Functional Status of Somatodendritic Serotonin 1A Autoreceptor after Long-Term Treatment with Fluoxetine in a Mouse Model of Anxiety/Depression Based on Repeated Corticosterone Administration

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ABSTRACT

Most preclinical studies investigating the effects and the mechanism of action of antidepressants have been performed in naive rodents. This is inappropriate because antidepressants act on specific symptoms of the pathological condition, such as distress and anxiety. We have developed a mouse model of anxiety/depression based on addition of corticosterone to drinking water. This model is highly reproducible and easy to set up compared with unpredictable chronic mild stress. The serotonin 1A (5-HT₁A) autoreceptor is known to play a role in mood disorders and their treatments. An increase in somatodendritic 5-HT₁A autoreceptor density in the dorsal raphe (DR) attenuates the therapeutic activity of selective serotonin-reuptake inhibitors (SSRIs), whereas their functional desensitization promotes activation of brain serotonergic transmission, thereby representing an adaptive change relevant to their therapeutic effect. Here we assessed the effects of sustained administration of the SSRI fluoxetine on 5-HT₁A autoreceptor sensitivity in mice administered with corticosterone. Fluoxetine attenuated hypothermia induced by the 5-HT₁A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin, decreased DR 5-HT neuronal activity, and decreased 5-HT release in both vehicle- and corticosterone-pretreated mice. However, such desensitization was more pronounced in corticosterone-pretreated mice. This change had an overall effect on serotonergic tone because we found a greater firing rate of 5-HT neurons associated with an enhancement of 5-HT outflow in the DR of corticosterone-pretreated mice in response to fluoxetine compared with the corresponding group of vehicle-pretreated mice. These results provide cellular explanations for the antidepressant effects produced by SSRIs in subjects with pathological conditions but not in naive animals or healthy volunteers.

Introduction

Alterations in multiple biological markers are implicated in the neurobiology of depression, based primarily on the characterization of antidepressant efficacy in naive rodents (Gourley and Taylor, 2009). However, it seems more appropriate to perform pharmacological studies in animal models that exhibit hallmark characteristics of anxiety/depression. Several animal models have been developed that are based mainly on stressful situations, such as unpredictable chronic mild stress (UCMS). Although UCMS has been efficiently...
used to assess antidepressant activity (Surget et al., 2009; Farley et al., 2010), it is notoriously difficult to reproduce consistently in rodents. An intriguing alternative may be to supply mice with exogenous corticosterone (David et al., 2009; Gourley and Taylor, 2009), a hormone produced in the adrenal glands in response to stress and found to be elevated in several animal models of depression and in depressed humans (Sterner and Kalynchuk, 2010). We have recently reported, in mice administered with corticosterone, some behavioral abnormalities that are indicative of anhedonia and hopelessness (David et al., 2009) and that mimic depressive symptoms observed in humans (Holsboer, 2000; Nemeroff and Vale, 2005). Therefore, corticosterone-treated mice provide a good preclinical model to investigate the interaction between hypothalamic-pituitary-adrenocortical axis dysfunction and antidepressant response.

