Fluoxetine-induced changes in body weight and 5-HT\textsubscript{1A} receptor-mediated hormone secretion in rats on a tryptophan-deficient diet

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D’Souza, D. N., Y. Zhang, F. Garcia, G. Battaglia, and L. D. Van de Kar. Fluoxetine-induced changes in body weight and 5-HT\textsubscript{1A} receptor-mediated hormone secretion in rats on a tryptophan-deficient diet. Am J Physiol Regul Integr Comp Physiol 286: R390–R397, 2004.—Tryptophan-depleting protocols are commonly used to study the role of serotonin in mood disorders. The present study examined the impact of a tryptophan-deficient diet and fluoxetine on the serotonergic regulation of neuroendocrine function and body weight. We hypothesized that the regulation of postsynaptic 5-HT\textsubscript{1A} receptors is dependent on the levels of 5-HT in the synapse. Rats on a control or a tryptophan-deficient diet received daily injections of saline or fluoxetine (5 or 10 mg kg\textsuperscript{-1}day\textsuperscript{-1} ip) from day 7 to day 21. The tryptophan-deficient diet produced a 41% reduction in the level of 5-HT but no change in the density of \textsuperscript{3}Hparoxetine-labeled 5-HT transporters. Treatment with fluoxetine increased the gain in weight in rats maintained on the control diet. The tryptophan-deficient diet produced a significant loss in body weight that was not significantly altered by treatment with fluoxetine. Treatment with fluoxetine produced a dose-dependent desensitization of hormone responses to injection of the 5-HT\textsubscript{1A} receptor agonist (±)8-hydroxy-2-(di-n-propylamino)tetralin ((±)8-OH-DPAT). The tryptophan-deficient diet produced an increase in the basal levels of corticosterone but did not alter the basal levels of ACTH or oxytocin. Also, this diet inhibited the magnitude of 8-OH-DPAT-induced increase in plasma levels of ACTH and oxytocin but did not impair the ability of fluoxetine to desensitize the 5-HT\textsubscript{1A} receptor-mediated increase in plasma hormones. These data suggest that a reserve of 5-HT enables fluoxetine to desensitize postsynaptic 5-HT\textsubscript{1A} receptors in the hypothalamus. In conclusion, the profound physiological changes induced by tryptophan depletion may complicate the interpretation of studies using this experimental approach.

SEROTONERGIC NEURONS play important roles in the regulation of food intake and neuroendocrine function (4, 11, 13, 25, 39, 57, 66). The present study used dietary and pharmacological approaches to examine the role of serotonin (5-HT) and 5-HT\textsubscript{1A} receptors in the regulation of body weight and neuroendocrine function.

Dysfunction of serotonergic neurotransmission is considered an underlying cause of mood and other neuropsychiatric disorders (9, 21, 44). Serotonin synthesis depends on the concentration of tryptophan, and a decrease in tryptophan can result in a significant inhibition of 5-HT synthesis and release (48, 49, 72). Studies in humans employing tryptophan depletion have increased dramatically in the past few years (from 2 in 1990 to 47 in 2002). These studies were accomplished by either giving patients a drink that lacks the essential amino acid l-tryptophan or by keeping them on a tryptophan-deficient diet or a combination of both. Tryptophan-deficient diets lead to a moderate reduction in the release of 5-HT from nerve terminals in the brain and might serve as an animal model of serotonin-associated disorders (26, 27). Additionally, tryptophan-deficient diets and tryptophan-depleting drinks have been tested in humans and were found to produce changes in sleep and mood, including a short-term reversal of the antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) (2, 8, 17, 46, 58, 77). Despite the recent popularity of tryptophan depletion as an experimental approach, there are gaps in our understanding of the physiological changes accompanying this protocol. Hence, the present study examined how feeding rats a tryptophan-deficient diet can affect neuroendocrine function and the maintenance of body weight.

Serotonergic neurons that innervate the hypothalamic paraventricular nucleus stimulate hypothalamic neurons to increase the secretion of several hormones, notably the hypothalamic pituitary adrenal axis (ACTH and corticosterone/cortisol) and oxytocin (3, 38, 56, 65, 78). A direct injection of the 5-HT\textsubscript{1A} receptor agonist (±)8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) into the hypothalamic paraventricular nucleus increases plasma levels of ACTH and corticosterone (50, 73). These and other observations promoted neuroendocrine challenge tests measuring plasma hormones as potential diagnostic peripheral markers of the function of hypothalamic 5-HT\textsubscript{1A} receptors in psychiatric patients (20, 51, 76).

