A Desensitization of Hypothalamic 5-HT₁A Receptors by Repeated Injections of Paroxetine: Reduction in the Levels of Gᵢ and Gₒ Proteins and Neuroendocrine Responses, but Not in the Density of 5-HT₁A Receptors

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ABSTRACT

The aim of the present study was to determine whether the previously observed desensitization of hypothalamic 5-hydroxytryptamine₁A (5-HT₁A) receptors, during daily injections of fluoxetine, is mediated by sustained blockade of 5-HT reuptake. In the present study, we examined the time course effects of another 5-HT uptake inhibitor, paroxetine. Paroxetine reduced the oxytocin, adrenal corticotropic hormone and corticosterone responses to a challenge with the 5-HT₁A agonist 8-hydroxy-2-(dipropylamino)tetralin. These reductions in hormone responses were significant after 3 daily injections and reached a maximum after 7 daily paroxetine injections. These hormone responses remained maximally suppressed after 14 daily injections of paroxetine. A single day of paroxetine treatment did not alter the hormone responses to 8-hydroxy-2-(dipropylamino)tetralin. Repeated injections of paroxetine did not reduce the density of 5-HT₁A receptors in any brain region but did produce a gradual reduction in the levels of Gᵢ and Gₒ proteins in a region-specific manner. The time course of the paroxetine-induced reduction in the level of Gᵢ and Gₒ proteins in the hypothalamus was similar to the effect previously observed with fluoxetine and was also similar to the time course of paroxetine-induced reductions in oxytocin and adrenal corticotropic hormone responses to 8-hydroxy-2-(dipropylamino)tetralin. In conclusion, these results suggest that blockade of 5-HT uptake sites produces a delayed and gradual desensitization of 5-HT₁A receptors in the hypothalamus. This desensitization is not due to changes in the density of hypothalamic 5-HT₁A receptors. Reduction in the hypothalamic level of Gₒ proteins may play a role in the desensitization of 5-HT₁A receptor systems. However, reductions in G₁ or Gₒ proteins cannot be excluded as potential mediators of the desensitization of 5-HT₁A receptor systems.

5-HT uptake inhibitors, such as fluoxetine and paroxetine, are a new class of drugs that were initially introduced to treat depression. 5-HT uptake inhibitors are also effective in additional disorders, such as obsessive compulsive disorder and premenstrual syndrome, for which older classes of drugs such as tricyclic antidepressants and MAO inhibitors are ineffective or less effective (Wong et al., 1995; Eriksson et al., 1995). This difference in therapeutic effects between 5-HT uptake inhibitors and older antidepressants may be due to different adaptive changes that they produce in brain 5-HT receptors (Blier and de Montigny, 1994; Duman et al., 1994; Gardier et al., 1996). One difference observed in our previous studies is that repeated injections of the 5-HT uptake inhibitor fluoxetine produce a gradual desensitization of hypothalamic 5-HT₁A receptors, whereas the tricyclic antidepressant and specific norepinephrine uptake inhibitor desipramine did not produce this effect (Li et al., 1996b; Li et al., 1994; Li et al., 1993). A desensitization of hypothalamic 5-HT₁A receptors was also observed in humans treated with fluoxetine (Lesch et al., 1991). Desipramine may not be able to desensitize 5-HT₁A receptors because it does not inhibit 5-HT uptake and thus cannot induce adaptive changes in serotonergic neurotransmission, leading to desensitization of postsynaptic 5-HT₁A receptors. These observations suggest that the desensitization of hypothalamic 5-HT₁A receptors may play a role in some of the therapeutic effects of 5-HT uptake inhibitors.

The purpose of the present study was to determine whether the effect we previously observed of fluoxetine on the 5-HT₁A receptors in the hypothalamus is mediated by blockade of 5-HT uptake sites. We examined the effects of another 5-HT uptake inhibitor, paroxetine, on hypothalamic 5-HT₁A receptors. Paroxetine has a chemical structure and functional properties similar to fluoxetine, suggesting it may also produce a desensitization of hypothalamic 5-HT₁A receptors.
pharmacokinetic profile different from those of fluoxetine (Lane et al., 1995; DeVane, 1994; Nemeroff, 1993). Because both fluoxetine and paroxetine inhibit 5-HT uptake sites, a common effect of these two drugs is likely to be mediated by blockade of 5-HT uptake sites. In other words, if both fluoxetine and paroxetine induce a desensitization of 5-HT₁₅ receptors, then it is likely that the desensitization of 5-HT₁₅ receptors is mediated by blockade of 5-HT uptake sites.