The activity of the serotonergic system is regulated by several extrinsic and intrinsic factors. In the dorsal raphe (DR), there is a negative feedback control driven by the serotonin-1A (5-HT_{1A}) autoreceptor located in the soma and dendrites of 5-HT neurons. Activation of this presynaptic autoreceptor inhibits the firing rate of 5-HT neurons, the amount of 5-HT released per action potential, and the synthesis of 5-HT (Blier and de Montigny, 1987; Richardson-Jones et al., 2010). Clinical and preclinical studies have clearly established the role of this autoreceptor in mood disorders and their treatments. For example, enhanced radiigand binding of an agonist to the inhibitory 5-HT_{1A} autoreceptor in the human DR provided pharmacological evidence of diminished activity of serotonergic neurons in suicide victims afflicted with major depression (Stockmeier et al., 1998). In addition, a functional polymorphism in the promoter region of the human HTR1A gene was reported (Lemonde et al., 2003), suggesting that an increase in the density of 5-HT_{1A} autoreceptor in the DR may predispose to depression (Lemonde et al., 2003; Le François et al., 2008). The role of 5-HT_{1A} autoreceptor in the mechanism of action of selective serotonin-reuptake inhibitors (SSRIs) has also been studied extensively. It is believed that overactivation and/or overexpression of 5-HT_{1A} autoreceptor would delay the onset of antidepressant effect, whereas the functional desensitization of this receptor after sustained administration of SSRIs is an adaptive change relevant to their therapeutic activity (Gardier et al., 1996). By using a new strategy to manipulate somatodendritic 5-HT_{1A} autoreceptors in raphe nuclei without affecting 5-HT_{1A} heteroreceptors, it has been shown that mice with a low expression of autoreceptor display a greater behavioral response to fluoxetine, thus establishing a causal relationship between 5-HT_{1A} autoreceptor levels and the response to SSRIs (Richardson-Jones et al., 2010).

Despite the importance of somatodendritic 5-HT_{1A} autoreceptor desensitization in the appearance of the therapeutic effects of SSRIs, such functional inactivation, surprisingly, was also reported in several animal models of stress, including chronic sleep restriction (Evraud et al., 2006; Novati et al., 2008) and maternal separation (van Riel et al., 2004), but also in animal models of anxiety/depression (i.e., chronic mild stress) (Lanfumey et al., 1999; Froger et al., 2004; Grippo et al., 2005; Bambico et al., 2009). These results are consistent with findings showing an attenuation of 5-HT_{1A} receptor functions in a dysregulated hypothalamic-pituitary-adrenocortical axis in both animals (Lanfumey et al., 1999; Fairchild et al., 2003; Leitch et al., 2003; Hensler et al., 2007) and humans (Young et al., 1994; McAllister-Williams et al., 2007). However, these results in animal models of stress have been challenged by recent findings showing, on the contrary, that the sensitivity and/or the density 5-HT_{1A} autoreceptor in the DR was increased in rat or mouse models of depression (El Yacoubi et al., 2003; Greenwood et al., 2003; Pineda et al., 2011). Thus modeling stress or depression status in animals leads to distinct changes in 5-HT_{1A} autoreceptor sensitivity.

The present study was aimed at determining the nature and intensity of changes in 5-HT_{1A} autoreceptor function in a mouse model of anxiety/depression undergoing long-term exposure to corticosterone given either alone or in combination with the SSRI fluoxetine. Ultimately, such data obtained in an animal model of anxiety/depression may help shed light on the fact that studying the mechanism of action of SSRIs is more relevant in a disease state.

**Materials and Methods**

**Animals.** Adult male C57BL/6J mice were purchased from Elevage Janvier (Le Genest St. Isle, France). All corticosterone-administered mice were 7 to 8 weeks old and weighed 20 to 24 g at the beginning of the treatment. They were maintained on a 12-h light/dark cycle schedule (lights on at 6:00 AM) and housed in groups of five. Food and water were provided ad libitum. Behavioral testing occurred during the light phase between 8:00 AM and 5:00 PM. Separated groups were used for the behavioral, electrophysiological, and neurochemical studies. All testing was conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee (Council directive 87-848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; permission 92-256B to D.J.D.).

**Drugs.** In place of normal drinking water, group-housed mice were presented for 7 weeks with vehicle (0.45% hydroxypropyl-β-cyclodextrin) or corticosterone (35 μg/ml) and, during the last 4 weeks of the corticosterone regimen, in the presence or absence of the SSRI fluoxetine (18 mg/kg/day). 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) hydrobromide and (N-[2-[4-[(2-methoxyphenyl)-1-piperazinyl]ethyl]-N’-[2-(pyridyl)]cyclohexanecarboxamide tri-hydrochloride (WAY100635) were obtained from Sigma-Aldrich (L’Isle d’Abeau, France) and dissolved in saline solution (0.9% NaCl). These compounds were administered subcutaneously at doses of 100 to 300 μg/kg s.c. and 300 μg/kg s.c., respectively.

