Agonist-Induced Serotonin 2A Receptor Desensitization in the Rat Frontal Cortex and Hypothalamus


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ABSTRACT

This study examined the time course and possible mechanisms of agonist-induced desensitization of 5-hydroxytryptamine serotonin 2A receptors in the rat frontal cortex and hypothalamic paraventricular nucleus after 1, 4, and 7 days of treatment with (−)-1-(2,5-dimethoxy-4-iodophenyl)2-aminopropane HCl [(−)-DOI] (1 mg/kg i.p.), a selective 5-HT2A/2C receptor agonist. In the frontal cortex, 5-HT-mediated phospholipase C (PLC) enzyme activity decreased by 24 to 30% after 4 to 7 days of (−)-DOI treatment without any significant changes in the guanosine 5′-3-O-(thio)triphosphate-mediated PLC enzyme activity. Additionally, treatment with (−)-DOI did not significantly change the levels of Gα11, regulator of G protein signaling (RGS)4, or RGS7 proteins in the frontal cortex, whereas Gαq protein levels in the frontal cortex decreased (47%) only after 7 daily (−)-DOI injections. The functional status of 5-HT2A receptors in the hypothalamic paraventricular nucleus was examined using 5-HT2A receptor-mediated increases in plasma hormone levels. Plasma adrenocorticotropic hormone (ACTH) and oxytocin measurements showed that 5-HT2A receptor desensitization began after only 1 day of (−)-DOI treatment, and the desensitization continued to increase after 4 and 7 days of treatment (ACTH response decreased 64.2–67.7%; oxytocin response decreased 82.3–90.1%). There were no significant alterations in levels of Gαq or Gα11 proteins in the hypothalamic paraventricular nucleus. In conclusion, these results suggest that chronically administered (−)-DOI induces desensitization of 5-HT2A receptors in vivo, via a reduction in the ability of 5-HT2A receptors to activate G proteins without consistently altering levels of Gαq proteins or RGS proteins.

Changes in serotonin 2A (5-hydroxytryptamine; 5-HT2A) receptor signaling have been implicated in psychiatric disorders such as depression, schizophrenia, and anxiety (Roth, 1994; Naughton et al., 2000). 5-HT2A receptors are the targets of some of the treatments for these disorders (Baxter et al., 1995). 5-HT2A receptors desensitize after chronic agonist stimulation (Roth et al., 1995; Smith et al., 1999; Anji et al., 2000). Receptor desensitization can occur by down-regulation of the receptor, internalization of the receptor, or uncoupling of the receptor from its signaling proteins. Although several studies have examined the mechanisms underlying 5-HT2A receptor desensitization in cell culture, the exact mechanism by which 5-HT2A receptors desensitize in vivo is not yet known. We focused on agonist-induced 5-HT2A receptor desensitization in rats, using the prototypical 5-HT2A/2C receptor agonist (−)-1-(2,5-dimethoxy-4-iodophenyl)2-aminopropane HCl [(−)-DOI], to investigate the mechanisms underlying the homologous desensitization of 5-HT2A receptors in vivo.

5-HT2A receptors are coupled through Gαq/11 proteins to phospholipase C (PLC) (Hide et al., 1989; Roth et al., 1998). Upon activation of PLC, hydrolysis of phosphatidylinositol 4,5-bisphosphate generates diacylglycerol and inositol 1,4,5-trisphosphate (Berridge, 1987). Gαq/11 proteins stimulate PLC activity until the bound GTP is hydrolyzed to GDP. The intrinsic GTPase activity of Gαq/11 proteins is enhanced by regulators of G protein signaling proteins type 4 and 7 (RGS4 and RGS7) (Hepler et al., 1997; Xu et al., 1999). To examine possible mechanisms underlying agonist-induced desensitization of 5-HT2A receptor signaling, we measured the levels of Gαq, Gα11, RGS4, and RGS7 proteins after chronic (−)-DOI treatment.

