Sustained Administration of Trazodone Enhances Serotonergic Neurotransmission: In Vivo Electrophysiological Study in the Rat Brain

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ABSTRACT

Despite its clinical use for more than two decades, the mechanisms by which trazodone acts as an antidepressant are not clear, because it has affinity for a variety of 5-hydroxytryptamine (5-HT; serotonin) receptors and the 5-HT transporter. This study examined the effects of sustained trazodone administration on 5-HT neurotransmission. Electrophysiological recordings were conducted in anesthetized rats. Subcutaneously implanted minipumps delivered vehicle or trazodone (10 mg/kg/day) for 2 and 14 days. A 2-day trazodone administration suppressed the firing rate of raphe 5-HT neurons, which recovered to baseline after 14 days. This was attributable to 5-HT1A autoreceptor desensitization because the suppressive effect of the 5-HT autoreceptor agonist lysergic acid diethylamide was dampened in 14-day trazodone-treated rats. Prolonged trazodone administration did not change the sensitivity of postsynaptic 5-HT1A and α2-adrenergic receptors in hippocampus, but enhanced synaptic 5-HT levels because the 5-HT1A antagonist N-(2-[4-(2-methoxyphenyl)-1-piperazinyl(ethyl)]-N-(2-pyridyl)) cyclohexanecarboxamide trihydrochloride (WAY-100635) enhanced hippocampal firing in treated rats, but not in controls. Trazodone administration for 14 days increased the 50% recovery time value, an index of 5-HT transporter blockade in vivo, and decreased the inhibitory function of terminal 5-HT1B autoreceptors on the electrically evoked release of 5-HT. The agonistic action of trazodone at 5-HT1A receptors was characterized as being full because it did not attenuate the inhibitory action of 5-HT when coapplied locally. The enhanced 5-HT neurotransmission by trazodone is caused in part by reuptake blockade and activation of postsynaptic 5-HT1A receptors, which may account for its effectiveness in major depression.

Introduction

Since its introduction in the 1980s, trazodone hydrochloride, a triazolopyridine derivative, has been used in the treatment of major depression (Cunningham et al., 1994) and as a bedtime sedative. The structure of trazodone, which is unrelated to those of other major classes of antidepressants, results in a low toxicity profile (Al-Yassiri et al., 1981). Its mechanism of action in the treatment of depression has not been fully elucidated, largely in part to its affinity for a variety of receptors that may contribute to its clinical actions (Table 1). Although the antidepressant action of trazodone is partially attributed to the blockade of 5-hydroxytryptamine (5-HT; serotonin) reuptake sites, its potency to inhibit 5-HT transporters (5-HTTs) is considerably less than that of selective serotonin reuptake inhibitors (SSRIs) such as citalopram and fluoxetine (Owens et al., 1997). The action of SSRIs on 5-HT neurotransmission has been established over the years. Short-term administration of SSRIs attenuates the firing rate of dorsal raphe (DR) 5-HT neurons, due to overactivation of somatodendritic 5-HT1A autoreceptors because SSRIs

| ABBREVIATIONS: | 5-HT, 5-hydroxytryptamine (serotonin); 5-HTT, 5-HT transporter; SSRI, selective serotonin reuptake inhibitor; WAY-100635, N-(2-[4-(2-methoxyphenyl)-1-piperazinyl(ethyl)]-N-(2-pyridyl)) cyclohexanecarboxamide trihydrochloride; LSD, lysergic acid diethylamide; 6-OHDA, 6-hydroxy-2-(di-n-propylamino)tetralin; DR, dorsal raphe; DA, dopamine; mCPP, meta-chlorophenylpiperazine; NE, norepinephrine; nACh, nicotinic cholinergic receptors; RT50, 50% recovery time; SIL, silence; LC, liquid chromatography; BAY 12-3702, R-(+-)-2-[4-(2-[(chroman-2-ylmethyl)-amino]-butyl)-1,1-dioxo-benzoyl]isothiazoline hydrochloride. |
promptly inhibit 5-HT uptake, leading to an enhancement of 5-HT levels in the vicinity of the cell body. Prolonged administration of SSRIs, however, leads to complete recovery of the 5-HT firing rate. Such a phenomenon is attributable to the desensitization of 5-HT1A autoreceptors (see Píñeyro and Blier, 1999). Indeed, desensitization of somatodendritic and terminal autoreceptors, after chronic administration of SSRIs, enhances 5-HT levels in the synaptic cleft (see Píñeyro and Blier, 1999). The gradual adaptive changes of 5-HT neuronal elements, which correspond to the delayed onset of therapeutic action, are perhaps caused by alterations in receptor gene transcription and modifications of G protein coupled to the 5-HT1A autoreceptor (Hensler, 2003).