Fluoxetine and other SSRIs are very effective in treating eating disorders, depression, and various other mood disorders such as obsessive-compulsive disorder, premenstrual syndrome, and anxiety (28, 35, 37, 62, 63, 71, 75). While SSRIs have become exceptionally popular, their mechanisms of action are still unclear. Several studies suggest that the gradual improvement seen during SSRI therapy may be related to neuroadaptive changes leading to a gradual desensitization of postsynaptic 5-HT\textsubscript{1A} receptors (10, 42, 43, 53, 68). One objective of the present study was to use a dietary manipulation of serotonin levels to examine the hypothesis that the maintenance of high levels of serotonin in the synaptic gap is a prerequisite for the long-term neuroadaptive effects of fluoxetine.

Unlike tricyclic antidepressants, SSRIs reduce body weight gain both in humans (15, 52) and in adult rats (79). However, the contribution of brain vs. peripheral serotonergic mechanisms to the effects of fluoxetine on body weight is unclear.

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While serotonergic mechanisms in the hypothalamus play a central role in the regulation of feeding (11, 18), serotonergic systems also play a primary role in the gastrointestinal system (31, 54). In a previous study in rats (23), we found that destruction of serotonergic nerve terminals in the brain with the serotonin neurotoxin 5,7-dihydroxytryptamine reduces body weight and potentiates the ability of fluoxetine to inhibit body weight gain. These effects on body weight could be mediated either by direct actions of fluoxetine on postsynaptic receptors or by effects on peripheral serotonergic mechanisms, since the serotonergic nerve terminals were destroyed only in the brain (by intracerebroventricular injection of 5,7-dihydroxytryptamine) whereas 5-HT stores in the periphery remained intact. The tryptophan-deficient diet affects peripheral and central serotonin stores to the same extent (1, 27, 29). Thus the present study examined body weight gain to investigate the contribution of brain and peripheral serotonergic mechanisms to the impact of SSRIs on body weight gain.

Destruction of serotonergic nerve terminals (>90%) by intracerebroventricular injection of 5,7-dihydroxytryptamine completely blocks the ability of fluoxetine to produce a desensitization of the neuroendocrine responses to (±)-8-OH-DPAT (23). This observation indicates that the ability of fluoxetine to induce a desensitization of hypothalamic postsynaptic 5-HT1A receptors depends on the integrity of serotonergic nerve terminals and is likely mediated by a gradual increase in the levels of 5-HT in the synaptic cleft, leading to overactivation of postsynaptic receptors. The tryptophan-deficient diet does not affect the integrity of serotonergic nerve terminals but reduces the stores of serotonin. Therefore, this diet provides a tool to determine whether a moderate reduction of 5-HT stores, compared with complete destruction of serotonergic nerve terminals, can influence the SSRI-induced desensitization of postsynaptic 5-HT1A receptors in the hypothalamus.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (225–250 g) were purchased from Harlan Laboratories (Indianapolis, IN). The rats were housed two per cage in a room controlled for temperature, humidity, and lighting (12:12-h light-dark cycle; lights on 7 AM to 7 PM). Food and water were available ad libitum. 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. All drug solutions were made immediately before injections. (±)-8-OH-DPAT was purchased from Research Biochemicals (Natick, MA), dissolved in saline, and injected in a dose of 50 μg/kg sc in a volume of 1 ml/kg. Fluoxetine HCl was donated by Eli Lilly (Indianapolis, IN). A fresh fluoxetine solution was prepared daily by dissolving fluoxetine in the vehicle (0.9% saline). Fluoxetine was injected (intraperitoneally) in a volume of 2 ml/kg.