**Body Temperature.** Body temperature was assessed intrarecally, using a lubricated probe (BIO-BRET-3) inserted approximately 2 cm and monitored with a thermometer (Bioseb, Vitrolles, France). Three baseline body temperature measurements were taken as a control measure before stress-induced hyperthermia (Supplemental Table) according to Van der Heyden et al. (1997). Ten minutes after the third baseline measurement, animals received 8-OH-DPAT (100 μg/kg s.c.), and body temperature was measured 10 min after the injection.

**In Vivo Electrophysiology.** Mice were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame with the skull positioned horizontally. To maintain a full anesthetic, chloral hydrate supplements of 100 mg/kg i.p. were given as needed. The extracellular recordings were carried out using single glass micropipettes (Stoelting Europe, Dublin, Ireland) for recordings in the DR. Micropipettes were preloaded with fiberglass strands to promote capillary filling with a 2 M NaCl solution.

**Recording of DR 5-HT Neurons.** Single glass micropipettes pulled on a pipette puller (Narishige, Tokyo, Japan) with impedances ranging from 2.5 to 5 MΩ were positioned 0.2 to 0.5 mm posterior to the interaural line on the midline and lowered into the DR, usually attained at a depth between 2.5 and 3.5 mm from the brain surface.
(Franklin and Paxinos, 2007). The DR 5-HT neurons were identified using the following criteria: a slow (0.5–2.5 Hz) and regular firing rate and a long-duration positive action potential (Aghajanian and Vandermaelen, 1982). The total number of spontaneously active 5-HT neurons and their firing rates were determined by monitoring their average discharge frequency. In each mouse, several tracts were performed to measure the spontaneous firing rate of DR 5-HT neurons. At the end of the experiment, only one neuron was studied with 8-OH-DPAT (100–300 µg/kg s.c.) to assess the functional activity of somatodendritic 5-HT1A autoreceptor. WAY100635 was used to reverse the suppressant effect of 8-OH-DPAT on the firing activity of DR 5-HT neurons. Changes in the firing activity are expressed as percentage of baseline firing rate.

In Vivo Microdialysis. Concentric dialysis probes (active length of 1 mm) were made of cuprophane fibers and set up as described previously (Malagie t al., 1996). Animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame for probe implantation in the DR according to the mouse brain atlas of Franklin and Paxinos (2007): −4.5 mm anterior, 0 mm lateral, and 3.0 mm ventral to bregma. Animals were allowed to recover from surgery overnight. The next day, −20 h after the surgery, were continuously perfused with artificial cerebrospinal fluid (147 mM NaCl, 3.5 mM KCl, 1.26 mM CaCl2, 1.2 mM MgCl2, and 1.0 mM NaH2PO4. pH 7.4)

Results and Discussion

Because 5-HT1A autoreceptor is a key component in the regulation of serotonergic neurotransmission, the present study examined its functional status after long-term administration of fluoxetine in mice pretreated with corticosterone or vehicle. In mice, 8-OH-DPAT-induced hypothermia is mediated by 5-HT1A autoreceptor (Richardson-Jones et al., 2010). We used this response to assess the functional activity of somatodendritic 5-HT1A autoreceptors. WAY100635 was used to reverse the suppressant effect of 8-OH-DPAT on the firing activity of DR 5-HT neurons. Changes in the firing activity are expressed as percentage of baseline firing rate.