In addition to its inhibiting action at 5-HTT, trazodone shows moderate potency at blocking 5-HT2A/2C receptors (Table 1), which may contribute to the mechanism of the action of trazodone. Microdialysis studies have consistently shown that 5-HT2C antagonists enhance dopamine (DA) concentration in the prefrontal cortex (Di Matteo et al., 1999; Gobert and Millan, 1999), whereas the effect of 5-HT2A antagonists on DA levels remains controversial (Schmidt and Fadayel, 1995). The active metabolite of trazodone at 5-HT2A receptors may help compensate for a lack of 5-HT2C receptor antagonism of the parent compound. Another interesting feature of trazodone is its moderate affinity for 5-HT1A autoreceptors and its direct activation of normosensitive postsynaptic 5-HT1A receptors in hippocampus (Blier and de Montigny, 1990).

The various 5-HT agonist–antagonist properties of trazodone may, therefore, contribute to the net therapeutic benefits of this antidepressant. The present in vivo electrophysiological experiments were thus undertaken to examine the effects of sustained administration of trazodone on 5-HT neuronal elements in the DR and hippocampus of the rat brain.

### Materials and Methods

#### Experimental Preparations

All of the electrophysiological experiments were carried out in male Sprague-Dawley rats (Charles River, St. Constant, Q.C., Canada), weighing between 250 and 350 g, at the time of the recordings. The animals were kept under standard laboratory conditions (12:12 light/dark cycle with access to food and water ad libitum). Under isoflurane anesthesia, the rats were implanted subcutaneously with an osmotic Alzet minipump (Alza, Palo Alto, CA) to ensure slow and steady release of 5, 10, and 20 mg/kg/day of trazodone hydrochloride or vehicle (2% hydroxy propyl-β-cyclodextrin used to dissolve the drug) for 2 or 14 days. On the day of recording, the rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Additional doses of chloral hydrate were given to maintain constant anesthesia. Body temperature was maintained at 37°C throughout the experiment by using a thermostatically controlled heating pad. For system injection of pharmacological agents a catheter was inserted in a lateral tail vein before the electrophysiological recordings. The electrophysiological experiments were carried out with the minipumps in place. All of the experiments were approved by the local Animal Care Committee and conducted in accordance with the Canadian Council on Animal Care for the care and use of laboratory animals.

#### In Vitro Determination of [3H]5-HT Uptake

To determine the degree of 5-HT reuptake inhibition in hippocampus, after a 2-day administration of trazodone (5, 10, and 20 mg/kg/day) and the SSRI escitalopram (10 mg/kg/day; used as a positive control), hippocampal slices were incubated in oxygenated Krebs’ solution at 37°C to determine in vitro [3H]5-HT uptake. After a 3-min stabilization period, the slices were incubated with 20 nM [3H]5-HT for 3 min. The uptake process was stopped by transferring the slices into 5 ml of ice-cold Krebs’ solution. The slices were then solubilized in 0.5 ml of Soluene 350. A parallel experiment was carried out at 0°C as a control for passive diffusion. The radioactivity in the media and tissue was determined by liquid scintillation spectrometry (Beckman Coulter, Fullerton, CA). The reuptake activity was assessed by determining the tissue-medium ratio of radioactivity using the formula: 

\[ R = \frac{(T - C)}{C} \]

where \( R \) is the ratio of tissue to medium for the control slice, \( T \) is the ratio of tissue for the treated slice, and \( C \) is the ratio of tissue to medium for the control slice.

#### Extracellular Unitary Recording of DR 5-HT Neurons

In vivo extracellular recordings of 5-HT neurons were obtained by using single-barrel glass micropipettes. The impedance of the electrodes was between 4 and 6 MΩ. After securing the rats on the stereotaxic apparatus, a burr hole was drilled 1 mm anterior to lambda. The microelectrodes were positioned 0.9 to 1.2 mm anterior.
to lambda on the midline and lowered into the DR. The 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified by their slow (0.5–2.5 Hz), regular firing rate and a long duration (0.8–1.2 ms) positive action potential (Aghajanian, 1978).

**Assessment of Sensitivity of the DR 5-HT<sub>1A</sub> Autoreceptor**

To assess the sensitivity of the 5-HT<sub>1A</sub> autoreceptor, dose-response curves for the alteration of 5-HT neuronal firing activities were constructed by using systemic administration of the 5-HT autoreceptor agonist lisergic acid diethylamide (LSD) in control and treated rats. LSD is a more reliable probe than the 5-HT<sub>1A</sub> receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) for 5-HT<sub>1A</sub> autoreceptor because the latter also acts on 5-HT<sub>1A</sub> receptors of cortical neurons feeding back to DR 5-HT neurons. Furthermore, the responsiveness of DR 5-HT neurons to microiontophoretic application of LSD, 8-OH-DPAT, and systemic injection of LSD is decreased, whereas that to systemic 8-OH-DPAT is not altered after chronic administration of the 5-HT<sub>1A</sub> receptor agonist gepirone (Blier and de Montigny, 1990). The potent and selective 5-HT<sub>1A</sub> receptor antagonist N-(2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-(2-pyrindinyl) cyclohexanecarboxamide trihydrochloride (WAY-100635) (Khwaja et al., 1995) was injected, after the LSD-induced inhibition, to reverse 5-HT firing activity. This also served to validate the use of the agonist that is not entirely selective for 5-HT<sub>1A</sub> receptors. To avoid the drug residual effects, the dose-response curves were obtained by using only the last neuron in each rat. It is worth noting that the firing rate of all 5-HT neurons used in this set of experiments was within their average firing rate, and a 2-min period was allowed to obtain a stable baseline before the systemic injection of LSD. The rate and pattern of firing rate were typical for 5-HT neurons as described previously (Aghajanian, 1978).