Diet. The L-Amino Acid-Defined Rodent Diets with or without L-tryptophan were purchased from Dyets (Bethlehem, PA). Rats were given an L-tryptophan-deficient diet (no. 510056) or a control diet (no. 510025). The control diet had the following composition (for 1,000 g): L-alanine, 5 g; L-aspartic acid H2O, 5 g; L-cysteine, 4 g; L-glutamic acid, 30 g; L-glutamine, 5 g; glycine, 20 g; L-histidine (free base), 6 g; L-isoleucine, 8 g; L-leucine, 12 g; L-lysine HCl, 14 g; L-methionine, 6 g; L-phenylalanine, 8 g; L-proline, 5 g; L-serine, 5 g; L-threonine, 8 g; L-tryptophan, 2 g; L-tyrosine, 4 g; L-valine, 8 g; corn starch, 419.086 g; dextrose, 140 g; sucrose 100 g; soybean oil (stabilized with TBHQ), 70.014 g; cellulose (microcrystalline), 50 g; salt mix (no. 210030), 35 g; vitamin mix (no. 310025), 10 g; choline bitartrate, 2.5 g; and sodium bicarbonate, 6.4 g. The L-tryptophan-deficient diet had an identical composition, except that it contained no L-tryptophan and 31.44 g of L-glutamic acid (instead of 30 g). The animals were started on the diet 7 days before the commencement of the fluoxetine injections, and this diet regimen was maintained throughout the treatment period until the day of death.

Experimental procedure. Cage mates were assigned to the same treatment group. The rats received daily injections of saline or fluoxetine (5 or 10 mg/kg ip) for 14 days. Eighteen hours after the last injection, the rats received a challenge injection of (±)-8-OH-DPAT (50 μg/kg sc) or saline and were decapitated 15 min after the injection. Trunk blood was collected in centrifuge tubes containing 0.5 ml of a 0.3M EDTA (pH 7.4) solution. Plasma was stored at −70°C until assayed for plasma hormone levels, and brains were immediately removed. The frontal cortex and hypothalamus were dissected and saved at −70°C for subsequent HPLC assays to ascertain the levels of 5-HT and 5-hydroxyindole acetic acid (HIAA) and to determine the density of the 5-HT transporters using radioligand binding assays, respectively.

Radioimmunoassay for plasma hormone concentrations. Plasma ACTH, corticosterone, and oxytocin concentrations were measured by radioimmunoassays as described in detail in our previous reports (41, 42). Radioimmunoassays for each hormone were conducted on all the samples at one time to avoid interassay variability.

Radioisogand binding assays for 5-HT uptake sites in the hypothalamus. Hypothalamic homogenates from rats that were maintained on the tryptophan-deficient or the tryptophan control diet and that received daily saline injections were prepared as follows: frozen tissues were placed in 30 vol of ice-cold 50 mM Tris-HCl (pH 7.7, 25°C) buffer and homogenized using a Tekmar Tissumizer (2 × 5 s). The homogenates were centrifuged at 37,000 g for 15 min at 4°C. Tissues were then washed and centrifuged once more. Finally, the pellets were resuspended to a concentration of 30 mg wet wt/ml in cold 50 mM Tris-HCl (pH 7.7) (40). The protein concentration of the homogenates was measured using the BCA protein assay kit (purchased from Pierce, Rockford, IL) and bovine serum albumin (BSA) as a standard. [3H]Paroxetine (20.3 Ci/mmol, DuPont NEN, Boston, MA) was incubated for 120 min at room temperature with hypothalamic homogenates, at a final concentration of 0.4 nM in 5 ml total volume of a buffer containing 50 mM Tris-HCl, 120 mM NaCl, and 5 mM KCl (pH = 7.7) as described in a previous report (6). The reaction was stopped by immediate filtration over polyethyleneimine (PEI)-treated Whatman GF/C filters, washed three times with 5 ml of a 50 mM Tris buffer (pH 7.7), and the radioactivity of the filters was counted in 5 ml of scintillation liquid at 5°C.