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; (−)-DOI, (−)-1-(2,5-dimethoxy-4-iodophenyl)2-aminopropane HCl; PLC, phospholipase C; RGS, regulator of G protein signaling; GTP-γS, 5′-3-O-(thio)triphosphate; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; IOD, integrated optical density; ANOVA, analysis of variance; MDL 100,907, (−)-2,3-dimethoxyphenyl-1-[2-4-(piperidine)-methanol.
We used two methods to measure the function of 5-HT$_{2A}$ receptor signaling: 1) a comparison of 5-HT- versus GTPγS-stimulated PLC activity, and 2) agonist-induced increases in plasma hormone levels. PLC activity is the most direct functional measure of 5-HT$_{2A}$ receptor signaling because PLC is one of the first effector enzymes that G$_{q/11}$ can activate. Unfortunately, the hypothalamic paraventricular nucleus contains insufficient amounts of protein to perform this assay. Hence, we used PLC activity as a functional assay of 5-HT$_{2A}$ receptor signaling only in the frontal cortex. The function of 5-HT$_{2A}$ receptor signaling in the hypothalamic paraventricular nucleus was measured by agonist-induced increases in plasma adrenocorticotropic hormone (ACTH), corticosterone, and oxytocin levels. Oxytocin and corticotropin-releasing hormone (CRH) are expressed by neurons in the hypothalamic paraventricular nucleus (Swanson and Sawchenko, 1983). Magnocellular neurons in the hypothalamic paraventricular nucleus project to the posterior pituitary and secrete oxytocin directly into the circulation. Parvocellular cells in the hypothalamic paraventricular nucleus secrete CRH into the pituitary portal vessels. CRH is then transported to the anterior pituitary gland where it stimulates the secretion of ACTH from the anterior pituitary; ACTH, in turn, stimulates the adrenal cortex to release corticosterone in rats (cortisol in humans).

DOI is a prototypical 5-HT$_{2A/2C}$ receptor agonist (Van Wijngaarden et al., 1990; Leonhardt et al., 1992). We used (−)-DOI because it is a more potent isomer than (+)-DOI (unpublished data). 5-HT$_{2A}$ receptors in the hypothalamic paraventricular nucleus have been shown to mediate the neuroendocrine responses to a peripheral injection of (−)-DOI, because intraparaventricular and peripheral injections of the selective 5-HT$_{2A}$ receptor antagonist MDL 100,907 dose dependently inhibit the effect of (−)-DOI (1 mg/kg s.c.) on hormone secretion (Zhang et al., 2002). Thus, plasma hormone levels are an index of the function of 5-HT$_{2A}$ receptor signaling in the hypothalamic paraventricular nucleus. The dose of (−)-DOI for the chronic and acute treatment was 1 mg/kg i.p., a submaximal dose for ACTH, corticosterone, and oxytocin responses (unpublished data).

Previous studies investigating DOI-induced 5-HT$_{2A}$ receptor desensitization in rats indicate that DOI treatment for 8 days produces a tolerance to the discriminative stimulus properties of DOI (Smith et al., 1999). Autoradiographic analysis (Smith et al., 1999) and binding studies indicate that rat cortical 5-HT$_{2A}$ receptors were down-regulated after 8 days of (±)-DOI treatment without a change in their affinity ($K_d$) (McKenna et al., 1989; Valdez et al., 2002). Although agonist-induced desensitization of 5-HT$_{2A}$ receptors has been documented, the mechanism and time course for induction of this desensitization have not been previously described. The present study used (−)-DOI to determine the time course and the mechanism by which chronic agonist treatment produces 5-HT$_{2A}$ receptor desensitization in vivo.

Materials and Methods

Animals

Male Sprague-Dawley rats (250–275 g; Harlan, Indianapolis, IN) were housed two per cage in an environment controlled for temperature, humidity, and lighting (7:00 AM–7:00 PM). Food and water were provided ad libitum. Eight rats were used per experimental group. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by the Loyola University Institutional Animal Care and Use Committee.

Drugs

(−)-DOI was purchased from Sigma/RBI (Natick, MA). It was dissolved in 0.9% saline and injected at a dose of 1 mg/kg i.p., when used for chronic treatment and injected at a dose of 1 mg/kg s.c. for the challenge injections.

Experimental Procedures

The rats were handled for at least 7 days before the challenge injection and acclimated to a quiet environment to minimize stress and prevent plasma hormones from exceeding basal levels. Rats were randomly assigned to the various experimental groups, cage mates being placed within the same experimental groups. The body weight of each rat was recorded every other day.