**Extracellular Recording and Microiontophoresis of CA<sub>3</sub> Dorsal Hippocampus Pyramid Neurons**

Extracellular recording and microiontophoresis of CA<sub>3</sub> pyramidal neurons were performed with five-barreled glass micropipettes. The central barrel, used for the unitary recording, was filled with a 2 M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 MΩ. The side barrels were filled with the following solutions: 5-HT creatinine sulfate (10 mM in 200 mM NaCl, pH 4), (±)NE bitartrate (10 mM in 200 mM NaCl, pH 4), trazodone hydrochloride (20 mM in 200 mM NaCl, pH 4), or quissqualic acid (1.5 mM in 200 mM NaCl, pH 8), and the last barrel was filled with a 2 M NaCl solution used for automatic current balancing. The micropipettes were lowered into the dorsal hippocampus CA<sub>3</sub> region by using the following coordinates: 4 mm anterior to lambda and 4.2 mm lateral (Paxinos and Watson, 1998). A small current of quisqualate (+2 to −5 nanoamperes (nA)) was used to activate the pyramidal neurons within their physiological firing range (10–15 Hz; Ranck, 1975) because these neurons do not discharge spontaneously in chloral hydrate anesthetized rats. The hippocampal CA<sub>3</sub> pyramidal neurons were found at a depth of 4.0 ± 0.5 mm below the surface of the brain and identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges (Kandel and Spencer, 1961). 5-HT and NE were microiontophoretically applied for 50 s. The duration of local application of the 5-HT and the ejection currents (nA) were kept constant before and after each intravenous injection of the 5-HT<sub>1A</sub> antagonist WAY-100635. The responsiveness of CA<sub>3</sub> pyramidal neurons to the microiontophoretic application of 5-HT and NE, before and after intravenous injections, was assessed by determining the number of spikes suppressed per nA for the 50-s ejection period.

**Assessment of the Tonic Activation of Postsynaptic 5-HT<sub>1A</sub> Receptors in Hippocampus**

The degree of tonic activation of hippocampus CA<sub>3</sub> 5-HT<sub>1A</sub> receptors was assessed by using systemic injection of the selective 5-HT<sub>1A</sub> antagonist WAY-100635 (Haddjeri et al., 1998). Such disinhibition of the neuronal activity is best assessed when the firing rate is low. Indeed, a low stable firing baseline was obtained by lowering the ejection current of quisqualate. The baseline firing was recorded for at least 2 min before the administration of WAY-100635. WAY-100635 (100 μg/kg) was systemically administered in incremental doses of 25 μg/kg at time intervals of 2 min to detect the changes in the firing activity of hippocampal pyramidal neurons in rats treated with vehicle and trazodone (10 mg/kg/day) for 14 days. Such curves represent stable changes in the firing rate of CA<sub>3</sub> pyramidal neurons as percentages of baseline firing after each systemic drug administration. To avoid residual drug effects, only one neuron in each rat was studied.

**In Vivo Determination of 5-HT Uptake**

To assess the relative degree to which trazodone blocks the 5-HTT, 50% recovery time (RT<sub>50</sub>) values were determined after microiontophoretic application of 5-HT in hippocampus CA<sub>3</sub> region. The RT<sub>50</sub> values correspond to the time in seconds elapsed from the cessation of microiontophoretic application of 5-HT to obtain a 50% recovery of the initial firing rate (de Montigny et al., 1980). It is a reliable index of 5-HT reuptake process in vivo. Indeed, previous experiments showed that acute systemic injection of the SSRI paroxetine significantly increased RT<sub>50</sub> values. Furthermore, this phenomenon was also observed in rats after the lesion of 5-HT neurons, thereby eliminating 5-HTT (Piñeyro et al., 1994).