HPLC determination of concentration of 5-HT, 5-HIAA, and dopamine in the cortex. The cortex was dissected and measured of 5-HT, 5-HIAA, and dopamine was carried out by an adaptation of the method of Ref. 74. Briefly, the mobile phase was a 0.1 M sodium acetate, 0.1 M citric acid, 0.55 M Na octane sulfonic acid, 0.15 mM EDTA, and 1 mM di-N-butylamine buffer, pH 3.7, containing 10% methanol, filtered, and degassed under vacuum. The stationary phase was equipped with a Waters Resolve 5-μm spherical C18 column (3.9 × 300 mm). The flow rate was set at 0.8 ml/min. Electrochemical detection was achieved using a Waters 460 electrochemical detector. The oxidation potential was set at +0.77 V. Raw data were collected using the Waters Millennium acquisition software, and the amount of 5-HT, 5-HIAA and dopamine was quantified by comparison of peak heights in samples relative to standards.

Statistical analyses. All the data are expressed as the group means ± SE. The body weight data were analyzed using a three-way ANOVA with repeated measures. Hormone data were analyzed by a 3-way ANOVA. Group means were compared by Newman-Keuls multiple range test (61). For levels of 5-HT, 5-HIAA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and dopamine as well as [3H]paroxetine binding, the analysis was performed using
RESULTS

Effect of fluoxetine on body weight. Rats placed on a tryptophan-deficient diet had a sustained and significant reduction in body weight compared with the control diet group throughout the 3 wk of the experiment (Fig. 1). Treatment with fluoxetine produced a dose-dependent reduction in body weight, both in rats maintained on the tryptophan-deficient diet and in rats maintained on the tryptophan control diet (Fig. 1).

The statistical analysis revealed significant main effects of the tryptophan-deficient diet \([F(1,1511) = 3910.59; \ P < 0.0001]\), fluoxetine \([F(2,1511) = 33.21; \ P < 0.0001]\), and duration of injections \([F(7,1511) = 214.92; \ P < 0.0001]\). There was a significant interaction between tryptophan-deficient diet and duration of injections \([F(7,1511) = 5953.85; \ P < 0.0001]\) as well as between fluoxetine and duration of injections \([F(14,1511) = 114.57; \ P < 0.0001]\). There was also a significant interaction among the tryptophan-deficient diet, fluoxetine, and duration of injections \([F(14,1511) = 50.88; \ P < 0.0001]\).

A post hoc Newman Keuls test revealed that for the groups maintained on the tryptophan control diet on day 21, the rats that received fluoxetine (10 mg kg\(^{-1}\) day\(^{-1}\)) had a significantly lower body weight than the group that received saline injections. However, no significant difference was observed in rats that were maintained on the tryptophan-deficient diet between rats that were treated with fluoxetine and rats that received daily injections of saline.

Neurochemical effects of the tryptophan-deficient diet. The tryptophan-deficient diet produced a significant reduction in 5-HT (41%, \(P < 0.01\)) and 5-HIAA (66%, \(P < 0.01\)) levels in the cortex but did not significantly alter the levels of dopamine, DOPAC, or HVA (Table 1). This tryptophan-deficient diet did not produce a reduction in the density of \(^{[3]}\)Hparoxetine-labeled 5-HT transporters in the hypothalamus (Table 2), suggesting that the depletion of 5-HT was not accompanied by destruction of serotonergic nerve terminals and/or downregulation of serotonin transporters.

![Fig. 1. Effect of the tryptophan-deficient (Tryp def) diet and fluoxetine on body weight. Arrow shows the 1st day of fluoxetine treatment (day 7 on the diet). Data represent means ± SE of 16–18 rats per group. *Significant effect of fluoxetine, \(P < 0.01\) (2-way ANOVA and Newman Keuls test). 3F5x and 10F5x, 5 or 10 mg kg\(^{-1}\) day\(^{-1}\) ip fluoxetine; Sal, saline.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00414.2003)

<table>
<thead>
<tr>
<th>Diet</th>
<th>(^{[3]})HParoxetine Binding, fmol/mg protein</th>
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</thead>
<tbody>
<tr>
<td>Control tryptophan</td>
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<tr>
<td>Tryptophan deficient</td>
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Data represent means ± SE. The rats were killed 3 wk after the diet was started.