Rats were injected with (−)-DOI (1 mg/kg i.p.) for 1, 4, and 7 days or 0.9% saline (1 ml/kg i.p.) for 7 days. Rats receiving (−)-DOI injections for 1 and 4 days were given injections of 0.9% saline (1 ml/kg i.p.) on the days before the commencement of (−)-DOI treatment. Thus, every group received injections for a total of 7 days, which allowed us to control for injection effects. Twenty-four hours after the last DOI treatment, a challenge injection of (−)-DOI (1 mg/kg s.c.) was administered 15 or 30 min before sacrifice. Control rats received a 0.9% saline (1 ml/kg s.c.) challenge injection 15 min before sacrifice. The trunk blood was collected in centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. The plasma samples for radioimmunoassays were stored at −80°C. Whole brains were removed, frozen on dry ice, and stored at −80°C for biochemical and molecular analyses.

PLC Activity

Tissue from the treatment groups that received the saline challenge was used for the measurement of PLC activity. PLC activity was measured by the amount of inositol 1,4,5 trisphosphate produced by PLC in the membrane fraction of the isolated tissue, as described previously (Wolf and Schutz, 1997). Briefly, 30 μg of membrane protein from frontal cortex was diluted into 100 μl of total volume with a buffer containing 25 mM Hepes-Tris, 3 mM EGTA, 10 mM LiCl, 12 mM MgCl$_2$, 1.44 mM sodium deoxycholate with 1 μM GTPγS, 300 nM free Ca$^{2+}$, 3 μM 5-HT, and 1 mM unlabeled phosphatidyl inositol. The reaction tubes were kept on ice until the incubation period (20 min at 37°C) was started with 100 μM [3H]phosphatidyl inositol. The reaction was stopped by addition of 0.9 ml of CHCl$_3$/MeOH (1:2) and 0.3 ml of chloroform. The tubes were shaken vigorously for 90 s and centrifuged at room temperature for 90 s at 21,238g. Then, 0.3 ml of the upper aqueous phase was mixed with 6 ml of scintillation cocktail and counted by a scintillation counter for 5 min. Protein concentrations in these membrane preparations were measured using the BCA protein assay kit (Pierce Chemical, Rockford, IL).

Immunoblot Analysis of G$_{q/11}$, G$_{11}$, RGS4, and RGS7 Proteins

Tissue Preparation. Frontal cortex from the treatment groups that received the 1 mg/kg (−)-DOI challenge was used for the measurement of G$_{q/11}$, G$_{11}$, RGS4, and RGS7 proteins. Punches of the hypothalamic paraventricular nucleus from the treatment groups
that did not receive a challenge injection were used for the measurements of G\(_{out}\) and G\(_{q11}\) protein. The paraventricular nucleus was dissected from a 700-μm coronal section obtained using a cryostat (−10°C) as described previously (Serres et al., 2000). Both tissues were homogenized in 10 mM Tris buffer containing 0.1 M NaCl, 0.1 M EDTA, and a protease inhibitor cocktail (1:1000) (Sigma-Aldrich, St. Louis, MO). Protein concentrations in these homogenates were measured as described above.

**Quantification of Protein Levels: General Procedure.** Solubilized proteins were loaded in the following amounts for the frontal cortex: 10 μg/lane for G\(_{out}\), G\(_{q11}\), and RGS7, and 15 μg/lane for RGS4 proteins; for the paraventricular nucleus, 4 μg/lane for G\(_{out}\) and G\(_{q11}\) proteins. These proteins were resolved by SDS-polyacrylamide gel electrophoresis containing 0.1% SDS, 12.5% acrylamide/bisacrylamide (30:0.2), 4.6 M urea, and 375 mM Tris, pH 8.7 (Blank and Exton, 1994). Gels were designed to contain six samples from the control group (saline treatment) and six samples from one of the (−)-DOI treatment groups. The proteins were electrophoretically transferred for 2 h to nitrocellulose membranes. Membranes were incubated with a blocking buffer for 1 h at room temperature (0.2% I-Block; Tropix, Bedford, MA and 0.1% Tween 20 detergent in phosphate-buffered saline buffer). The membranes were incubated overnight at 4°C with a primary antibody. Next, the membranes were washed in phosphate-buffered saline (containing 0.05% Tween 20) and incubated for 1 h at room temperature with a horseradish peroxidase-conjugated secondary antibody. The membranes were incubated with the ECL chemiluminescence substrate solution (Amersham Biosciences Inc., Piscataway, NJ) before exposure to Kodak blue-sensitive X-ray film (Midwest Scientific, Valley Park, MO).