5-HT₁₅ receptors can be classified into somatodendritic (5-HT₁₅ autoreceptors) and postsynaptic receptors (De Vry, 1985; Hoyer et al., 1994). Somatodendritic 5-HT₁₅ autoreceptors are located on 5-HT neurons in the dorsal and median raphe nuclei in the midbrain (Kia et al., 1996). Activation of somatodendritic 5-HT₁₅ autoreceptors by 5-HT or 5-HT₁₅ agonists decreases the firing rate of neurons and subsequently reduces the release of 5-HT from nerve terminals (Artigas et al., 1996; Briley and Moret, 1993; Adell et al., 1993; Hjorth and Auerbach, 1995). Somatodendritic 5-HT₁₅ autoreceptors may be coupled to Gi, proteins, which increase the opening of K⁺ channels and consequently suppress the activity of 5-HT neurons (Sprouse and Aghajanian, 1988; Innis and Aghajanian, 1987; Innis et al., 1988; Clarke et al., 1996; Romero et al., 1994). It has been proposed that 5-HT₁₅ autoreceptors may be partly responsible for the delay in the therapeutic effects of 5-HT uptake inhibitors. The rationale for this hypothesis is as follows: The administration of 5-HT uptake inhibitors induces an increase in 5-HT concentration in the synaptic cleft. This increase in 5-HT concentration activates somatodendritic 5-HT₁₅ autoreceptors in the raphe nuclei and consequently decreases 5-HT release in forebrain regions. Therefore, clinical symptoms cannot be improved until somatodendritic 5-HT₁₅ autoreceptors are desensitized by repeated administration of 5-HT uptake inhibitors. Several studies support this hypothesis, mainly with data obtained using microdialysis and/or electrophysiological recording. However, data regarding changes in the density of 5-HT₁₅ receptors in the midbrain raphe are inconsistent (Welner et al., 1989; Hensler et al., 1991; Le Poul et al., 1995; Li et al., 1994). Our previous study demonstrated that the 5-HT uptake inhibitor fluoxetine reduces the levels of Gi₁ and Gi₁₂ proteins in the midbrain, which may be related to the desensitization of somatodendritic 5-HT₁₅ autoreceptors (Li et al., 1996b). The current study examined whether paroxetine will reduce the levels of these G proteins in the midbrain.

Postsynaptic 5-HT₁₅ receptors are distributed in many forebrain regions that receive serotonergic input, such as the hippocampus, hypothalamus, amygdala and cortex (Kia et al., 1996; Gozlan et al., 1995; Khawaja, 1995). Activation of postsynaptic 5-HT₁₅ receptors by 5-HT produces physiological responses that depend on the function of the target cells. Stimulation of 5-HT₁₅ receptors in the hypothalamic paraventricular nucleus increases the secretion of several hormones, including ACTH, corticosterone and oxytocin (Budgy, 1995; Van de Kar and Brownfield, 1993; Bagdy and Kologer, 1993). Therefore, the magnitude of the hormone responses to 5-HT₁₅ agonists can reflect the function of 5-HT₁₅ receptor systems in the hypothalamus (Bagdy, 1995; Bagdy and Makara, 1994). 5-HT₁₅ receptors can couple to Gi₁ and Gi₆ proteins. Their affinity for Gi₆ and Gi₃ proteins is higher than for Gi₁ and Gi₆ proteins. (Raymond et al., 1993; Mulheron et al., 1994; Bertin et al., 1992; Butkerait et al., 1995; Fargin et al., 1991). Our previous studies indicate that daily injections of fluoxetine produce a gradual reduction in the levels of Gi₁ and Gi₆ proteins, but not Gi₂ proteins, in the hypothalamus.

We hypothesized that repeated injections of paroxetine also will produce a delayed and gradual desensitization of 5-HT₁₅ receptors in the hypothalamus. The time course of the effect of paroxetine on the hormone responses to 8-OH-DPAT was examined to assess the function of 5-HT₁₅ receptors in the hypothalamus. To determine which components of the 5-HT₁₅ receptor system may be involved in the desensitization, we further examined the time course of the effect of paroxetine on the density of 5-HT₁₅ receptors and on the levels of Gi₁ and Gi₆ proteins in the hypothalamus, midbrain and frontal cortex.

