a significant genotype \times drug interaction

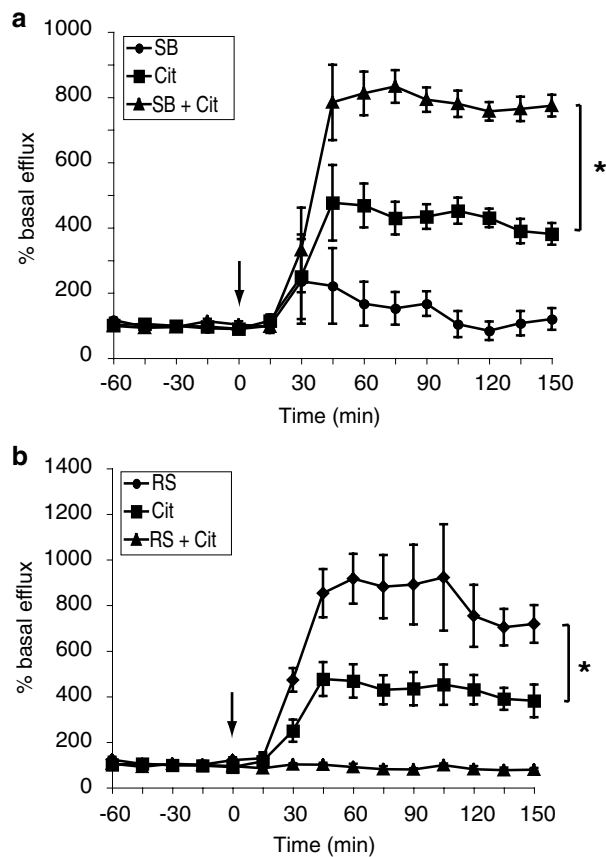


Figure 3 Coadministration of 5-HT_{2C} receptor antagonists potentiated SSRI-induced hippocampal serotonin release in freely moving rats. All drugs were administered at time 0. (a) Whereas the selective 5-HT_{2C} receptor antagonist SB 242084 (0.4 mg/kg s.c.) did not alter serotonin levels when administered alone ($n = 5$), it significantly augmented the serotonergic neurochemical response to 3 mg/kg s.c. citalopram ($F(1,117) = 9.19$, $*p < 0.05$, $n = 5$; citalopram alone $n = 7$). (b) Another 5-HT_{2C} receptor antagonist, RS 102221 (0.6 mg/kg s.c.) did not alter serotonin levels when administered alone ($n = 5$), but significantly augmented the serotonergic neurochemical response to 3 mg/kg s.c. citalopram ($F(1,120) = 12.14$, $**p < 0.005$, $n = 5$; citalopram alone $n = 7$).

($F(3,27) = 8.7$, $p < 0.01$; Figure 5a). In an independently conducted experiment, a similar effect was observed in wild-type mice treated with the 5-HT_{2C} receptor antagonist SB 206553. Although neither citalopram nor SB 206553 produced significant effects on immobility, coadministration of these compounds (SB 206553 5 mg/kg, s.c., 30 min prior to testing; citalopram 16 mg/kg s.c.) produced a significant anti-immobility response ($F(3,28) = 4.35$, $p < 0.02$) (Figure 5b).

DISCUSSION

Our findings indicate that serotonin 5-HT_{2C} receptors have substantial influence on both neurochemical and antidepressant-like behavioral responses to SSRIs. The observed inability of ketanserin administered alone to alter central serotonin levels was consistent with previous studies indicating that 5-HT₂ receptor agonists and antagonists produce at most small effects on serotonin levels when administered alone (Gobert and Millan, 1999; Gobert et al, 2000). We were therefore surprised to find that ketanserin

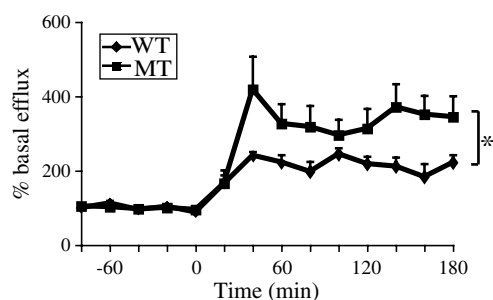


Figure 4 Neurochemical effects of fluoxetine are potentiated in 5-HT_{2C} receptor null mutant mice. Fluoxetine was administered at time 0. Basal extracellular serotonin levels were (mean \pm SEM) 5.8 ± 0.7 fmol/sample in the PFC of 5-HT_{2C} receptor mutant mice and 5.6 ± 0.6 fmol/sample in the PFC of wild-type animals and did not significantly differ between the two groups. Fluoxetine-induced (18 mg/kg i.p.) increases in serotonin levels were potentiated in mutant mice ($F(1,18) = 6.018$, $*p < 0.05$, $n = 9$ WT and 11 MT).