Table 1. Effect of the tryptophan-deficient diet on the levels of 5-HT, 5-HIAA, dopamine, DOPAC, and HVA in the frontal cortex

<table>
<thead>
<tr>
<th>Diet</th>
<th>fmol/µg protein</th>
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<tbody>
<tr>
<td>Control-Tryptophan</td>
<td></td>
</tr>
<tr>
<td>Tryptophan-Deficient</td>
<td></td>
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<tr>
<td>%Decrease</td>
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<tr>
<td>5-HT</td>
<td>57.8±6.6</td>
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<tr>
<td>5-HIAA</td>
<td>73.1±3.2</td>
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<tr>
<td>Dopamine</td>
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</tr>
<tr>
<td>DOPAC</td>
<td>2.4±0.4</td>
</tr>
<tr>
<td>HVA</td>
<td>3.2±0.4</td>
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</table>

Data represent means ± SE of 6 rats challenged with saline per control-tryptophan group and tryptophan-deficient group. The rats were killed 3 wk after the diet was started. *Significant difference from control-tryptophan diet group, \(P < 0.01\) (Student’s \(t\)-test). 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindole acetic acid; HVA, homovanillic acid; DOPAC, dihydroxyphenylacetic acid.

Hormone responses to a challenge with a 5-HT\(_{1A}\) receptor agonist. Basal levels of oxytocin (in rats challenged with a saline injection) were not altered by fluoxetine treatment or the tryptophan-deficient diet. Administration of \((±)\)-8-OH-DPAT (50 µg/kg sc) to rats maintained on a control diet produced a significant increase in plasma levels of oxytocin (1,128%) compared with saline-challenged rats (Fig. 2). Daily injections of 5 mg/kg fluoxetine, for 14 days, produced a reduction (−76%) in the \((±)\)-8-OH-DPAT-induced oxytocin response (Fig. 2). Daily injections of 10 mg/kg fluoxetine induced an even greater reduction (−93%) in the \((±)\)-8-OH-DPAT-induced oxytocin response. The tryptophan-deficient diet significantly reduced (60%) the oxytocin response to \((±)\)-8-OH-DPAT compared with rats given the control tryptophan diet. Yet, treatment with fluoxetine produced a dose-dependent desensitization of the oxytocin response to \((±)\)-8-OH-DPAT in rats that were given the tryptophan-deficient diet. Daily injections of 5 mg/kg fluoxetine, for 14 days, produced a reduction of (−59%) in the \((±)\)-8-OH-DPAT-induced oxytocin response (Fig. 2). Daily injections of 10 mg/kg fluoxetine induced an even greater reduction (−80%) in the \((±)\)-8-OH-DPAT-induced oxytocin response. The three-way ANOVA indicated that there were significant main effects of the tryptophan-deficient diet \([F(1,68) = 18.25; \ P < 0.0001]\), fluoxetine \([F(2,68) = 54.70; \ P < 0.0001]\), and \((±)\)-8-OH-DPAT \([F(1,68) = 87.20; \ P < 0.0001]\). There was a significant interaction between the tryptophan-deficient diet and fluoxetine \([F(2,68) = 12.70; \ P < 0.0001]\), the tryptophan-deficient diet and \((±)\)-8-OH-DPAT \([F(1,68) = 17.78; \ P < 0.0001]\), and fluoxetine and \((±)\)-8-OH-DPAT \([F(2,68) = 49.71; \ P < 0.0001]\). There was a significant interaction among the trypto-
Fig. 2. Effect of tryptophan-deficient diet on fluoxetine-induced desensitization of 5-HT₁₆ receptor-mediated increase in plasma oxytocin levels. Data represent means ± SE of 8–10 rats per group. *Significant difference compared with the saline [0 dose of (±)-8-hydroxy-2-(di-n-propylamino)tetralin ((±)-8-OH-DPAT)] group, $P < 0.05$. **Significant difference compared with the saline [0 dose of (±)-8-OH-DPAT] group, $P < 0.01$. #Significant difference compared with saline [0 dose of fluoxetine] group, $P < 0.05$. ##Significant difference compared with saline [0 dose of fluoxetine] group, $P < 0.01$ (3-way ANOVA and Newman-Keuls multiple range test). Fluo-5 and Fluo-10, 5 or 10 mg/kg –1 day–1 ip fluoxetine.