Materials and Methods

Animals. Male Sprague-Dawley rats (225–275 g) were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN). The rats were housed two per cage in a light- (12-hr light/dark; lights on at 7 A.M.), humidity- and temperature-controlled room. Food and water were available ad libitum. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by the Loyola University Institutional Animal Care and Use Committee.

Experimental procedure. The rats were injected with paroxetine (10 mg/kg, ip) once daily for 1, 3, 7 or 14 days. The control rats received saline injections for 14 days. Eighteen hours after the last injection, the rats were challenged with saline or 8-OH-DPAT (50 or 500 µg/kg, sc) and decapitated 15 min later. These doses represent the ED₅₀ and Eₘₐₓ doses of 8-OH-DPAT-induced increase in plasma oxytocin (Li et al., 1993). Trunk blood was collected in centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. After centrifugation at 2500 rpm, 4°C, for 15 min, plasma aliquots were stored at −70°C until they were used for hormone assays. The brains from saline-challenged rats were quickly and carefully removed and frozen on powdered dry ice until the brains were completely frozen. The brains were then wrapped with parafilm, aluminum foil and stored at −70°C until they were sectioned for autoradiography. The brains of the other rats were dissected, and the hypothalamus, midbrain and frontal cortex were stored at −70°C for immunoblotting of Gi₁ and Gi₆ proteins.

Drugs and reagents. The following drugs and chemicals were used in this study: 8-OH-DPAT (Research Biochemicals Inc., Natick, MA), ACTH antisera (IgG1) (IgG Corp, Nashville, TN). ACTH (1–39) standards were obtained from Calbiochem (San Diego CA). Bovine serum albumin and aprotinin (Sigma Chemical Co., St. Louis, MO), normal rabbit serum and goat anti-rabbit-γ-globulin (Calbiochem, San Diego, CA), [3H]-ACTH (INCSTAR, Stillwater, MN), corticosterone antiserum (INC Biochemicals, Irvine, CA), ultima gold scintillation fluid (Packard Instrument Co., Inc., Downers Grove, IL), acetone (Spectranalyzed A-19) and petroleum ether (Fisher, Pittsburgh, PA), [3H]-corticosterone, [3H]-oxytocin, [3H]-8-OH-DPAT and anti Gi₁₂ (AS/7) and anti Gi₅ (GC/2) sera (DuPont-NEN, Boston, MA), anti Gi₆ serum (Upstate Biotechnology Inc., Lake Placid, NY), rabbit peroxidase-anti-peroxidase (Organon Teknika Co., Durham, NC), the chemiluminescence substrate solution LumioGlue (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) and NP-40 (Nonidet P-40 or Igepal CA-630) (Sigma Chemical Co., St. Louis, Mo). Paroxetine was a gift from Smith-Kline Beecham (Philadelphia, PA). All the drugs were dissolved in saline and injected in a volume of 2 ml/kg for paroxetine and 1 ml/kg for 8-OH-DPAT.

Radioimmunoassay for plasma hormone concentrations. Plasma ACTH and corticosterone were measured by radioimmunoassays as described in detail in our previous paper (Li et al., 1993). Plasma oxytocin was assayed by a method modified from Keil et al.
Plasma was first extracted before it was used in the radioimmunoassay. Briefly, rat plasma (1 ml) was mixed with 2 ml ice-cold acetone (Spectranalyzed, Fisher, Pittsburgh, PA) and centrifuged at 2000 rpm, 4°C, for 30 min. The supernatant was then added to 5 ml cold petroleum ether and mixed immediately. After centrifugation at 2000 rpm, 4°C, for 15 min, the top layer was aspirated and discarded. The remaining solution was dried by blowing air into the tubes at 4°C. The dried extract was then dissolved in 1 ml of assay buffer (0.05 M phosphate buffer, pH 7.4, containing 0.125% bovine serum albumin, 0.01% sodium azide and 0.001 M EDTA) and was used for the oxytocin radioimmunoassay. This assay is a double-antibody assay. The plasma extract (20 or 200 μl) and oxytocin standard (0–1 ng) were incubated in triplicate with 0.1 ml of rabbit anti-oxytocin serum (1:50,000 dilution) in a total volume of 0.4 ml for 24 hr at 4°C. 125I-oxytocin (Du Pont-NEN, Boston, MA, 3000 cpm, 0.1 ml) was then added to the tubes and incubated for 72 hr at 4°C. To the tubes was then added 0.1 ml goat anti-rabbit γ-globulin (1:12.5 dilution), followed by 0.1 ml of normal rabbit serum (1:120 dilution). After incubation for 24 hr, the tubes were centrifuged at 15,000 × g, at 4°C, for 20 min. The supernatant was decanted, and the radioactivity in the pellet was counted for 5 min by a Micromedic 4/200 plus g counter and analyzed from the standard curve using the RIA-AID computer program (Robert Maciel Associates, Arlington, MA). The concentration of plasma oxytocin was calculated with a correction factor based on the recovery of the extraction. The sensitivity limit of this assay is 1 pg/tube, and the intra- and interassay variabilities are 8.1% and 8.6%, respectively.