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Data Analysis and Statistics. Results from data analyses were expressed as mean ± S.E.M. of body temperature (behavior), of DR 5-HT firing rate (electrophysiology), and of [5-HT]H in the DR (neurochemistry). Two-way ANOVAs were applied for the statistical analyses of the data with pretreatment (vehicle versus corticosterone) and treatment (vehicle versus fluoxetine) as main factors. In all cases, when appropriate, pairwise comparisons were performed using the protected least-significant difference post hoc test using the computer software StatView 5.0 (SAS Institute, Cary, NC). Accepted level of significance was set at p < 0.05.

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The spike firing rate of 5-HT neurons in rats submitted to UCMS was lower than that of the control group (Bambico et al., 2009). Nevertheless, our results concur with reports that the basal firing rate of DR 5-HT neurons in rats is not affected by long-term corticosterone treatment despite the desensitization of 5-HT1A autoreceptor (Fairchild et al., 2003). It thus seems that the degree of stress may play a major role in the regulation of DR 5-HT neuronal activity. Consistent with this hypothesis, Bambico et al. (2009) reported that long-term unpredictable stress reduced the firing rate of DR 5-HT neurons whereas short-term restraint stress failed to do so. In the present study, the observation that the basal DR 5-HT neurons’ firing rate was not altered in corticosterone mice despite the desensitization of 5-HT1A autoreceptor may be attributable to the lack of tonic activation of this receptor in vivo (Bortolozzi et al., 2004; Guillon et al., 2006). It is also possible that compensatory mechanisms, such as a hypersensitization of the terminal 5-HT1B autoreceptor, occurred (Gur et al., 2001). After 28 days of treatment with fluoxetine, a significant decrease in the firing activity of 5-HT neurons was observed in vehicle-pretreated mice (1.1 ± 0.1 Hz) but was no longer present in corticosterone-pretreated mice (2.3 ± 0.2 Hz) compared with corresponding groups of mice administered with vehicle (Fig. 2, C and D). Thus, the recovery in firing rate of DR 5-HT neurons returned here to baseline after 28 days of fluoxetine treatment specifically in corticosterone-pretreated mice. Hence, one of the most remarkable results obtained herein is the observation that the combination of both agents produced additional effects allowing 5-HT neurons to regain their baseline more rapidly when they have been first sensitized (or activated) by the elevated corticosterone levels. This result is in agreement with the findings of Bambico et al. (2009).
with a robust desensitization of 5-HT$_{1A}$ autoreceptor and suggests that pathological conditions are necessary for fluoxetine to produce its maximal electrophysiological effects. With respect to the number of spontaneous active DR 5-HT neurons, a two-way ANOVA revealed no significant effect of pretreatment [$F_{1,13} = 1.1, p > 0.05$] and treatment [$F_{1,15} = 0.7, p > 0.05$] factors. The number of neurons recorded per electrode descent is known as a valid, indirect index of the percentage of neurons spontaneously discharging (active) during in vivo electrophysiological recordings. Nevertheless, in the present study, the number of spontaneously active DR 5-HT neurons was not modified either by corticosterone and/or fluoxetine treatments (Table 1).