Fig. 3. Effect of tryptophan-deficient diet on fluoxetine-induced desensitization of 5-HT₁₆ receptor-mediated increase in plasma ACTH (A) and corticosterone (B) levels. Data represent means ± SE of 8–10 rats per group. *Significant difference compared with the saline [0 dose of (±)-8-OH-DPAT] group, $P < 0.05$. **Significant difference compared with the saline [0 dose of (±)-8-OH-DPAT] group, $P < 0.01$. #Significant difference compared with saline [0 dose of fluoxetine] group, $P < 0.05$. ##Significant difference compared with saline [0 dose of fluoxetine] group, $P < 0.01$. + Significant difference compared with the control diet group, $P < 0.05$ (3-way ANOVA and Newman-Keuls multiple range test).

Basal levels of ACTH (in rats challenged with a saline injection) were not altered by either daily injections of fluoxetine or by the tryptophan-deficient diet (Fig. 3A). Administration of (±)-8-OH-DPAT (50 μg/kg sc) to rats maintained on a control tryptophan diet produced a significant increase in plasma levels of ACTH (624%) compared with saline-challenged rats (Fig. 3A). Daily injections of 5 mg/kg fluoxetine, for 14 days, produced a significant reduction in the (±)-8-OH-DPAT-induced increase in ACTH levels (−62%). Daily injections of 10 mg/kg fluoxetine induced an even greater reduction (−87%) in the (±)-8-OH-DPAT-induced ACTH response. In contrast with rats given a control-tryptophan diet, injection of (±)-8-OH-DPAT to rats maintained on a tryptophan-deficient diet produced a much smaller increase (194%) in plasma ACTH that was not statistically significant. The three-way ANOVA for ACTH indicated that there were significant main effects of the tryptophan-deficient diet [$F(1,73) = 22.95; P < 0.0001$], fluoxetine [$F(2,73) = 31.99; P < 0.0001$], and (±)-8-OH-DPAT [$F(1,73) = 44.68; P < 0.0001$]. There was a significant interaction between the tryptophan-deficient diet and fluoxetine [$F(2,73) = 8.09; P < 0.001$], the tryptophan-deficient diet and (±)-8-OH-DPAT [$F(1,73) = 22.99; P < 0.0001$], and fluoxetine and (±)-8-OH-DPAT [$F(2,73) = 19.90; P < 0.0001$]. There was a significant interaction among the tryptophan-deficient diet, (±)-8-OH-DPAT, and fluoxetine [$F(2,73) = 9.02; P < 0.0005$].

Basal levels of corticosterone (in rats challenged with a saline injection) were not altered by treatment with fluoxetine but were increased in rats maintained on the tryptophan-deficient diet (Fig. 3B) compared with the control diet. Administration of (±)-8-OH-DPAT (50 μg/kg sc) to saline-treated rats produced a significant increase in plasma levels of corticosterone (663%) compared with saline-challenged rats (Fig. 3B). Daily injections of 5 mg/kg fluoxetine, for 14 days, produced a reduction (−32%) in the (±)-8-OH-DPAT-induced corticosterone response. Daily injections of 10 mg/kg fluoxetine induced an even greater reduction (−56%) in the (±)-8-OH-DPAT-induced corticosterone response. In rats given a tryptophan-deficient diet, injection of 5 mg/kg fluoxetine and 10 mg/kg of fluoxetine produced 24 and 42% reduction in the (±)-8-OH-DPAT-induced corticosterone response, respectively. However, this effect was not statistically significant. The three-way ANOVA indicated that there were significant main effects of the tryptophan-deficient diet [$F(1,71) = 11.52; P < 0.001$], fluoxetine [$F(2,71) = 5.9; P < 0.01$], and (±)-8-OH-DPAT [$F(1,71) = 23.87; P < 0.0001$]. There was a significant interaction between the tryptophan-deficient diet and (±)-8-OH-DPAT [$F(1,71) = 14.53; P < 0.005$] and fluoxetine and (±)-8-OH-DPAT [$F(2,71) = 5.98; P < 0.05$]. However, the interaction between the tryptophan-deficient diet and fluoxetine was not statistically significant. There was no significant interaction among the tryptophan-deficient diet, (±)-8-OH-DPAT, and fluoxetine.
DISCUSSION

Treatment with SSRIs produces a gradual desensitization of postsynaptic 5-HT$_{1A}$ receptors. The main observations of the study are that placing rats on a tryptophan-deficient diet, while producing a modest reduction in serotonin stores, results in profound changes in the regulation of body weight and neuroendocrine function. Therefore, it is doubtful that the data in the psychiatric literature, which rely on modification of tryptophan in the diet, are reflective only of changes in serotonin stores in the brain. Nevertheless, the modest reduction in serotonin stores does not prevent the neuroadaptive effects of SSRIs on postsynaptic 5-HT$_{1A}$ receptors.