 Autoradiographic analysis of 3H-8-OH-DPAT binding. The brains from the rats that received a saline challenge were cut into 15-μm coronal sections using a cryostat at −21°C. The sections were thaw-mounted on chromalum/gelatin-coated slides and stored at −20°C. Sections were collected from the following levels: frontal cortex (Bregma + 3.70 mm), medial hypothalamus (Bregma − 1.80 mm), caudal hypothalamus (Bregma − 3.14 mm) and midbrain (Bregma − 7.8 mm) according to the atlas of Paxinos and Watson (1986). Sections from these levels were used for autoradiographic analysis (fig. 1).

The autoradiographic assay for 3H-8-OH-DPAT-labeled 5-HT_{1A} receptors was performed as described by Pazos and Palacios (1985). Briefly, after a preincubation in the assay buffer (containing 0.17 M Tris, 4 mM CaCl, 10 μM pargyline and 0.01% ascorbic acid, pH 7.6), slide-mounted sections were incubated with 3H-8-OH-DPAT (2 nM, 135 Ci/mmol) at room temperature for 1 hr. This concentration of 3H-8-OH-DPAT is equal to its K_d in hypothalamic homogenates (Li et al., 1993). Nonspecific binding was defined in the presence of 10^{-6} M 5-HT. After being washed twice with Tris buffer at 4°C for 5 min, the slides were dipped in cold H₂O and then blow-dried immediately. Then they were exposed to tritium-sensitive Hyperfilm-3H for either 2 months or 2 weeks (for sections containing a high density of the binding sites). A set of 3H microscales (Amersham, Arlington Heights, IL) was exposed to each film together with the slides to calibrate the optical density to fmol/mg tissue equivalent. The films were developed by a Kodak developing procedure for X-ray films.

 Autoradiograms were analyzed densitometrically using the NIH.
image analysis program for Macintosh computers. The gray scale density readings were calibrated to fmol/mg tissue equivalent using the \(^{3}H\)microscales. Brain regions were identified according to the atlas of Paxinos and Watson (1986), as shown in figure 1. The layers of the cortex were identified according to the atlas of Zilles (1985). The density of \(^{3}H\)-8-OH-DPAT binding in each brain region was measured and expressed as fmol/mg tissue equivalent. An area outside of the section was measured as the background of the film, which was subtracted from each measurement in the section. Specific \(^{3}H\)-8-OH-DPAT binding sites in each brain region were determined by subtracting the nonspecific binding sites from the total binding sites in each region. The data for a brain region of each rat represents the mean of four adjacent brain sections.

**Immunoblots.** The levels of G\(_{i1}\), G\(_{i2}\), G\(_{i3}\), and G\(_{o}\) proteins in the hypothalamus, midbrain and frontal cortex were measured using immunoblots as described in detail in our previous paper (Li et al., 1996b). Briefly, the solubilized proteins (10–35 \(\mu g\) of protein) that were extracted from the membranes of the hypothalamus, midbrain and frontal cortices (Sterneweis and Robinshaw, 1984; Okuhara et al., 1996) were resolved by SDS-polyacrylamide gel electrophoresis (containing 0.1% SDS, 12% acrylamide/bisacrylamide (30:0.2), 4 M urea and 375 mM Tris, pH 8.4 (Mullaney and Miligan, 1990)). The proteins were then electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with polyclonal antisera for G\(_{i1}\) (AS/7, 1:2500 dilution), G\(_{i2}\) (Anti-G\(_{i2}\), 1:2000 dilution) and G\(_{o}\) (GO/2, 1:2000 dilution), followed by a secondary antibody (goat anti-rabbit serum, 1:10,000 dilution) and then rabbit peroxidase-antiperoxidase (1:10,000 dilution). After several washes, the membranes were incubated with the chemiluminescence substrate (LumiGlo) and then exposed to Kodak X-ray film. Films were analyzed densitometrically using the NIH image analysis program for Macintosh computers as detailed in our previous paper (Li et al., 1996b).