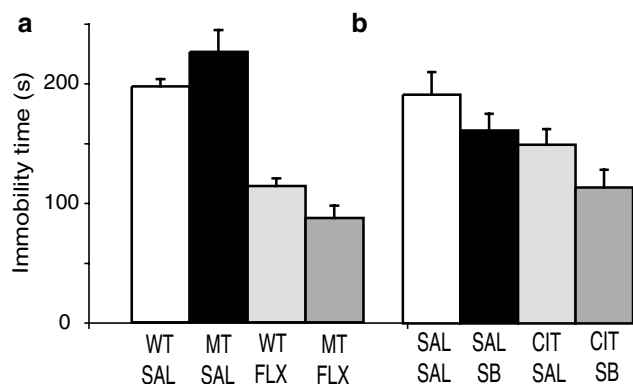


Figure 5 Potentiation of behavioral effects of citalopram and fluoxetine by 5-HT_{2C} receptor inactivation. (a) Immobility times of 5-HT_{2C} receptor mutant and wild-type mice during tail suspension assay. No significant effect of genotype on immobility time was observed in saline-treated animals. However, fluoxetine administration (20 mg/kg i.p.) elicited a significantly greater effect on immobility time in 5-HT_{2C} receptor mutant mice than in wild-type controls ($F(1,27) = 8.7$, $**p < 0.01$; $n = 6-10$ mice per group). (b) Coadministration of a 5-HT_{2C} receptor antagonist potentiates the effect of fluoxetine on immobility time in the tail suspension test. Administration of the 5-HT_{2C} receptor antagonist SB 206553 (5 mg/kg s.c.) produced no significant alteration in behavioral responses to tail suspension. However, when coadministered with citalopram (16 mg/kg s.c.), SB 206553 significantly potentiated the anti-immobility effects of the SSRI ($F(3,28) = 4.35$, $*p < 0.01$, $n = 8$ per group).

produced a robust augmentation of citalopram-induced elevations of serotonin levels. This effect of ketanserin was also observed with the SSRIs fluoxetine and sertraline, indicating a general ability of ketanserin to potentiate the neurochemical effects of serotonin reuptake blockade.

Determination of the mechanism through which ketanserin potentiates SSRI action required consideration of the potential contributions of the multiple receptor subtypes for which the ketanserin has substantial antagonist affinity, such as the 5-HT_{2A}, 5-HT_{2C}, histamine H₁, and α ₁-adrenergic receptors (Leysen *et al*, 1992; Roth *et al*, 2000). When corresponding subtype-selective antagonists were coadministered with citalopram, only the 5-HT_{2C} receptor antagonists SB 242084 and RS 102221

reproduced the effects of ketanserin. However, neither antagonist altered extracellular serotonin levels when administered alone.

The potentiation of SSRI-induced serotonin release by 5-HT_{2C} receptor inactivation was observed in both the hippocampal formation and prefrontal cortex, two regions that have been strongly implicated in the pathophysiology of depressive disorders and in responses to antidepressant drugs (Stockmeier, 1997; Davidson *et al*, 2002). The potential antidepressant efficacy of drug treatments has been associated with reductions of immobility time in a tail suspension assay (Steru *et al*, 1985; Porsolt, 2000). Since the enhancement of brain serotonin function is thought to be central to the antidepressant effects of SSRIs, we hypothesized that the antidepressant-like behavioral effects of SSRIs would be potentiated by 5-HT_{2C} receptor inactivation. Accordingly, pharmacological blockade of 5-HT_{2C} receptors produced a small potentiation of citalopram's effect in the TST, yet produced no observable effects in this test in the absence of SSRIs. Additional studies are required to determine the extent to which behavioral responses to the pharmacological blockade of 5-HT_{2C} receptors generalizes to additional SSRIs.

Similar phenomena were uncovered independently by one of our groups (Tecott, American), in studies employing a line of mice bearing a null mutation of the 5-HT_{2C} receptor gene. As this work was performed independently, a number of procedural differences exist between the European and American data sets. These include the use of different species and SSRIs for examining influences of 5-HT_{2C} receptors. Thus, 5-HT_{2C} receptor influences on extracellular serotonin levels were examined using 5-HT_{2C} receptor antagonists in citalopram-treated rats (European group) and using 5-HT_{2C} receptor mutant mice treated with fluoxetine (American group). Analogously, mouse tail suspension testing was performed using 5-HT_{2C} receptor antagonists in citalopram-treated animals (European group) and using 5-HT_{2C} receptor mutant mice treated with fluoxetine (American group). However, despite these procedural differences, a striking degree of concordance exists between the results obtained independently by our groups: (1) both the 5-HT_{2C} receptor mutation and the 5-HT_{2C} receptor antagonists enhance fluoxetine-induced extracellular serotonin levels in prefrontal cortex, (2) neither the mutation nor the antagonists themselves alter extracellular serotonin levels. Taken together, these complementary pharmacological and genetic studies provide strong evidence for the potentiating effects of 5-HT_{2C} receptor inactivation on SSRI action.