**The 5-HT Pathway Stimulation**

The CA<sub>3</sub> region of hippocampus receives extensive projections from DR 5-HT neurons. To electrically stimulate the ascending 5-HT pathway, a bipolar electrode (NE-100; David Kopf) was implanted 1 mm anterior to lambda on the midline with a 10° backward angle in the ventromedial tagmentum and 8.0 ± 0.2 mm below the surface of the brain. Two hundred square pulses with duration of 0.5 ms were delivered by a stimulator (S48; Grass Instruments, Quincy, MA) at an intensity of 300 μA and a frequency of 1 and 5 Hz. The stimulation of the 5-HT pathway induces a brief suppressant period caused by the release of 5-HT in the synapse. The effects of stimulation of ascending 5-HT pathway were assessed at 1 and 5 Hz, on the same neuron, to determine the function of terminal 5-HT<sub>1B</sub> autoreceptors (Chapat et al., 1986). The two series of stimulations, 1 and 5 Hz, were carried out because previous studies showed that the activation of terminal 5-HT<sub>1B</sub> autoreceptors decreases the 5-HT release in the terminal areas and increasing the frequency of stimulation from 1 to 5 Hz induces a greater activation of 5-HT<sub>1B</sub> autoreceptors and consequently a greater negative feedback on the release of 5-HT (Chapat et al., 1986). As a result, the smaller release of the neurotransmitter in the synapse, obtained at 5 Hz, induces a smaller period of suppression compared with that of the 1-Hz stimulation. The stimulation pulses and the firing activity were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, Cambridge, UK). Peristimulus time histograms of CA<sub>3</sub> pyramidal neurons were generated to determine the suppression of firing measured in absolute silence (SIL) value in ms. The SIL value corresponds to the duration of a total suppression of the CA<sub>3</sub> hippocampal pyramidal neuron. This parameter was calculated by computer by dividing the total number of events, suppressed by the stimulation, by the frequency of firing of the recorded neuron.

**Quantification of Trazodone and mCPP in the Brain**

**Standards Preparation.** Trazodone, mCPP, and domperidone were initially prepared at 1 mg/ml in dimethyl sulfoxide and subsequently diluted in acetonitrile. For analysis in brain, traz-
Domperidone and mCPP were added to 100 mg/ml control brain homogenate (5–2500 pg/mg tissue) and extracted as described below. Domperidone was prepared at 1 μg/ml in acetoneitrile to be used as an internal standard.

**Sample Preparation.** Tissue samples were homogenized at 100 mg/ml in deionized water and 100 μl of tissue homogenate was used for the extraction. To each sample, 10 ng (10 μl of 1 μg/ml) of domperidone was added followed by 1 ml of ethyl acetate containing 0.1% (v/v) ammonium hydroxide (30%, w/v) and extraction by vortexing for 10 min. Organic and aqueous layers were separated by centrifugation (10 min, 20,000g) and the organic layer was removed, evaporated by vacuum centrifugation at room temperature, and reconstituted in 200 μl of beginning mobile phase (20% acetoneitrile/80% 0.1% formic acid in water) for liquid chromatography (LC)-double mass spectrometry analysis.

**Mass Spectrometry**
Positive ion electrospray ionization mass spectra were obtained with an AB Sciex 3200 QTRAP triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) with a turbo V ion source interfaced to a Shimadzu (Kyoto, Japan) high-performance LC system. Samples were chromatographed with a Waters (Milford, MA) Sunfire C18, 2.5 μm, 50 mm × 4.6 mm column. The LC was a gradient elution using 100% acetonitrile as the organic phase and 0.1% formic acid in water as the aqueous as follows: 20% acetonitrile for 0.1 min, linearly ramp to 90% acetonitrile at 1.5 min, hold at 90% for 30 s, return to 20% acetonitrile over 30 s, and equilibrate column for 30 s at 20% acetonitrile. The flow rate was 1 ml/min, and sample injection volume was 60 μl. The analysis time was 3 min. The mass spectrometer settings were: temperature, 550°C; spray needle, 5500 V; curtain gas, 10; collision gas, N2 (collisionally activated dissociation), 3; and ion source gas 1 and 2, 55 and 45, respectively. The compound-dependent settings for trazodone, mCPP, and domperidone were as follows, respectively: declustering potential, 57, 24, and 63; excitation potential, 4, 10, and 9; collision cell entrance potential, 21, 26, and 36; and collision cell exit potential, 3, 3, and 2. Samples were quantified by the internal standard method in the MRM mode by monitoring the transition m/z 372 → 176 for trazodone, m/z 197 → 154 for mCPP, m/z 426 → 175 for the internal standard domperidone. Each ion transition was integrated for 250 ms. Quantitation of trazodone and mCPP in the brain were based on standard curves in spiked matrix by using the ratio of either analyte peak area to domperidone peak area using 1/x² weighting for both analytes.

**Drugs**
Trazodone HCl (Lobapharm, Montreal, QC, Canada) was dissolved in 20% hydroxy propyl-β-cyclodextrin. Escitalopram was obtained from Lundbeck (Copenhagen, Denmark). LSD was purchased from Health Canada (Ottawa, ON, Canada). WAY-100635, 5-HT creatinine sulfate, and (-)-NE bitartrate and quisqualic acid were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in distilled water. [3H]5-HT was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). For quantification experiments trazodone, mCPP, and domperidone were obtained from Sigma-Aldrich. All other chemicals and solvents were obtained from Thermo Fisher Scientific (Waltham, MA).