**Statistics.** The data from the hormone analyses were extrapolated from standard curves using the RIA-AID computer program (Robert Maciel Associates, Arlington, MA). The data are presented as group means ± S.E.M. The data from the hormone assays were analyzed by a two-way ANOVA, and the data obtained from autoradiographic analysis of \(^{3}H\)-8-OH-DPAT binding and from immunoblots for G proteins were analyzed by a one-way ANOVA. Group means were compared by Newman-Keuls multiple-range test (Steel and Torrie, 1960). A computer program (NWA STATPAK, Portland, OR) was used for all the statistical analyses.

**Results**

**Hormone responses to 8-OH-DPAT.** Both doses of 8-OH-DPAT (50 and 500 \(\mu g/kg\)) significantly increased plasma oxytocin, ACTH and corticosterone concentrations. Pretreatment with paroxetine blunted the increase in the levels of these hormones induced by a low dose (50 \(\mu g/kg\)) of 8-OH-DPAT as detailed below (figs. 2–4). However, paroxetine had different effects on the oxytocin, ACTH and corticosterone responses to the high dose (500 \(\mu g/kg\)) of 8-OH-DPAT (table 1).

Paroxetine significantly decreased the oxytocin response to both low (50 \(\mu g/kg\)) and high (500 \(\mu g/kg, sc\)) doses of 8-OH-DPAT after 3 daily injections (fig. 2, table 1). The reduction in the oxytocin response to 8-OH-DPAT reached a maximum after 7 and 14 daily paroxetine injections (fig. 2). A single daily injection of paroxetine did not produce a significant reduction in the effect of the low dose of 8-OH-DPAT (50 \(\mu g/kg\)) on plasma oxytocin concentration. However, the oxytocin response to the high dose (500 \(\mu g/kg\)) of 8-OH-DPAT was significantly reduced by 1 day after a single injection of paroxetine (table 1).

Repeated injections of paroxetine significantly reduced the ACTH response to the 50-\(\mu g/kg\) dose of 8-OH-DPAT (fig. 3). A partial reduction of the ACTH response to 8-OH-DPAT occurred after 3 daily injections of paroxetine, and this inhibition was maximal after 7 and 14 daily injections. The ACTH response to 8-OH-DPAT was not altered one day after a single injection of paroxetine (fig. 3). Furthermore, repeated injections of paroxetine did not decrease the effect of the high dose of 8-OH-DPAT (500 \(\mu g/kg\)) on ACTH secretion (table 1).

Repeated injections of paroxetine significantly inhibited the increased in corticosterone concentration induced by the 50-\(\mu g/kg\) dose of 8-OH-DPAT (fig. 4). Unlike the ACTH response, the reduced corticosterone response to 8-OH-DPAT was statistically significant only after 7 and 14 daily injections of paroxetine (fig. 4). Injections of paroxetine for 1 or 3 days did not decrease the corticosterone response to 8-OH-DPAT.
Although paroxetine did not alter the levels of G13 and G0 proteins in the frontal cortex, the levels of G11 and G3 proteins were significantly decreased in the frontal cortex 1 day after a single injection of paroxetine and remained below control levels until 14 daily injections (fig. 8A and B).

Discussion

The effects of paroxetine described here are very similar to those obtained with fluoxetine, which suggests that sustained blockade of 5-HT uptake produces a delayed and gradual desensitization of 5-HT1A receptors in the hypothalamus. This desensitization is not due to a down-regulation of hypothalamic 5-HT1A receptors and may be due to mechanisms downstream from the receptor level. Because the time course of paroxetine-induced reduction in the level of G13 proteins in the hypothalamus is similar to the time course of reduction in the hormone responses to 8-OH-DPAT, it is possible that the reduction in the level of G13 proteins may play a role in the desensitization of hypothalamic 5-HT1A receptors. However, the possibility of a role for G11 or G0 proteins cannot be excluded.

