Reduced 5-HT$_{1A}$- and GABA$_B$ Receptor Function in Dorsal Raphe´ Neurons Upon Chronic Fluoxetine Treatment of Socially Stressed Rats

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Cornelisse LN, Van der Harst JE, Lodder JC, Baarendse PJ, Timmerman AJ, Mansvelder HD, Spruijt BM, Brussaard AB. Reduced 5-HT$_{1A}$- and GABA$_B$ receptor function in dorsal raphe neurons upon chronic fluoxetine treatment of socially stressed rats. J Neurophysiol 98: 196–204, 2007. First published April 25, 2007; doi:10.1152/jn.00109.2007. Autoinhibitory serotonin 1A receptors (5-HT$_{1A}$) in dorsal raphe nucleus (DRN) have been implicated in chronic depression and in actions of selective serotonin reuptake inhibitors (SSRI). Due to experimental limitations, it was never studied at single-cell level whether changes in 5-HT$_{1A}$ receptor functionality occur in depression and during SSRI treatment. Here we address this question in a social stress paradigm in rats that mimics anhedonia, a core symptom of depression. We used whole cell patch-clamp recordings of 5-HT- and baclophen-induced G-protein-coupled inwardly rectifying potassium (GIRK) currents as a measure of 5-HT$_{1A}$- and GABA$_B$ receptor functionality. 5-HT$_{1A}$- and GABA$_B$ receptor-mediated GIRK-currents were not affected in socially stressed rats, suggesting that there was no abnormal (auto)inhibition in the DRN on social stress. However, chronic fluoxetine treatment of socially stressed rats restored anticipatory behavior and reduced the responsiveness of 5-HT$_{1A}$ receptor-mediated GIRK currents. Because GABA$_B$ receptor-induced GIRK responses were also suppressed, fluoxetine does not appear to desensitize 5-HT$_{1A}$ receptors but rather one of the downstream components shared with GABA$_B$ receptors. This fluoxetine effect on GIRK currents was also present in healthy animals and was independent of the animal’s “depressed” state. Thus our data show that symptoms of depression after social stress are not paralleled by changes in 5-HT$_{1A}$ receptor signaling in DRN neurons, but SSRI treatment can alleviate these behavioral symptoms while acting strongly on the 5-HT$_{1A}$ receptor signaling pathway.

INTRODUCTION

5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (DRN) have been implicated in mood disorders and suicide although their direct role remains unclear. In the DRN of patients suffering from major depression, both increased (Stockmeier et al. 1998) and reduced levels (Arango et al. 2001) of 5-HT$_{1A}$ autoreceptors have been observed. Changes in 5-HT$_{1A}$ autoreceptors may result from alterations in 5-HT$_{1A}$ receptor gene transcription (Parsey et al. 2006) due to polymorphisms in the 5-HT$_{1A}$ receptor promoter region (Albert and Lemonde 2004; Lemonde et al. 2003; Ou et al. 2003).

Moreover, SSRI treatment in patients may have long-term effects on 5-HT$_{1A}$ receptor levels in the DRN because depressed patients treated with antidepressants appear to have lower 5-HT$_{1A}$ receptors levels than antidepressant-naïve patients (Parsey et al. 2006). Also in rodents, desensitization of 5-HT$_{1A}$ autoreceptors as a result of chronic SSRI administration has been observed (Blier et al. 1998; Czachura and Rasmussen 2000; Elena Castro et al. 2003; Hensler 2002; Le Poul et al. 1995; Pejchal et al. 2002; Shen et al. 2002; Subhash et al. 2000). Although it remains unclear by which mechanism SSRIs exert their antidepressant action, there is a clear relation between reduced 5-HT$_{1A}$ receptor function and increased 5-HT levels in DRN innervated forebrain areas after SSRI treatment in rodents. If SSRIs are acutely applied, locally enhanced 5-HT levels in the DRN inhibit serotonergic neurons through activation of the 5-HT$_{1A}$ autoreceptor. This results in decreased 5-HT release in the projection areas (Artigas et al. 1996b). However, after chronic SSRI treatment, 5-HT$_{1A}$ receptors are desensitized, and firing of DRN cells is restored resulting in increased 5-HT levels in the frontal cortex (Artigas et al. 1996b; Bel and Artigas 1993). Co-application of 5-HT$_{1A}$ receptor antagonists appears to prevent the initial decrease of 5-HT release in the forebrain during SSRI treatment and may accelerate antidepressant responses in patients (Artigas et al. 1996b; Blier et al. 1998).