**Statistical Analysis**
All data are reported as mean values ± S.E.M. The n values represent the number of neurons recorded, unless otherwise indicated. Data were obtained from three to eight rats per experimental group. Statistical comparisons were carried out by using one-way analysis of variance (treatment as the main factor), and Bonferroni post hoc analysis was conducted when significant analysis of variance results were obtained. Dose-response curves were constructed by using linear regression analysis with Prism 5 software (GraphPad Software Inc., La Jolla, CA). Statistical comparisons were carried out with two-tailed Student’s t test when a parameter was studied in control and treated rats. The effects of changing the frequency of stimulation from 1 to 5 Hz on the SIL value of the same neuron were assessed by paired Student’s t test. Analysis of covariance was used to assess the statistical significance of the difference in the degree of reduction in the response of hippocampus neurons when the frequency of stimulation was increased from 1 to 5 Hz in control and trazodone-treated rats. Statistical significance was taken as P < 0.05.

**Results**

**Effects of 2- and 14-Day Administration of Trazodone on the Firing Rate of DR 5-HT Neurons.** In comparison to the vehicle group, the 2-day administration of trazodone at the dose of 10 and 20, but not 5, mg/kg/day significantly decreased the spontaneous firing rate of DR 5-HT neurons by 40 and 37%, respectively (vehicle: 1.11 ± 0.08 Hz, p < 0.01 for both trazodone 10 and 20 mg/kg/day; Fig. 1A). Although trazodone at the dose of 20 mg/kg/day inhibited the 5-HT firing rate to the extent of 10 mg/kg/day, it significantly reduced the number of spontaneously active 5-HT neurons by 65% after 2 days once compared with the vehicle group (p < 0.01; Table 2). Therefore, the average firing rate for the 2-day trazodone dose of 20 mg/kg/day is an underestimated value because this regimen markedly decreased the number of spontaneously active 5-HT neurons.

It is noteworthy that systemic administration of the potent and selective 5-HT1A receptor antagonist WAY-100635 (100 μg/kg) normalized the 5-HT firing-inhibition induced by trazodone at 10 and 20 mg/kg/day (Fig. 1, A and B) and restored the number of spontaneously active neurons in rats treated with trazodone at 20 mg/kg/day (Table 2). It is important to note that our previous studies and those of others have shown that systemic administration of WAY-100635 does not significantly alter the average spontaneous firing rate of DR 5-HT neurons in naive rats (Gartshe et al., 1995; Haddjeri et al., 2004).

Because trazodone at the dose of 10 mg/kg/day induced a marked physiological action on 5-HT neurons, this dose was used to study the effects of 14-day administration of trazodone on the 5-HT system. In contrast to the 2-day regimen, the firing rate of DR 5-HT neurons completely recovered to the baseline after 14 days of trazodone administration (10 mg/kg/day; p > 0.05; Fig. 1C).

**Brain Concentration of Trazodone and mCPP After 2-Day Regimen.** The steady-state concentrations of trazodone and its major metabolite mCPP were 25 ± 2.7 and 4 ± 0.25 ng/ml, respectively, after 2-day administration of trazodone (10 mg/kg). These levels are in the same range as those reported by DeVane et al. (1999).

**Assessment of Sensitivity of Somatodendritic 5-HT1A Autoreceptors.** The responsiveness of somatodendritic 5-HT1A autoreceptor, after 14-day trazodone (10 mg/kg/day) regimen, was assessed by using the 5-HT autoreceptor agonist LSD probe (Blier and de Montigny, 1990). The firing of DR 5-HT neurons in all of the naive rats was completely inhibited at the dose of 20 μg/kg (ED50 = 9.0 ± 1.0 μg/kg; Fig. 2A). In contrast, the effect of LSD at this dose was markedly attenuated in suppressing the neuronal activity of 5-HT neurons in rats treated with trazodone.
for 14 days. In fact, higher doses of LSD were needed to induce a complete suppression of the 5-HT firing (ED50/H11005 16.0/H11006 1.0/H9262 16.0 g/kg; Fig. 2B). A complete dose-response relationship between the suppression of DR 5-HT firing rate and different doses of LSD showed a significant 2-fold shift to the right in the 14-day trazodone-treated rats. In addition to the altered ED50 values, the slopes of the two dose-response curves were significantly different (5.2/H11569/H11569 0.5/H11021/H11021 versus 2.3/H11006 0.1 for the vehicle and trazodone groups, respectively; F1,18 = 5.46, p < 0.05; Fig. 2C), indicating that the long-term trazodone regimen resulted in 5-HT1A autoreceptor desensitization.

Effects of Sustained Administration of Trazodone for 14 Days on the Responsiveness of Dorsal Hippocampus CA3 Pyramidal Neurons to Exogenous 5-HT and NE. Microiontophoretic application of 5-HT and NE suppressed the firing rate of dorsal hippocampal CA3 pyramidal neurons in the vehicle and trazodone-treated rats for 14 days. In fact, higher doses of LSD were needed to induce a complete suppression of the 5-HT firing (ED50 = 16.0 ± 1.0 µg/kg; Fig. 2B). A complete dose-response relationship between the suppression of DR 5-HT firing rate and different doses of LSD showed a significant 2-fold shift to the right in the 14-day trazodone-treated rats. In addition to the altered ED50 values, the slopes of the two dose-response curves were significantly different (5.2 ± 0.5 versus 2.3 ± 0.1 for the vehicle and trazodone groups, respectively; F1,18 = 5.46, p < 0.05; Fig. 2C), indicating that the long-term trazodone regimen resulted in 5-HT1A autoreceptor desensitization.