In the present study, we used the elevation in plasma levels of oxytocin, ACTH and corticosterone after an injection of 8-OH-DPAT to assess the function of hypothalamic 5-HT1A receptors. Several studies have demonstrated that 8-OH-DPAT increases the plasma concentrations of ACTH, corticosterone and oxytocin in a dose-dependent manner. The ACTH and corticosterone responses to 8-OH-DPAT can be inhibited by the 5-HT1A antagonists pindolol, spiperone, NAN-190, UH-301, WAY-100135 and WAY-100635 (Cowen et al., 1990; Lejeune et al., 1993; Pan and Gilbert, 1992; Fletcher et al., 1995; Przegalinski et al., 1989; Kelder and Ross, 1992; Vicent et al., 1996). The oxytocin response to 8-OH-DPAT can be inhibited by WAY-100635 and NAN-190 (Vicent et al., 1996; Bagdy and Kalogeris, 1993). A lesion in the hypothalamic paraventricular nucleus blunted the ACTH, corticosterone and oxytocin responses to another 5-HT1A agonist, ipsapirone (Bagdy and Makara, 1994; Bagdy, 1995). These data suggest that the 8-OH-DPAT-induced increase in plasma hormone concentrations is mediated by activation of 5-HT1A receptors in the hypothalamus, probably in the paraventricular nucleus. Therefore, the magnitude of hormone responses to 8-OH-DPAT can be used as a tool to assess the function of hypothalamic 5-HT1A receptor systems (Van de Kar, 1991; Van de Kar, 1989; Van de Kar and Brownfield, 1993). The results of the present study show that repeated injections of paroxetine decrease the hormone responses to 8-OH-DPAT, which suggests that paroxetine produces a desensitization of 5-HT1A receptor systems in the hypothalamus. The desensitization of hypothalamic 5-HT1A receptors appears after 3 daily injections and reaches a maximum after 7 and 14 days. These results are consistent with those observed after repeated injections of fluoxetine (Li et al., 1996b), which suggests that a similar adaptive mechanism leads to the desensitization of 5-HT1A receptors during blockade of 5-HT uptake sites.

A previous study on the time course of the effects of fluoxetine indicated that three daily injections produce a partial desensitization of hypothalamic 5-HT1A receptors. In the present study, we added a group that received a single injection of paroxetine to determine when the desensitization of
the hypothalamic 5-HT1A receptors would start. The results of the present study indicate that desensitization of hypothalamic 5-HT1A receptors does not occur 1 day after a single injection of paroxetine.

In a result similar to our previous observations with fluoxetine, daily injections of paroxetine decreased the ACTH and corticosterone responses to a low dose, but not a high dose, of 8-OH-DPAT, whereas the decrease in the oxytocin response was observed at both low and high doses of 8-OH-DPAT (table 1). This difference between ACTH and oxytocin responses might result from differences in receptor reserve for these hormones. There is a higher 5-HT1A receptor reserve for ACTH and corticosterone responses than for the oxytocin response to 5-HT1A agonists (Meller and Bohmaker, 1994; Pinto et al., 1994; unpublished data from W. Pinto, L. D. Van de Kar and G. Battaglia). These differences in receptor reserve may be related to an amplification of the signals in each stage of the hypothalamic-pituitary-adrenal axis for the ACTH and corticosterone responses to activation of 5-HT1A receptors, whereas oxytocin is released directly from cells in the hypothalamus via their nerve terminals in the neural lobe of the pituitary gland. However, it is also possible that the lack of change in the ACTH and corticosterone secretion and may be sensitized by repeated injections of paroxetine. For example, 8-OH-DPAT has about a 10-fold lower affinity for 5-HT7 receptors than for 5-HT1A receptors (Kawahara et al., 1994). However, a role for...
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**Fig. 5.** Example of immunoblots of G proteins in brain regions from rats that received daily injections of paroxetine. Top panel: G₁ and G₂ proteins in the frontal cortex; middle panel: G₃ proteins in the hypothalamus; bottom panel: G₆ proteins in the midbrain. The lanes from left to right contain membrane extracts from brain regions of rats that received vehicle and paroxetine for 1 day, 3 days, 7 days and 14 days.