These studies suggest a role for DRN 5-HT$_{1A}$ autoreceptors in etiology of depression as well as in the antidepressant action of SSRIs. However, in these studies, radioligand binding essays and in vivo recordings were used that do not allow detection of changes in 5-HT$_{1A}$ receptor function at the cellular level. Moreover, the experiments were performed in normal animals only and not in animal models for depression.

Here we set out to quantify the 5-HT$_{1A}$ receptor function at the cellular level in the DRN, using 1) control rats chronically treated with the SSRI fluoxetine, 2) a social stress animal model for depression, and 3) socially stressed rats chronically treated with fluoxetine. Whole cell patch-clamp recordings of 5-HT and baclophen (BAC)-induced GIRK currents were used as a measure of 5HT$_{1A}$ and GABA$_B$ receptor functionality (Bayliss et al. 1997; Williams et al. 1988). With this approach, we directly tested if 5-HT$_{1A}$-mediated inhibition is changed and whether this change is exclusive for the 5-HT$_{1A}$ pathway or that also the GABA$_B$ pathway is affected. Thereby, we discriminate between changes in receptor levels and changes downstream of the receptor because 5-HT$_{1A}^{-}$ and GABA$_B$...
receptors share the same intracellular pathway through coupling to a pertussis-toxin-sensitive G protein (Innis and Aghajanian 1987; Innis et al. 1988; Williams et al. 1988).

A social stress paradigm in Wistar rats was used, consisting of 1 wk of daily social defeat encounters with a dominant intruder followed by ~3 mo of social isolation. This paradigm induces symptoms resembling anhedonia in depressed patients (Auriaombe et al. 1997), measured as the absence of anticipatory behavior in response to sucrose rewards in these animals (Von Frijtag et al. 2000, 2002). The anhedonia in rodents is a long-term effect that correlates with impaired social memory and interactions (Rygula et al. 2005; Von Frijtag et al. 2000) and is counteracted by imipramine (Von Frijtag et al. 2002) or fluoxetine (Rygula et al. 2006) as also shown in this study. Using this animal model for depression, we investigated the putative relationship among social stress, the effects of chronic fluoxetine, and 5-HT1A and GABA receptors function in the DRN.

Methods

Animals, housing, and conditioning

Male Wistar rats (~8 wk old; HsdCpb:WU, Harlan, the Netherlands) were used in experiments all approved by the Animal Ethical Committee of the Vrije Universiteit in Amsterdam, which act in accordance to European law. The social stress procedure has been described previously (Von Frijtag et al. 2000–2002). In short, the social defeat procedure consisted of daily resident-intruder sessions on five consecutive days. Each defeat session consisted of a forced introduction of an adult male rat (intruder) in the territory of a dominant male rat (resident) and was divided into a pre (5 min), fight (5–10 min), and post (5 min) phase. To minimize the risk of severe injuries, residential male Long-Evans rats (LE/CpbHsd, Harlan, UK) were selected for their fighting tactics. Only those rats that terminated their attacks when the intruder showed submissive behaviors were allowed to the defeat procedure. As part of the social stress paradigm, defeated animals were housed individually in macrolon type III cages (30 cm long, 25 cm wide, and 15 cm high) after the first defeat session, and control rats were housed socially (2 per cage). Three months later, the chronic fluoxetine treatment started, consisting of daily oral gavage with fluoxetine (10 mg/kg in tap water) or vehicle (tap water) only, during a subsequent period of 21 days before the behavioral testing or slice recordings started (fluoxetine HCl was a generous gift from Trifarma SpA (Milan, Italy)). Different animals were used for the behavioral assay and slice recordings to avoid interference of experimental procedures. The behavioral assay consisted of classical Pavlovian conditioning as previously described (Von Frijtag et al. 2000; Van der Harst 2005). A brief conditioning stimulus (CS, 2 × 60 W light-input with 1-s interval followed by an auditory signal) was repeatedly paired with an unconditioned stimulus (US, free access to 5% sucrose solution for 5 min and of which the consumption was measured). During a training period of 10 days, the offset of the CS and the onset of the US were progressively increased from 30 s to 10 min over 32 trials. Behavior was observed and analyzed from videotape using the computer program ‘The Observer’ (Noldus Information Technology, Wageningen, the Netherlands). Behavioral activity displayed in the CS-US interval, reflected by the frequency of behavioral transitions displayed in the time interval between announcement and presentation of the reward, was used as a parameter for anticipation and reflects the level of reward sensitivity (Von Frijtag et al. 2000, 2002). Significant differences within groups between baseline and posttreatment testing was analyzed by paired t-test.