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>2 Days</th>
<th>Post-WAY-100635</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.9 ± 0.5 (15)</td>
<td></td>
</tr>
<tr>
<td>Trazodone (5 mg/kg)</td>
<td>5.5 ± 0.6 (14)</td>
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</tr>
<tr>
<td>Trazodone (10 mg/kg)</td>
<td>4.2 ± 0.7 (10)</td>
<td></td>
</tr>
<tr>
<td>Trazodone (20 mg/kg)</td>
<td>1.7 ± 0.3 (11)*</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.01.
In Vivo Determination of [3H]5-HT Uptake by Trazodone. After the 2-day regimens, the ex vivo experiments from hippocampal slices revealed that trazodone, in a dose-dependent manner, at doses of 5 and 10 mg/kg/day significantly blocked the 5-HT reuptake process by 57% (p < 0.05) and 62% (p < 0.01), respectively. It is worth noting that trazodone at the dose of 20 mg/kg/day also markedly inhibited 5-HTT in hippocampus by 70% (p < 0.01). The SSRI escitalopram, used as a positive control, at the dose of 10 mg/kg/day significantly inhibited the 5-HT reuptake process by 81% (p < 0.001; Fig. 4).

Assessment of the Overall Serotonergic Tone after 14-Day Administration of Trazodone as Determined with the Tonic Activation of Postsynaptic 5-HT1A Receptors. As illustrated in Fig. 5, cumulative administration of 5-HT1A antagonist WAY-100635 failed to modify the firing activity of dorsal hippocampus CA3 pyramidal neurons in the vehicle group (p > 0.05; Fig. 5). On the other hand, the second dose and subsequent doses of systemic injection of WAY-100635 markedly enhanced the firing activity in rats administered with trazodone for 14 days (p < 0.05 for all doses except the first one; Fig. 5). Indeed, the final injection of WAY-100635 increased the neuronal activity in the CA3 region by approximately 160% compared with control rats. The marked increment in the firing activity of pyramidal neurons in the trazodone-treated rats reflects the degree to which WAY-100635 disinhibits neuronal activity caused by the tonic activation of postsynaptic 5-HT1A receptors by extracellular 5-HT in the hippocampus. It is important to emphasize that the inhibitory effect of microiontophoretic application of 5-HT was significantly blocked in all rats after the last injection of WAY-100635 (vehicle: pre-WAY-100635, 55 ± 5 spikes suppressed/nA, post-WAY-100635, 29 ± 4 spikes suppressed/nA, n = 6, p < 0.001; 14-day trazodone: pre-WAY-100635, 46 ± 3, post-WAY-100635, 12 ± 2, n = 8, p < 0.001; Fig. 5), confirming that the inhibitory action was mediated by 5-HT1A receptors.

Effects of Sustained Administration of Trazodone for 14 Days on the Efficacy of the Electrical Stimulation of the 5-HT Afferent Fibers to the Hippocampus. To assess the amount of 5-HT fibers released per electrical impulse in the 5-HT pathway, the 5-HT afferent fibers to the hippocampus were electrically stimulated. The responsiveness of terminal 5-HT1B autoreceptors was also evaluated by increasing the frequency of stimulation from 1 to 5 Hz on the same neuron. The stimulation of the 5-HT pathway at 1 Hz
enhanced the period of suppression in rats administered with trazodone for 14 days by 20% once compared with the corresponding SIL value in the vehicle group ($p < 0.01$; Fig. 6). Increasing the frequency of stimulation from 1 to 5 Hz significantly reduced the period of suppression in the vehicle group by 25% ($p < 0.01$; Fig. 6), an effect caused by the greater degree of activation of terminal 5-HT1B autoreceptors (Chaput et al., 1986). Furthermore, unlike in the vehicle group, the decremental effect obtained by enhancing the frequency of stimulation from 1 to 5 Hz was abolished in rats treated with trazodone, and the period of suppression at 5 Hz was greater than the corresponding value in the vehicle group ($p < 0.01$; Fig. 6), indicating that the inhibitory function of the terminal autoreceptor was diminished.

Effects of Microiontophoretic Application of Trazodone on Hippocampus CA3 Pyramidal Neurons. Trazodone was locally applied in the dorsal hippocampus CA3 region to characterize its effects at postsynaptic 5-HT1A receptors. The firing activity of pyramidal neurons was markedly reduced by local application of 5-HT (Fig. 7, A and B). Microiontophoretic application of trazodone also markedly inhibited the CA3 neuronal activity (Fig. 7, A and C). The inhibitory effects of both 5-HT and trazodone were significantly blocked after the systemic administration of WAY-100635 (100 µg/kg), indicating that these inhibitory actions were mediated by 5-HT1A receptors ($p < 0.01$; Fig. 7, A, C, and D). The agonistic action of trazodone at postsynaptic 5-HT1A receptors was further characterized as being full because it did not attenuate the inhibitory action of the endogenous agonist 5-HT when coapplied by microiontophoresis in hippocampus ($p > 0.05$; Fig. 7, A and B).