Prior investigations of the influence of 5-HT_{2C} receptors in assays of 'behavioral despair' have yielded inconsistent results. In one previous study, several 5-HT₂ receptor agonist and antagonist compounds failed to influence immobility in the mouse forced swim test (Redrobe and Bourin, 1997). By contrast, in a study utilizing the rat forced swim test, several 5-HT_{2C} receptor agonists, but not mCPP, reduced immobility in the forced swim test; these effects were blocked by 5-HT_{2C} receptor antagonists (Cryan and Lucki, 2000). Differences between these findings and those in the present study may relate to differences in the assays (forced swim vs TST), drug doses, and the species (rats vs mice) used.

The observed influence of 5-HT_{2C} receptors on extracellular serotonin levels during SSRI treatment, but not under baseline conditions was not anticipated. The possibility that 5-HT₂ receptor antagonism could influence forebrain serotonin levels had been suggested by a prior study with the nonspecific 5-HT_{2C} receptor antagonist mianserin (Kreiss and Lucki, 1995). However, the high affinity of mianserin for 5-HT_{2C} receptors, α adrenoceptors, and histamine receptors precludes attribution of this effect to its activity at 5-HT_{2C} receptors (NIMH Psychoactive Drug Screening Program Database: <http://kidb.cwru.edu/pdsp.php>). To our knowledge, 5-HT_{2C} receptors have not been demonstrated on the presynaptic elements of serotonergic neurons and have not been considered to have a classic autoreceptor function. The ability of both pharmacological and genetic inactivation of 5-HT_{2C} receptors to alter serotonin levels during SSRI treatment, but not under baseline conditions, suggests that 5-HT_{2C} receptors may contribute to a negative feedback mechanism recruited under conditions of elevated serotonergic tone. Such a role for these receptors in the homeostatic regulation of serotonin levels could account for prior observations that 5-HT_{2C} receptor inactivation enhances sensitivity to the motoric effects of the serotonin-releasing agents dexfenfluramine and MDMA (Vickers et al, 1999; Bankson and Cunningham, 2002).

Several possible mechanisms could underlie the augmentation of SSRI-induced serotonin release by 5-HT_{2C} receptor inactivation. Although an autoreceptor function has not been demonstrated for 5-HT_{2C} receptors, it is notable that these receptors are expressed in the vicinity of mesencephalic raphe serotonergic neurons that project widely to the forebrain (Clemett et al, 2000). Neurochemical and electrophysiological studies indicate that these neurons are subject to GABAergic inhibitory regulation (Gervasoni et al, 2000; Tao and Auerbach, 2003). Notably, nonspecific 5-HT receptor agonists suppress serotonin neuronal firing rates (Boothman et al, 2003), and bath application of serotonin to mesencephalic slices increases the frequency of inhibitory postsynaptic potentials in these serotonergic neurons (Liu et al, 2000). These effects are attenuated by 5-HT_{2A} receptor antagonists, and to a much lesser extent, by 5-HT_{2C} receptor antagonists (Liu et al, 2000; Martin-Ruiz et al, 2001; Boothman et al, 2003). It is therefore possible that functional blockade of 5-HT_{2C} receptors in the DRN could reduce the magnitude of serotonin-mediated excitation of raphe GABA neurons. Alternatively, it is possible that serotonin release could be influenced by 5-HT_{2C} receptors expressed within interneurons of the PFC and hippocampus (Wright et al, 1995). Nonspecific 5-HT₂ receptor agonists have been found to enhance GABAergic neurotransmission in both the prefrontal cortex and CA1 region of the hippocampus (Shen and Andrade, 1998; Abi-Saab et al, 1999). The relative contributions of the 5-HT_{2A} and 5-HT_{2C} receptor subtypes to these effects remain to be determined.

Altogether, our results define a novel action of 5-HT_{2C} receptors in the modulation of serotonin levels within brain regions implicated in the regulation of affect. These results further suggest that coadministration of 5-HT_{2C} receptor antagonists with SSRIs warrants consideration as a novel approach for overcoming homeostatic mechanisms that attenuate the therapeutic efficacy of SSRIs.

In this regard, additional studies are now underway to determine whether this potentiation of SSRI action is maintained during chronic coadministration with 5-HT_{2C} receptor antagonists.

ACKNOWLEDGEMENTS

We thank Odette P Murphy for technical assistance and Dr Kevin Delucchi for advice regarding statistical analysis. LT is supported by a Staglin Music Festival Investigator Award from the National Alliance for Research on Schizophrenia and Affective Disorders.

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