Slice recordings

Rats were anesthetized in a closed compartment with 4 ml Isoflurane (Abbot Laboratories, Queenborough, Kent, UK) sprinkled on a tissue and decapitated using a guillotine. Coronal midbrain slices (400 μm) were prepared in ice-cold slice solution containing (in mM) 3.5 KCl, 2.4 CaCl2, 1.3 MgSO4, 1.2 KH2PO4, 212.5 sucrose, 26 NaHCO3, and 10 nM-glucose, carboxygenated in 5% CO2-95% O2. Slices were transferred to artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 3 KCl, 1.2 NaH2PO4, 2.4 CaCl2, 1.3 MgSO4, 10 nM-glucose, and 25 NaHCO3, supplemented with 20 μM DNQX to prevent overexcitation by glutamatergic inputs. Slices were kept at 37°C for 1 h to recover and were subsequently stored at room temperature. The recording chamber was continuously perfused with ACSF, 20 μM DNQX, and 20 μM bicuculline to block AMPA and GABA receptors. Cells were recorded in the whole cell configuration in voltage- and current-clamp mode. To measure the I-V relation of GIRK currents, slow descending and ascending voltage ramps were applied between −60 and −120 mV in Figs. 1–4 and between −60 and −100 mV in Fig. 5. Patch pipettes had a resistance of 2–2.5 MΩ. The internal solution contained (in mM) 5 NaCl, 1 MgCl2, 130 C6H12O6·K (K-Glucurate), 10 HEPES, 2 Na2-ATP, 0.5 Tris-GTP, and 0.02 EGTA. Series resistance was not compensated. Average access resistance at the end of the recording was 9.6 ± 0.3 (SE) MΩ.

Large DRN neurons were filled through the recording pipette with 120 μM Alexa 594 (Molecular Probes, Eugene, OR) dissolved in intracellular current-clamp medium. After fully loading with dye (15–30 min after break-in), these cells were visualized with a Leica MP-LS 2 photon laser microscope (Leica, Mannheim, Germany), and image projections (at 1 μm intervals) were obtained with Leica LCS software and ImageJ (National Institutes of Health) as described previously (de Rover et al. 2004).

The agents used in this study (5-HT, baclophen, DNQX, bicuculline) were obtained from RBI (Natick, MA). Order of application in Figs. 2–5 was 5 μM 5-HT, wash, 100 μM fluoxetine, or 1 mM BAC. For this study, we performed our recordings in the absence of tryptophan loading (Liu et al. 2005). These conditions, which are unfavorable for sustaining endogenous 5-HT synthesis, make it unlikely that endogenous 5-HT secretion through spontaneous firing of 5-HT have contributed to basal current responses in the absence of exogenous 5-HT application.

Data analysis

Current-voltage (I-V) curves were obtained by averaging the I-V responses during the negative and positive slope of the voltage-clamp protocol in Fig. 1C. Conductances were calculated from the slope of the straight line fitted to the linear part of the I-V curves. 5-HT- and BAC-induced conductances shown in Figs. 2–5 were corrected for basal conductance by subtraction of the basal conductance level at the start of each experiment (reanalyzing the data by normalizing the induced GIRK conductances to the basal conductance in each recording did not change the results; not shown). Basal conductances were not different between the experimental groups shown in Figs. 2–5 (Fig. 2: control + water 1.06 ± 0.07 nS, control + fluoxetine 1.20 ± 0.14 nS; Fig. 3: control + 1.43 ± 0.18 nS, social stress 1.65 ± 0.16 nS; Fig. 4: social stress + water 1.25 ± 0.09 nS, social stress + fluoxetine 1.26 ± 0.07 nS; Fig. 5: control + fluoxetine 1.73 ± 0.54 nS, social stress + fluoxetine 1.65 ± 0.61 nS). For statistical analysis of the effects of different pharmacaa within a given experimental group (i.e., comparison of the effect of 5 vs. 100 μM 5-HT within the control for instance), we used a two sample paired t-test assuming unequal variance (2 tailed). For comparison between experimental groups of the induced effect of 5-HT or BAC at any given concentration and the conductance levels after wash, we used a regular ANOVA followed by a post hoc test (Bonferroni mode) for multiple comparisons. In some instances, to increase statistical power for the data presented, we
pooled the data if experiments were conducted under identical experimental conditions; this was done in particular for data shown in Fig. 2, where the control + fluoxetine data were pooled with those also shown in Fig. 5. Data of Fig. 3 and Fig. 4 could not be pooled or compared because the animal-handling conditions were not identical, i.e., in Fig. 3 experimental animals were not treated orally either with water or fluoxetine, whereas for data obtained for Fig. 4, they were.