**Specific Protein Antibodies.** The G\(_{out}\) protein (−42 kDa) was detected using a rabbit polyclonal G\(_{out}\) antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1:1000 dilution for frontal cortex, 1:500 dilution for paraventricular nucleus). The G\(_{q11}\) protein (−40 kDa) was detected using a rabbit polyclonal G\(_{q11}\) antibody (Santa Cruz Biotechnology, Inc.; 1:1000 dilution for frontal cortex, 1:500 dilution for paraventricular nucleus). The antibodies for G\(_{out}\) and G\(_{q11}\) proteins recognized two bands, both of which were included in the quantitation of the protein levels. The specificity of these antibodies was verified with preabsorption studies (unpublished data). The RGS4 protein (−35 kDa) was detected using a goat polyclonal RGS4 antibody (N-16; Santa Cruz Biotechnology, Inc.; 1:2000 dilution). The RGS7 protein (−56 kDa) was detected using a rabbit polyclonal RGS7 antibody generously donated by Drs. Philip Jones and Kathleen Young at Wyeth-Ayerst Research (Princeton, NJ).

After incubation with the RGS4 protein antibody, anti-goat secondary antibody and peroxidase anti-peroxidase reagents were used. All the other primary antibodies (G\(_{out}\), G\(_{q11}\), and RGS7 proteins) were followed by a goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Inc., 1:10,000 dilution for frontal cortex, 1:20,000 dilution for paraventricular nucleus). All of these secondary antibodies were conjugated to horseradish peroxidase.

**Western Blot Data Analysis.** Films were analyzed densitometrically using the Scion Image program (Frederick, MD). Gray scale density readings were calibrated using a transmission step wedge standard. The integrated optical densities (IODs) of each band were calculated as the sum of the densities of all of the pixels within the area of the band outlined. An area adjacent to the band was used to calculate the background density of the band. The background IOD was subtracted from the IOD of each band. Each experimental sample was measured in triplicate. Due to lack of tissue, this was only measured in duplicate.

**Statistical Analyses**

All data are presented as group mean ± S.E.M. Body weight data were analyzed by a one-way analysis of variance (ANOVA) with repeated measures and hormone data (ACTH, corticosterone, and oxytocin) were analyzed by a two-way ANOVA; both were followed by a Newman-Keuls multiple range test. PLC activity was analyzed using one-way ANOVA, followed by a Newman-Keuls post hoc analysis. G\(_{out}\), G\(_{q11}\), RGS4, and RGS7 protein levels were analyzed using a Student’s t test. GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD) was used for all statistical analyses. All p values less than 0.05 were reported as significant.

**Results**

**Body Weights across Treatments (Fig. 1).** The body weight of the rats progressively increased across time during the experiment (Fig. 1). Daily injections of (−)-DOI for 1, 4, and 7 days inhibited the rate of weight gain compared with the saline controls [\(F_{1,15,340}=26.30; \ p<0.0001\)].

**Agonist-Induced Desensitization of 5-HT-Stimulated PLC Activity in Frontal Cortex (Fig. 2).** Treatment with (−)-DOI decreased 5-HT-stimulated PLC activity in the frontal cortex of rats [\(F_{3,26}=12.96; \ p<0.0001\)]. 5-HT-stimulated PLC activity decreased 24% after 4 days (\(p<0.01\)) and 30% after 7 days (\(p<0.01\)) of (−)-DOI treatment (Fig. 2A). (−)-DOI treatment did not affect GTPγS-stimulated PLC activity in the frontal cortex [\(F_{3,30}=1.3; \ p=0.296\)] (Fig. 2B). (−)-DOI treatment decreased the ratio of 5-HT- to GTPγS-stimulated PLC activity [\(F_{3,26}=12.13\) by 4% after 1 day (\(p<0.05\)), 29% after 4 days (\(p<0.01\)), and 34% after 7 days (\(p<0.01\)) (Fig. 2C).

**Levels of 5-HT2A Signaling Proteins in Frontal Cortex (Fig. 3).** Treatment with (−)-DOI did not significantly reduce the levels of G\(_{out}\) proteins after 1 day (\(p=0.91\)) and 4 days (\(p=0.57\)) of (−)-DOI treatment (Fig. 3). Treatment with (−)-DOI for 7 days resulted in decreased levels of G\(_{out}\) proteins by 47% in homogenates of the frontal cortex (\(p<0.05\); Fig. 3). Levels of G\(_{q11}\) protein in homogenates of the frontal cortex were not significantly altered after 4 days (\(p=0.84\)) or 7 days (\(p=0.68\)) of treatment with (−)-DOI (Table 1).