Mood disorders have long been regarded as a manifestation of lowered serotonergic function (34). Therefore, dietary manipulation of serotonin stores could be considered a model of some of the mechanisms underlying mood disorders (69). We used the tryptophan-deficient diet protocol, which exerts its effects by depleting the precursor of 5-HT, i.e., tryptophan, and thus lowers 5-HT stores (45). A study using a continuous cerebrospinal fluid sampling in healthy human subjects after tryptophan depletion demonstrated that cerebrospinal fluid levels of the 5-HT metabolite 5-HIAA were decreased to a similar extent as seen in the present study (12). The tryptophan depletion paradigm was thus considered an important tool in studying the role of serotonin in the therapeutic effects of SSRIs and other antidepressants (7, 30, 46, 60). However, the results of the present study suggest that the tryptophan depletion protocol produces a complex of physiological changes and thus cannot be considered a simple model of depleting serotonin stores.

The reduction of body weight in rats maintained on the tryptophan-deficient diet was expected in light of the fact that destruction of serotonergic neurons also reduces body weight (23). Both diets had an identical composition except for the amount of l-tryptophan and glutamate. Because the tryptophan-deficient diet had a significant effect on body weight, it could mean that tryptophan in the food is an important component needed to maintain body weight. Serotonergic nerve terminals in the hypothalamus also play a central role in the regulation of feeding behavior (11). As tryptophan is the essential precursor in the synthesis of 5-HT, the data also could suggest that the reduction in body weight is due to reduced levels of serotonin. Glucocorticoids increase feeding and reduce energy expenditures (33). Hence, the rats maintained on the tryptophan-deficient diet should not have lost body weight as they had high basal levels of corticosterone. However, evidence suggests that serotonergic mechanisms mediate the effects of glucocorticoids on energy balance (33). Thus the reduction in body weight in rats maintained on a tryptophan-deficient diet could be mediated through both neural and endocrine mechanisms.

The measurement of the density of [3H]paroxetine-labeled 5-HT transporters served to determine whether the integrity of serotonergic nerve terminals was affected by the tryptophan-deficient diet. In our previous study (23), destruction of serotonergic neurons by intracerebroventricular injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine produced a 95% reduction in the density of [3H]paroxetine-labeled 5-HT transporters, indicating a significant disruption in the integrity of serotonergic nerve terminals. However, in the present study, the tryptophan-deficient diet produced no loss of 5-HT transporters, suggesting that the serotonergic nerve terminals were not destroyed by the diet. Because 5-HT transporters are the target of fluoxetine, the tryptophan-deficient diet still retained the substrate for fluoxetine.

The 5-HT$_{1A}$ receptor agonist (±)8-OH-DPAT activates 5-HT$_{1A}$ receptors in the hypothalamic paraventricular nucleus to increase the secretion of hormones such as oxytocin, ACTH, and corticosterone (3, 32, 70). This effect can be inhibited by 5-HT$_{1A}$ receptor antagonists and by destruction of the paraventricular hypothalamic nucleus (16, 70). Hence neuroendocrine challenge tests are a useful tool in assessing the function of hypothalamic 5-HT$_{1A}$ receptors. Although the magnitude of increases in plasma levels of ACTH and oxytocin after injection of 8-OH-DPAT were largely interpreted as indications of changes in 5-HT$_{1A}$ receptor signaling, other interpretations related to changes in the regulatory mechanisms governing the secretion of these hormones must be kept in mind.