Discussion
The present data showed that trazodone potently suppressed the firing rate of DR 5-HT neurons after 2 days of sustained administration. This firing rate, however, recovered to baseline after administration of trazodone for 14 days. The complete recovery of 5-HT firing is attributable to decreased sensitivity of 5-HT1A autoreceptors because the suppressive effect of the 5-HT autoreceptor agonist LSD was decreased by the long-term trazodone regimen. With respect to 5-HT neurotransmission in the forebrain, although the sensitivity of postsynaptic 5-HT1A and α1-adrenergic receptors was not changed after its prolonged administration, trazodone increased tonic activation of postsynaptic 5-HT1A receptors in hippocampus, as indicated by disinhibition of neuronal activity by WAY-100635. This study, therefore, indicates that sustained administration of trazodone enhances 5-HT neurotransmission, at least in part, by desensitizing the inhibitory function of 5-HT1A and 5-HT1B autoreceptors that are present on the cell body and terminals, respectively, in the presence of 5-HT reuptake inhibition.

The in vitro data showed that trazodone inhibited 5-HTT in a dose-dependent manner in the hippocampus, a phenomenon that was also observed with the SSRI escitalopram (Fig. 4), suggesting that trazodone modulates the 5-HT system, in part, by inhibiting 5-HT reuptake sites. Although trazodone at the dose of 5 mg/kg/day significantly blocked the 5-HTT, it did not alter the firing rate of 5-HT neurons after 2 days (Fig. 1), indicating that the degree of blockade was below the threshold to induce a net physiological action. This result is akin to what has been reported in humans: an 80% occupancy of 5-HTT is required to obtain an antidepressant effect with a variety of reuptake inhibitors (Meyer et al., 2004). Trazodone at 10 and 20 mg/kg/day, on the other hand, suppressed the firing rate of 5-HT neurons after 2 days of administration. In parallel to the 2-day trazodone regimen, acute administration of trazodone dose-dependently reduced the firing rate of 5-HT neurons (Scuée-Moreau and Dresse, 1982), perhaps because of its blocking property at 5-HTT and/or α1-adrenoceptors (Table 1). Although the 2-day administration of trazodone at the dose of 20 mg/kg/day reduced the firing rate of 5-HT neurons to the same extent as 10 mg/kg/day, the number of spontaneously active 5-HT neurons was reduced only by the highest dose. It is well established that short-term
administration of SSRIs potently suppresses 5-HT neurons because of the activation of somatodendritic 5-HT_{1A} autoreceptors by enhancing 5-HT levels in the vicinity of the cell body. In line with this, systemic administration of the 5-HT_{1A} antagonist WAY-100635 reversed the trazodone-induced suppression of 5-HT firing rate to their baseline (Fig. 1) and normalized the number of spontaneously active 5-HT neurons (Table 2), indicating that the suppressant effect may be mediated via 5-HT_{1A} receptors and not by blocking {alpha}_1-adrenoceptors. In contrast to the 2-day effect, the firing rate of 5-HT neurons showed a full recovery after 14 days of trazodone administration. The recovery of 5-HT firing activity was concurrent with desensitization of 5-HT_{1A} autoreceptors (Fig. 2), which may account for firing normalization, a physiological adaptation that was consistently observed with long-term SSRI administration (see Pinéyro and Blier, 1999).

Even though long-term administration of trazodone did not alter the sensitivity of postsynaptic 5-HT_{1A} and {alpha}_2-adrenergic receptors (Fig. 3), it induced a robust tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus after 14 days, as revealed by disinhibition of the neuronal firing activity induced by WAY-100635 (Fig. 5). Enhanced tonic activation is consistent with previous microdialysis experiments showing increased extracellular concentration of 5-HT in terminal brain areas after administration of antidepressants with serotonergic action (Romero et al., 1996). It has been shown that the presumed enhanced 5-HT levels in hippocampus may partially be caused by the inhibition of 5-HTT by prolonged, but not a 2-day, administration of the SSRI paroxetine (Besson et al., 2000). The observation that prolonged administration of trazodone considerably increased the overall net effect of 5-HT in hippocampus suggests similar mechanism of action as the SSRIs. In line with this, microdialysis experiments showed that administration of trazodone markedly enhanced the extracellular 5-HT concentration in the frontal cortex of freely moving rats (Pazzaglia et al., 1999), an effect that was higher than that of the SSRI sertraline (Garzone et al., 2009). Vilazodone, which like trazodone is a SSRI/5-HT_{1A} agonist, enhances extracellular 5-HT concentration in the rat frontal cortex more than the SSRI paroxetine alone (Hughes et al., 2005).