**Fig. 1.** Effect of daily (−)-DOI injections (1, 4, and 7 days) on rat body weight. The data represent mean ± S.E.M. of 24 rats per group. A significant effect of chronic (−)-DOI treatment compared with its respective chronic saline treatment is indicated by * (\(p<0.05\)) and ** (\(p<0.01\)).
The levels of RGS4 or RGS7 proteins were not significantly changed in whole tissue homogenates of the frontal cortex after (-)-DOI treatment for 1 day (RGS4, p = 0.92; RGS7, p = 0.26), 4 days (RGS4, p = 0.45; RGS7, p = 0.23), or 7 days (RGS4, p = 0.98; RGS7, p = 0.24) (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>G_{q11} Protein</th>
<th>RGS4 Protein</th>
<th>RGS7 Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>NA</td>
<td>100 ± 30.7</td>
<td>100 ± 4.88</td>
</tr>
<tr>
<td>DOI, 1 day</td>
<td>NA</td>
<td>96.3 ± 13.8</td>
<td>113.5 ± 10.4</td>
</tr>
<tr>
<td>DOI, 4 days</td>
<td>100 ± 10.0</td>
<td>100 ± 16.6</td>
<td>100 ± 23.1</td>
</tr>
<tr>
<td>DOI, 7 days</td>
<td>93.3 ± 29.9</td>
<td>65.5 ± 8.6</td>
<td>67.2 ± 11.8</td>
</tr>
<tr>
<td>Saline</td>
<td>100 ± 23.3</td>
<td>100 ± 14.9</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>DOI, 7 days</td>
<td>81.3 ± 37.1</td>
<td>99.5 ± 13.7</td>
<td>74.8 ± 17.6</td>
</tr>
</tbody>
</table>

NA, data not available due to insufficient amounts of tissue.

**Agonist-Induced Desensitization of (-)-DOI-Mediated Hormone Secretion (Fig. 4).** Basal plasma ACTH, corticosterone, and oxytocin levels were not significantly altered after one, four, and seven daily injections of (-)-DOI. An acute dose (i.e., a challenge dose) of (-)-DOI was administered 15 or 30 min before the measurement of plasma...
chronic saline treatment is indicated by hormone levels. The (-)-DOI challenge injection increased oxytocin levels above the levels of saline controls after 7 days of saline treatment and after 1 day of (-)-DOI treatment, but not after 4 and 7 days of (-)-DOI treatment \(F_{2,78} = 26.0; p < 0.0001\). The (-)-DOI challenge injection alone, without any (-)-DOI pretreatment, increased plasma levels of oxytocin by 390% at 15 min and 414% at 30 min \(p < 0.01\) (Fig. 4A). The (-)-DOI challenge injection increased ACTH levels above the levels of saline controls after 7 days of saline treatment and 1, 4, and 7 days of (-)-DOI treatment \(F_{2,78} = 96.72; p < 0.0001\). The (-)-DOI challenge injection increased plasma levels of ACTH by 932% at 15 min and 1185% at 30 min \(p < 0.01\) (Fig. 4B). The (-)-DOI challenge injection increased corticosterone levels above the levels of saline controls after 7 days of saline treatment and 1, 4, and 7 days of (-)-DOI treatment \(F_{2,78} = 78.81; p < 0.0001\). Plasma corticosterone levels were increased 255% at 15 min and 382% at 30 min \(p < 0.01\) (Fig. 4C).

Treatment with (-)-DOI did not alter basal hormone levels, but it significantly reduced the oxytocin \(F_{3,78} = 11.1; p < 0.0001\) and ACTH responses to a (-)-DOI challenge \(F_{3,78} = 18.65; p < 0.0001\) at both 15 and 30 min postinjection (Fig. 4, A and B). Treatment with (-)-DOI did not alter the corticosterone response to the (-)-DOI challenge injection at any time \(F_{3,78} = 6.15; p < 0.0008\) (Fig. 4C).