Having observed electrophysiological differences on the sensitivity of 5-HT$_{1A}$ autoreceptor in corticosterone- and vehicle-pretreated mice administered with fluoxetine, we next sought to determine how these differences are reflected at the neurochemical level by using in vivo microdialysis at the somatodendritic level (i.e., in the DR). A two-way ANOVA on the neurochemical level by using in vivo microdialysis at the somatodendritic level (i.e., in the DR). A two-way ANOVA on the percentage of inhibition of 5-HT extracellular levels in-duced by 8-OH-DPAT, revealed a significant effect of pre-treatment [$F_{1,16} = 12.1, p < 0.01$] and treatment factors [$F_{1,16} = 5.1, p < 0.05$]. Our results show that the decrease in the extracellular levels of 5-HT in the DR induced by 8-OH-DPAT was significantly lower in corticosterone- than in vehicle-pretreated mice administered with fluoxetine (Fig. A and B). Once again, our results emphasize the fact that the functional desensitization of 5-HT$_{1A}$ autoreceptor in response to fluoxetine is potentiated in corticosterone-pretreated mice. It is noteworthy that area under the curve (AUC) values on extracellular levels of 5-HT in the DR parallel hypothymia data suggesting that such response may be an indirect measure of changes in basal 5-HT outflow in this brain region. Regarding basal extracellular levels of 5-HT in the DR, a two-way ANOVA revealed no significant effect of pretreatment [$F_{1,16} = 1.7, p > 0.05$] but a significant effect of treatment factors [$F_{1,16} = 28.6 p < 0.001$]. The absence of differences on the basal extracellular levels of 5-HT in the DR between vehicle- and corticosterone-pretreated mice is consistent with electrophysiological data. As expected, fluoxetine significantly increased basal extracellular levels of 5-HT in the DR in both vehicle- and corticosterone-pretreated mice (Table 2), with a trend of higher increase in the corticosterone group of mice. These findings raise the possibility that the degree of increase in 5-HT levels near 5-HT cell bodies might account for the differential degree of desensitization of 5-HT$_{1A}$ autoreceptor. This neurochemical observation corresponds with a higher firing rate of DR 5-HT neurons and a more pronounced desensitization of 5-HT$_{1A}$ autoreceptor in corticosterone- compared with vehicle-pretreated mice in response to fluoxetine. However, previous microdialysis data failed to detect differences in basal corti-

### TABLE 1
Effect of sustained corticosterone ± fluoxetine on the number of spontaneously active DR 5-HT neurons.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Neurons per Tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (β-CD 0.45%)</td>
<td>Vehicle</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Vehicle (β-CD 0.45%)</td>
<td>Fluoxetine (18 mg/kg/day)</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Corticosterone (35 μg/ml/day)</td>
<td>Vehicle</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Corticosterone (35 μg/ml/day)</td>
<td>Fluoxetine (18 mg/kg/day)</td>
<td>2.9 ± 0.3</td>
</tr>
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β-CD, β-cyclodextrin.
autoreceptor without affecting the basal firing rate or extracellular levels of 5-HT in the DR and 2) potentiates fluoxetine-induced desensitization of 5-HT\textsubscript{1A} autoreceptor. Such mechanism is in favor of a greater enhancement of brain serotonergic neurotransmission and would thus represent a plausible explanation of the antidepressant-like effects of SSRI, specifically in animal models of anxiety/depression. Several hypotheses may explain the fact that corticosterone desensitized 5-HT\textsubscript{1A} autoreceptor and potentiated the behavioral, electrophysiological, and neurochemical effects of fluoxetine. It is possible that corticosterone directly downregulated the expression of 5-HT\textsubscript{1A} autoreceptor. In situ hybridization and immunocytochemical studies have revealed the presence of glucocorticoid receptor mRNA or protein specifically within 5-HT cell bodies in the DR (Härfstrand et al., 1986), although the 5-HT\textsubscript{1A} receptor gene includes a glucocorticoid-responsive element (Ou et al., 2001). Therefore, corticosterone might have desensitized somatodendritic autoreceptor through a mechanism independent of an increase in extracellular 5-HT levels in the DR. It is noteworthy that it was shown that corticosterone significantly reduced the expression of mRNA encoding G-protein linked inwardly rectifying K\textsuperscript{+} channel (Fairchild et al., 2003), suggesting that the desensitization of 5-HT\textsubscript{1A} autoreceptor induced by corticosterone could result from an alteration in their coupling property. Another possibility would be that corticosterone increased the activity of the tryptophan hydroxylase. The activation of tryptophan hydroxylase would thus result in an increase in 5-HT release at somatodendritic autoreceptors in the mechanism of action of serotonergic antidepressant drugs: recent findings from in vivo microdialysis studies. Fundam Clin Pharmacol 10: 16–27.


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