Previous studies have reported that long-term administration of agents that potently inhibit 5-HTT, such as citalopram, fluoxetine, paroxetine, and fluvoxamine, induce desensitization of 5-HT_{1B} autoreceptors on 5-HT terminals (Pinéyro and Blier, 1999). Thus, it was deemed crucial to assess the sensitivity of terminal autoreceptors because these autoreceptors exert an inhibitory role on 5-HT release (Chaput et al., 1986). Compared with the vehicle group, stimulation of the 5-HT bundle at 1 Hz increased the effectiveness of stimulation in rats administered with trazodone for 14 days, as for the abovementioned SSRIs. Increasing the frequency of stimulation from 1 to 5 Hz reduces the period of suppression in the vehicle group, because of greater activation of terminal 5-HT_{1B} autoreceptors, as reported previously (Chaput et al., 1986). In trazodone-treated rats, however, this decremental effect was abolished when the frequency of stimulation was increased from 1 to 5 Hz, suggesting that the inhibitory action of terminal autoreceptors was diminished (Fig. 6). In addition, long-term administration of YM992,
which like trazodone blocks 5-HTT and 5-HT₁A receptors, desensitized terminal 5-HT autoreceptors (Dong et al., 1999). Desensitization of terminal 5-HT₁B autoreceptors may thus be the driving force for the enhanced 5-HT release in the synapse after 14-day administration of trazodone. Indeed, 5-HT reuptake blockade per se could not explain the increased 5-HT tone in hippocampus, because acute administration of the SSRIs citalopram or fluoxetine does not enhance the effectiveness of stimulation (Pinéyro and Blier, 1999). Furthermore, Gross et al. (1987) showed that trazodone increased the electrically induced [³H]5-HT release from cortical slices, an effect that was independent of 5-HT reuptake inhibition. Moreover, it is worth mentioning that desensitization of the terminal 5-HT₁B autoreceptor after long-term administration of trazodone is not likely caused by the agonistic action of its major metabolite mCPP on these receptors or its potent antagonistic effect at 5-HT₃ receptors, because its concentration is low in the brain, as reported previously at the steady-state level (DeVane et al., 1999).

Microiontophoretic application of trazodone showed that, similar to 5-HT, it acts as an agonist at postsynaptic 5-HT₁A receptors in the hippocampus CA₃ region because administration of the 5-HT₁A receptor antagonist WAY-100635 markedly blocked the inhibitory actions of both 5-HT and trazodone (Fig. 7). The agonistic action of trazodone at 5-HT₁A receptors was further characterized as being full because it did not antagonize the inhibitory action of the endogenous agonist 5-HT when coapplied by microiontophoresis in hippocampus (Fig. 7). In addition, when trazodone was administered for 14 days and the responsiveness to 5-HT was examined with the minipump in the animal delivering the drug, there was no alteration in the sensitivity of the pyramidal neurons to 5-HT (Fig. 3). On the other hand, the 5-HT₁A partial agonist gepirone (Blier and de Montigny, 1990) significantly offsets the inhibitory effect of 5-HT on the hippocampus firing rate when coapplied locally. Thus far, R(−)-2-[4-[(chroman-2-ylmethyl)-amino]-butyl]-1,1-dioxo-benzo[d]isothiazolone hydrochloride (BAY 3702) is the only 5-HT₁A agonist reported to act as a full agonist, like trazodone, at 5-HT₁A receptors in the CA₃ region (Dong et al., 1998). Even though both agents behave as full agonists at these postsynaptic 5-HT₁A receptors, they did not alter the sensitivity of these receptors after prolonged administration, in contrast to that of the somatodendritic autoreceptors. Furthermore, previous in vitro studies showed that trazodone has moderate affinity at 5-HT₁A receptors in the frontal cortex of human brain and rat hippocampus (Cusack et al., 1994; Owens et al., 1997) and that it acts as an agonist at human 5-HT₁A receptors (Odagaki et al., 2005). The agonistic action of trazodone at 5-HT₁A receptors, in addition to its modulatory action on 5-HT neurotransmission, may contribute to enhance DA release in the medial prefrontal cortex (Chung et al., 2004).

Enhanced 5-HT transmission by prolonged administration of trazodone may be clinically relevant. Indeed, trazodone, similar to other classes of antidepressants including tricyclics, monoamine oxidase inhibitors, SSRIs, mirtazapine, gepirone,
and electroconvulsive seizures, shares the property of enhancing the overall 5-HT neurotransmission in hippocampus after long-term administration (Haddjeri et al., 1998). The therapeutic effect of trazodone in the treatment of major depression may thus be, at least in part, caused by the enhancement of 5-HT neurotransmission via 5-HT inhibition and 5-HT1A receptor agonism. This medication, however, has not been used as first-line treatment for depression because of its daytime sedative action. The latter effect is probably caused by a combination of H3, 5-HT1A, and α1-adrenergic receptor antagonism (Table 1), properties that individually can contribute to hypnotic and/or sedative effects. The development of a slow release preparation of trazodone, thereby eliminating sharp plasma/brain peaks, has, however, helped diminish daytime drowsiness, while maintaining the therapeutic benefit on depressed mood (Sheehan et al., 2009).

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