The ANOVA indicated that the interaction between the (-)-DOI treatment and (-)-DOI challenge was significant for the ACTH response \(F_{2,78} = 5.71; p < 0.0001\). The interaction between the (-)-DOI treatment and the (-)-DOI challenge was not significant for oxytocin \(F_{2,78} = 3.52; p < 0.004\) or corticosterone responses \(F_{2,78} = 1.60; p < 0.16\). The post hoc Newman-Keuls test indicated that (-)-DOI treatment for 1 day significantly reduced the (-)-DOI-induced increase in plasma oxytocin levels by 26.8% [15 min post (-)-DOI challenge injection] and 51.9% [30 min post (-)-DOI challenge injection; \(p < 0.01\)]. Daily (-)-DOI injections for 4 days reduced the oxytocin response to (-)-DOI by 60.4% [15 min post (-)-DOI challenge injection; \(p < 0.01\)] and 67.3% [30 min post (-)-DOI challenge injection; \(p < 0.01\)]. Daily (-)-DOI injections for 7 days reduced oxytocin response to (-)-DOI by 90.1% [15 min post (-)-DOI challenge injection; \(p < 0.01\)] and 82.3% [30 min post (-)-DOI challenge injection; \(p < 0.01\)] (Fig. 4A). There were no significant differences between the 15 and 30 min (-)-DOI-challenged groups.

(-)-DOI treatment for 1 day significantly reduced the effect of (-)-DOI on plasma ACTH levels by 25.1% [15 min post (-)-DOI challenge injection; \(p < 0.05\)] and 50.6% [30 min post (-)-DOI challenge injection; \(p < 0.01\)]. Daily (-)-DOI injections for 4 days reduced ACTH response to (-)-DOI by 44.4% [15 min post (-)-DOI challenge injection; \(p < 0.01\)] and 47.2% [30 min post (-)-DOI challenge injection; \(p < 0.01\)]. Daily (-)-DOI injections for 7 days reduced the ACTH response to (-)-DOI by 64.2% [15 min post (-)-DOI challenge injection; \(p < 0.01\)] and 67.7% [30 min post (-)-DOI challenge injection; \(p < 0.01\)] (Fig. 4B). There was no significant difference between the 15- and 30-min (-)-DOI-challenged groups within the same treatment group. The (-)-DOI-induced increase in plasma corticosterone levels was not affected by chronic (-)-DOI treatment \(F_{6,89} = 1.60; p > 0.1\) (Fig. 4C).

**Levels of 5-HT<sub>2A</sub> Signaling Proteins in the Hypothalamic Paraventricular Nucleus.** Treatment with (-)-DOI did not produce a significant change in levels of G<sub>11</sub> proteins after 1 day \(p = 0.77\), 4 days \(p = 0.21\), or 7 days \(p = 0.87\) (Table 2). Levels of G<sub>9α</sub> proteins in the hypothalamic para-
ventricular nucleus were not significantly altered after treatment with \(-\)-DOI for 1 day \((p = 0.9)\), 4 days \((p = 0.95)\), or 7 days \((p = 0.98)\) (Table 2).

### Discussion

Daily injections of \(-\)-DOI induce a gradual decrease in the rate of weight gain and induce a gradual desensitization of 5-HT
2A receptor signaling in the frontal cortex and the paraventricular nucleus of the hypothalamus after 1 to 7 days of treatment. There are minor differences in the rate courses of desensitization between the cortex and hypothalamus, which may be an intrinsic difference between 5-HT
2A receptor signaling systems in the frontal cortex and hypothalamic paraventricular nucleus.

Daily treatment with \(-\)-DOI did not reduce body weight but rather inhibited the normal, progressive gain in body weight. This inhibition in the rate of weight gain starts after only 1 day of \(-\)-DOI treatment and is more pronounced as the duration of \(-\)-DOI treatment increases. This reduction in the rate of weight gain is consistent with previous results showing that DOI causes a reduction in food intake (De Vry and Schreiber, 2000). Both 5-HT
2A and 5-HT
2C receptors have been shown to regulate eating behavior (De Vry and Schreiber, 2000). DOI-induced adipogenesis is not solely mediated by 5-HT
2A receptors because the 5-HT
2A receptor-selective antagonist MDL 100,907 is unable to completely block the effect of DOI on eating behavior (De Vry and Schreiber, 2000). Because DOI has a similar affinity for 5-HT
2A and 5-HT
2C receptors (Van Wijngaarden et al., 1990; Zifa and Fillion, 1992), the hypophagia could be a phenomenon mediated by 5-HT
2A receptors, 5-HT
2C receptors, or both. Thus, at this time, we are unable to state whether this DOI-mediated decrease in the rate of weight gain is solely mediated by 5-HT
2A receptors.