The significance found is indicated in the figures with NS, *, ** or *** for not significant, $P < 0.05, 0.01$, and $0.005$, respectively. Error bars show SE. Not shown is the fact that all “wash conductances” were not significantly different between experimental groups and that all 5-HT- or BAC-induced conductances in Figs. 2–5 were significantly different from conductances before application (at $P < 0.01$) unless mentioned otherwise explicitly (see Figs. 4 and Figs. 5, responses to 1 mM BAC). In addition, we used a multivariate ANOVA to test the putative interaction between social defeat and SSRI treatment on GIRK current activation by either 5-HT or BAC. Reanalyzing the data by normalizing the induced GIRK conductances to the basal conductance in each recording did not change the results (not shown).

RESULTS
Assessing 5-HT$_{1A}$ receptor function in DRN neurons: GIRK currents

5-HT-mediated auto-inhibition of serotonergic cells is extensively studied in rodents. 5-HT induces a hyperpolarizing current by activating the 5-HT$_{1A}$ receptor that is coupled to a GIRK channel (Bayliss et al. 1997; Katayama et al. 1997;...
Penington et al. 1993a,b; Williams et al. 1988). Besides 5-HT1A receptors, GABA_B receptors also are coupled to GIRK channels in raphe nucleus 5-HT cells (Bayliss et al. 1997; Innis and Aghajanian 1987; Innis et al. 1988; Williams et al. 1988). This allows an accurate quantification on the cellular level of the 5-HT1A receptor-mediated inhibition relative to the GIRK response.

In whole cell recordings from neurons in the ventral portion of the DRN, we distinguished between 5-HT-containing neurons and non-5-HT neurons based on morphology and electrophysiological characteristics. The putative 5-HT neurons had a morphology of magnocellular neurons (Fig. 1A) and showed membrane potential profiles in response to a series of current steps that were typical of 5-HT neurons (Fig. 1B) (see Li et al. 2001). In our experiments, 89% (n = 196) of the cells displayed the characteristic electrophysiological profile of 5-HT neurons. The vast majority of these cells fired spontaneously at the onset of recordings, unless slices were exposed to tryptophan prior to recording (Liu et al. 2005) (n = 15, not shown). In that case, DRN neurons secrete 5-HT within the nucleus, which hyperpolarizes DRN neurons and reduce spontaneous activity (Johnson et al. 2002; Liu et al. 2005). In the absence of the 5-HT precursor tryptophan, endogenous paracrine 5-HT release is low or absent in DRN slices (Johnson et al. 2002), providing a good starting point for quantitative measures of the exogenous 5-HT-induced GIRK responses. Current responses were quantified by calculating the conductances from the measured I-V curves.

Application of 5-HT induced a robust hyperpolarization of putative 5-HT neurons (Fig. 1C) but not of non-5-HT neurons (not shown). Subsequently, in the same recording, we switched to voltage-clamp and applied descending and ascending voltage ramps, both in the absence and presence of various concentrations of 5-HT (Fig. 1C, bottom). In the presence of 5-HT, the slope of the current response during voltage ramps was much steeper, indicating that 5-HT activated a GIRK conductance as reported previously (Bayliss et al. 1997; Katayama et al. 1997; Penington et al. 1993a,b; Williams et al. 1988).

Subtraction of the control current from the current during 5-HT application yielded the 5-HT-activated current with a reversal potential of $-86 \pm 7$ (SD) mV (Fig. 1D, n = 38). This is in line with the Nernst equilibrium potential for GIRK potassium currents calculated from the intracellular and extracellular solutions used and corrected for the liquid junction potential ($-81$ mV). Comparing conductances at different 5-HT concentrations resulted in a dose-response relation with an EC50 of 31; 100 μM 5-HT, 6.0 ± 0.8, n = 11; wash, 2.0 ± 0.5, n = 10). All induced effects were significantly different from their control (P < 0.05) but not from the induced effects in the other experimental group. The 100 μM 5-HT effect was significantly different from the 5 μM 5-HT effect (*** P < 0.05) but not from the 1 mM BAC effect, within each experimental group.