1993; Li et al., 1996a) and also is in agreement with data reported by other investigators (Le Poul et al., 1995; Hensler et al., 1991). Other investigators have shown that neither fluoxetine nor paroxetine altered the density of 5-HT₁A receptors in the dorsal raphe nucleus or in the dentate gyrus of the hippocampus (Le Poul et al., 1995) and that repeated injections of sertraline or citalopram did not alter the density of 5-HT₁A receptors in several brain regions (Hensler et al., 1991). Together, these observations suggest that sustained inhibition of 5-HT reuptake does not lead to down-regulation of 5-HT₁A receptors. The lack of paroxetine-induced change in [³H]-8-OH-DPAT binding in tissues in which G protein levels have been decreased raises several questions. [³H]-8-OH-DPAT is a 5-HT₁A agonist and should bind with high affinity to the coupled state of 5-HT₁A receptors. Under the conditions of our assay, using concentrations equal to the Kᵰ (2 nM), binding is determined by both receptor number and affinity. If there is little surplus of G proteins, a reduction in G proteins should lead to reduced coupling to the receptors, and thus there should have been a reduction in [³H]-8-OH-DPAT binding. This clearly was not the case, which could suggest a surplus of G proteins. Yet there was a marked reduction in the functional responsiveness of hypothalamic 5-HT₁A receptors, which suggests that desensitization did occur. Therefore, the mechanism responsible for paroxetine-induced desensitization could occur downstream from the receptor G protein level.

Although the effects of paroxetine and fluoxetine on G protein levels are similar, they are not identical. Overall, paroxetine produces a greater and earlier reduction in the levels of G₁ and G₆ proteins, especially the levels of G₁₁ and G₁₂ proteins. For example, paroxetine reduced the levels of G₁₁ proteins in the hypothalamus after 3 daily injections, whereas fluoxetine did not significantly reduce the hypothalamic levels of G₁₁ protein until 7 daily injections (Li et al., 1996b). Also, fluoxetine did not significantly alter the frontal cortical levels of G₁₁ and G₁₂ proteins, but paroxetine pro-

**Fig. 6.** Paroxetine reduces the levels of G proteins in the hypothalamus. The data represent mean ± S.E.M. of 6 to 8 rats per group. A) Levels of G₁₁ proteins. One-way ANOVA: F(4, 24) = 3.7267, P < .05. B) Levels of G₁₂ proteins. One-way ANOVA: F(4, 25) = 1.892. C) Levels of G₃ proteins. One-way ANOVA: F(4, 27) = 2.6918, P < .05; D) Levels of G₆ proteins. One-way ANOVA: F(4, 28) = 3.3027, P < .05. * Significant difference from the vehicle group, P < .05 (Newman-Keuls multiple-range test).
Repeated injections of paroxetine significantly decreased the levels of Gi2 protein in the midbrain. The reduction in the levels of Gi2 proteins appeared after 1 day and remained during 14 daily injections of paroxetine. Paroxetine reduced the levels of Go proteins after 3 daily injections, and this reduction was maintained after 7 daily injections. These changes are similar to the results observed with fluoxetine injections (Li et al., 1996b). The levels of Go proteins returned to normal after 14 daily injections of paroxetine. This is in contrast with fluoxetine-induced reduction of Go proteins, which remained reduced during the 22 daily injections. Desensitization of somatodendritic 5-HT1A autoreceptors in the dorsal raphe nucleus has been detected after 3 to 14 daily injections of fluoxetine or paroxetine (Le Poul et al., 1995). However, no data are available on the effect of 1 daily injection of 5-HT uptake inhibitors on somatodendritic 5-HT1A autoreceptors. Therefore, it is difficult to determine which G proteins are involved in the desensitization of somatodendritic 5-HT1A autoreceptors in the raphe nuclei.

In conclusion, the results of the present study suggest that repeated injections of paroxetine produce a delayed and gradual desensitization of hypothalamic 5-HT1A receptor systems. This desensitization is similar to that induced by fluoxetine, which suggests that sustained blockade of 5-HT uptake sites mediates this effect. Because the density of 5-HT1A receptors in hypothalamic nuclei was not altered, it is unlikely that the desensitization of hypothalamic 5-HT1A receptors is due to their down-regulation. Similarities in the time course of the decrease in both Gi2 proteins and hormone responses to 8-OH-DPAT suggests that Gi3 proteins may be involved in the desensitization of 5-HT1A receptors.

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References
BAGDY, G. AND MAKARA, G. B.: Hypothalamic paraventricular nucleus lesions...


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