Treatment with \(-\)-DOI produces a desensitization of 5-HT
2A receptor signaling in the hypothalamic paraventricular nucleus as indicated by reduced levels of plasma oxytocin and ACTH after \(-\)-DOI challenge. A decrease in 5-HT
2A receptor-mediated ACTH secretion after DOI treatment was also shown by Mazzola-Pomietto et al. (1996). To determine whether \(-\)-DOI treatment alters the time course of agonist-induced hormone responses, 15- and 30-min challenge periods were chosen. The hormone responses to \(-\)-DOI were similar at 15- and 30-min post \(-\)-DOI injection. These results suggest that reduced hormone responses to the 5-HT
2A receptor agonist most likely represent true desensitization of 5-HT
2A receptors and not a mere shift in the time course of the hormone responses.

Treatment with \(-\)-DOI does not produce a measurable change in \(-\)-DOI-induced increase in plasma corticosterone levels, in concordance with previous studies (Mazzola-Pomietto et al., 1996). ACTH stimulates corticosterone release from the adrenal cortex. Previous studies have shown that plasma levels of ACTH exceeding 200 to 300 pg/ml produce a maximal increase in plasma corticosterone levels (Levy et al., 1992). Thus, in the present experiment, although the ACTH response to a \(-\)-DOI challenge injection was greatly reduced by pretreatment with \(-\)-DOI, even these ACTH levels were elevated to such an extent that a maximal stimulation of the secretion of corticosterone was evident across the entire duration of the \(-\)-DOI treatment. Hence, plasma corticosterone is the least sensitive peripheral marker of desensitized hypothalamic receptors.

5-HT-stimulated PLC activity in the frontal cortex is almost entirely mediated by 5-HT
2A receptors, because pretreatment with 5-HT
2A receptor antagonists (ketanserin, spiperone, or mianserin) inhibits most of the 5-HT-stimulated PLC activity in the frontal cortex of rats (Wolf and Schutz, 1997). GTPyS, a nonhydrolyzable form of GTP, irreversibly binds to G proteins and enables them to activate second messenger enzymes, such as PLC. In contrast to 5-HT-stimulated PLC activity, GTPyS-stimulated PLC activity measures only the ability of G
q/11 proteins to stimulate PLC activity. Comparison of the two stimuli allows us to determine whether the change in receptor signaling occurs at the receptor and/or the interface between the receptor and G protein or occurs at the interface of the G protein and effector enzyme (PLC) and/or the PLC enzyme. The ability of G
q/11 proteins to stimulate PLC activity did not change during the \(-\)-DOI treatment. This observation suggests that neither PLC nor G
q/11 proteins are altered in such a way that their functional interaction is hindered. The ability of 5-HT
2A receptors to stimulate PLC activity through G
q/11 proteins is hindered after the \(-\)-DOI treatment. Thus, our data suggest that the desensitization of 5-HT
2A receptor signaling is not due to a reduced ability of G
q/11 proteins to stimulate PLC activity but rather due to a change in 5-HT
2A receptors or in the coupling of 5-HT
2A receptors to G proteins.

Results from both the PLC activity assay and the immunoblot analyses suggest that the desensitization of 5-HT
2A receptor signaling is not likely due to an altered expression of G
q11, RGS4 or RGS7 proteins. Although our previous studies indicate that treatments with other serotonin-modulating drugs and certain pathologies result in significant and consistent changes in levels of G
q11, G
q11, and RGS4 proteins (Carrasco et al., 2003; Muma et al., 2003), our current data indicate that treatment with \(-\)-DOI does not produce significant alterations in the levels of G
q11, RGS4, or RGS7 proteins. The lack of change in G1 and RGS proteins after 4 days of \(-\)-DOI treatment is consistent with the PLC activity data, which suggest that \(-\)-DOI did not alter the ability of G1 proteins to stimulate PLC.

A decrease in G
q11 protein levels may be one mechanism by which 5-HT
2A receptors in the frontal cortex desensitize after 7 days of \(-\)-DOI treatment, but it is not likely involved in the desensitization of the 5-HT
2A receptors that occur after 4 days of \(-\)-DOI treatment. In addition, even though there was a 47% decrease in G
q11 protein levels after 7 days of \(-\)-DOI treatment, there was no change in the GTPyS-stimulated PLC activity. Although there is currently no definitive explanation for this phenomenon, it may be that G
q11 protein