Placing rats on a tryptophan-deficient diet resulted in inhibition of the effect of (±)8-OH-DPAT on ACTH release. This observation contrasts with our previous study (23) in which destruction of serotonergic neurons, by an intracerebroventricular injection of 5,7-dihydroxytryptamine, did not reduce the ACTH response to (±)8-OH-DPAT. The tryptophan-deficient diet also produced a significant increase in the basal levels of ACTH, corticosterone, which could exert feedback inhibition of the hypothalamic pituitary adrenal axis, explaining the difference between these two studies. Another possibility is that the 5-HT$_{1A}$ receptors have become less sensitive as a result of increased plasma levels of corticosterone. Glucocorticoids can induce a desensitization of 5-HT$_{1A}$ receptors in several brain regions (5, 22, 64). Thus the reduced ACTH response to 8-OH-DPAT might represent a desensitization induced by elevated plasma glucocorticoid levels. An alternative possibility is that the effects of the tryptophan-deficient diet are unrelated to the serotonergic system. Tryptophan is a critical component of ACTH and other melanocortin related peptides, and it plays a pivotal role in the ability of these peptides to interact with their (melanocortin) receptors (36). Hence, a reduction in tryptophan might affect the hypothalamic-pituitary-adrenal axis regardless of the serotonergic mechanisms affected by this tryptophan-deficient diet. Consistent with this possibility, depletion of serotonin with the synthesis inhibitor p-chloro-phenylalanine (which would not affect the composition of tryptophan in the amino acid sequence of ACTH or melanocortin receptors) does not alter the activity of the hypothalamic-pituitary-adrenal axis during exposure to immune-related stimuli (19).

The results for oxytocin are consistent with previous studies (67), in which destruction of serotonergic neurons using 5,7-dihydroxytryptamine produced an inhibition of the oxytocin response to (±)8-OH-DPAT. Depletion of serotonin in the brain can reduce the oxytocin response to the milk ejection reflex (47) and inhibits the increase in mRNA encoding oxytocin during osmotic stimulation (14). Destruction of serotonergic neurons also reduces the levels of oxytocin in the neural lobe of the pituitary gland (55). Thus the reduced oxytocin response to (±)8-OH-DPAT might be due to reduced stores of oxytocin (55). Serotonergic neurons are probably necessary to maintain the proper functioning of oxytocin neurons. This may
explain the reduction in the oxytocin response to (±)8-OH-DPAT after the tryptophan-deficient diet.

We also performed this experiment to determine whether a moderate reduction of serotonin stores could alter the neuroadaptive effects of SSRIs. The 5-HT reuptake system is the primary mechanism terminating the activation of postsynaptic serotonin receptors by synaptic 5-HT. Consequently, blockade of the 5-HT reuptake system leads to gradual increases in the levels of 5-HT in the synaptic cleft (24, 59). Patients suffering from mood disorders have deficits in brain serotonergic function. It is unclear whether the deficits in brain serotonin could become severe enough to prevent SSRIs from exerting their therapeutic effects. The tryptophan-deficient diet might provide a rat model to answer this question. The results of the present study demonstrate that a moderate 40% reduction in the levels of 5-HT in the brain does not compromise the ability of fluoxetine to desensitize postsynaptic 5-HT1A receptors in the hypothalamus. This observation is contrasted by a previous study (23) demonstrating that destruction of serotonergic nerve terminals blocks the fluoxetine-induced desensitization of neuroendocrine responses to a 5-HT1A receptor agonist, suggesting that the ability of fluoxetine to produce a desensitization of hypothalamic postsynaptic 5-HT1A receptor systems is critically dependent on the integrity of serotonergic nerve terminals. The current study demonstrates that when the nerve terminals are intact and the reduction of serotonin stores is incomplete, fluoxetine still retains its ability to desensitize postsynaptic 5-HT1A receptors. Thus a substantial reserve may exist in serotonergic nerve terminals enabling them to maintain serotonergic neurotransmission. This interpretation is consistent with the fact that SSRIs are effective in treating mood disorders that are associated with a deficit in serotonin.

In conclusion, the results of the present study suggest that a tryptophan-deficient diet produces complex physiological changes that might limit the interpretation of studies based on this experimental approach. Also, a moderate reduction of serotonin stores does not prevent fluoxetine from desensitizing postsynaptic 5-HT1A receptors in the hypothalamus, suggesting that SSRIs are capable of producing neuroadaptive changes in postsynaptic receptors in the presence of a moderate reduction of 5-HT stores.

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REFERENCES