### Table 2

| Treatment Type | G
q11 Protein | G
q11 Protein |
<table>
<thead>
<tr>
<th></th>
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<tr>
<td>Saline 1 day</td>
<td>100 ± 10.4</td>
<td>100 ± 17.8</td>
</tr>
<tr>
<td>DOI 1 day</td>
<td>93.3 ± 19.2</td>
<td>103.4 ± 20.7</td>
</tr>
<tr>
<td>Saline 4 days</td>
<td>131.4 ± 17.1</td>
<td>97.5 ± 27.1</td>
</tr>
<tr>
<td>DOI 7 days</td>
<td>100 ± 21.7</td>
<td>100 ± 33.0</td>
</tr>
<tr>
<td>Saline 100</td>
<td>94.4 ± 25.5</td>
<td>98.9 ± 29.3</td>
</tr>
</tbody>
</table>
levels are sufficient to sustain GTPγS-driven PLC activity because G_{a,11} protein levels were not reduced. Another explanation for this phenomenon is that there are two separate mechanisms of desensitization. One mechanism is activated within a few days of chronic treatment and is then succeeded by a decrease in G_{q} protein levels after one full week of chronic treatment. Furthermore, although we did observe a desensitization of the 5-HT_{2A} receptor in the hypothalamic paraventricular nucleus after (−)-DOI treatment, we did not observe a decrease in G_{q} protein levels at any time point after (−)-DOI treatment in this hypothalamic region. Thus, a decrease in G_{q} protein levels is not necessary for the desensitization of 5-HT_{2A} receptors in the hypothalamic paraventricular nucleus and is unlikely to be the primary mechanism by which desensitization of 5-HT_{2A} receptors occurs in the frontal cortex.

Previously published data from NIH-3T3 cells demonstrated that agonist-induced 5-HT_{2A} receptor desensitization can occur without receptor down-regulation (Roth et al., 1995). In contrast, previous in vivo studies have found (−)-DOI treatment to reduce cortical 5-HT_{2A} receptor density without altering their affinity (K_{d}) (McKenna et al., 1989; Anji et al., 2000;Valdez et al., 2002). Although down-regulation is one mechanism underlying receptor desensitization, other mechanisms may also account for the reduced density of 5-HT_{2A} receptors. For example, desensitization of 5-HT_{2A} receptors in C6 glioma cells is mediated by clathrin-mediated receptor internalization (Hanley and Hensler, 2002). Other studies indicate that 5-HT_{2A} receptor internalization is cell type-specific (Roth et al., 1995; Gray and Roth, 2001). For example, agonist-induced 5-HT_{2A} receptor desensitization was not accompanied by changes in [^{3}H]ketanserin-labeled 5-HT_{2A} receptors or changes in cellular distribution of 5-HT_{2A} receptor in NIH-3T3 cells, stably transfected with 5-HT_{2A} receptors (Roth et al., 1995).

Because our present data suggest that DOI disrupts the receptor-to-G protein interaction, one possible mechanism underlying this desensitization could be phosphorylation of the 5-HT_{2A} receptor and/or G_{a,11} proteins. In support of this theory, protein kinase C inhibitors and Ca^{2+} calcium modulin kinase inhibitors prevent agonist-induced 5-HT_{2A} receptor desensitization in Chinese hamster ovary cells stably transfected with 5-HT_{2A} receptors (Berg et al., 2001). Although 5-HT_{2A} receptors contain 11 potential phosphorylation sites, phosphorylation of the 5-HT_{2A} receptor has been a difficult phenomenon to demonstrate and therefore agonist-induced phosphorylation of 5-HT_{2A} receptors has not been definitively shown (Saltzman et al., 1991; Vuore-Cravari et al., 1995; Gray and Roth, 2001). Recently, two phosphorylation sites on serine residues have been identified as critical for 5-HT_{2A} receptor desensitization in cell culture (Gray et al., 2003). Furthermore, agonist-induced desensitization of 5-HT_{2A} receptors may possibly be mediated by phosphorylation of the G protein. Although the potential post-translational modification sites for G_{a,q} and G_{a,11} proteins have not been documented, their amino acid sequences contain many serine and threonine residues that are possible phosphorylation sites.

In conclusion, chronic systemic treatment with (−)-DOI produces a desensitization of 5-HT_{2A} receptors in the frontal cortex and the paraventricular nucleus of the hypothalamus. Our results suggest that the (−)-DOI-induced desensitization observed in the frontal cortex is seemingly not due to changes in expression of G_{a,11}, RGS4, or RGS7 proteins and may partially involve a decrease in G_{q} protein levels. The desensitization of 5-HT_{2A} receptors is most likely due to post-translational modifications of the 5-HT_{2A} receptor, G_{a,q} or G_{a,11} proteins altering the 5-HT_{2A} receptor-to-G_{a,q/11} protein interface.

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