FIG. 4. Chronic application of fluoxetine to socially stressed animals leads to reduction of both 5-HT- and GABA_B-mediated GIRK responses. A–D: example traces of 5-HT- and BAC-induced currents in socially stressed animals not treated (A and C) or treated with fluoxetine (B and D). E: 5-HT-activated GIRK conductances in socially stressed animals not treated (5 μM 5-HT, 4.7 ± 0.6, n = 19; wash, 0.3 ± 0.2, n = 19; 100 μM 5-HT, 6.3 ± 0.7, n = 11; wash, 0.9 ± 0.3, n = 10) and treated with fluoxetine (5 μM 5-HT, 2.7 ± 0.2, n = 31; wash, 0.3 ± 0.1, n = 31; 100 μM 5-HT, 2.7 ± 0.5, n = 15; wash, 1.4 ± 0.5, n = 8). F: BAC-activated GIRK conductances in socially stressed animals treated not treated (wash, 0.1 ± 0.1, n = 4; 1 mM BAC, 4.1 ± 0.3, n = 4) and treated with fluoxetine (wash, 0.2 ± 0.1, n = 14; 1 mM BAC, 0.4 ± 0.2, n = 13). All induced effects, with the exception of the BAC effect in socially stressed animals treated with fluoxetine, were significantly different from their control (P < 0.05) and from the induced effects in the other experimental group (** P < 0.01, *** P < 0.005). The 100 μM 5-HT effect was significantly different from the 5 μM 5-HT effect (P < 0.05) in the social stress–not treated with fluoxetine–group but not in the social stress–treated with fluoxetine–group. The BAC effect is significantly different from the 100 μM 5-HT effect (P < 0.05) in both groups.
2.7 and 100 μM 5-HT-induced maximal GIRK conductance (Fig. 1E).

**Chronic fluoxetine reduces 5-HT$_{1A}$- and GABA$_B$-induced GIRK-currents**

It has been suggested that during treatment with SSRIs, 5-HT$_{1A}$ receptors on DRN neurons desensitize (Elena Castro et al. 2003; Hensler 2002; Le Poul et al. 1995; Pejchal et al. 2002; Shen et al. 2002; Subhash et al. 2000). To test whether fluoxetine treatment would reduce 5-HT$_{1A}$ receptor-mediated inhibition, healthy rats were treated for 21 days with daily oral administrations of fluoxetine. The 5-HT-induced GIRK responses were probed with two 5-HT concentrations: 5 μM, close to the EC$_{50}$, and 100 μM. In line with data shown in Fig. 1E, in DRN slices from water-treated animals 100 μM 5-HT induced a significantly larger GIRK conductance than 5 μM 5-HT (Fig. 2, A, B, and E). In contrast, in fluoxetine-treated animals, paired comparison of the GIRK responses induced by these two 5-HT concentrations showed that they were not significantly different (Fig. 2, A, B, and E). Moreover, whereas the response to 5 μM 5-HT on chronic fluoxetine was similar as in the water-treated group, the 100 μM 5-HT response was significantly reduced. This implies that 5-HT$_{1A}$ receptor functionality is reduced after chronic fluoxetine treatment.

To examine whether the effect of fluoxetine treatment was unique to the 5-HT$_{1A}$ receptor pathway, we also tested whether the GABA$_B$ receptor-induced GIRK currents were affected. GIRK responses induced by a maximal dose of the GABA$_B$ receptor agonist BAC (1 mM) were significantly reduced in 5-HT neurons after fluoxetine treatment (Fig. 2, C, D, and F). This suggests that fluoxetine treatment affects 5-HT$_{1A}$ receptor functionality downstream of the 5-HT$_{1A}$ receptor and at a level of the signaling pathway that is shared with the GABA$_B$ receptor to the GIRK pathway.

Recently, it was claimed (Takahashi et al. 2006) that fluoxetine may suppress basal GIRK conductance acutely and directly. To test whether the suppression of the maximal GIRK current activation in our experiments was due to a chronic, rather than to an acute or direct effect of fluoxetine, we measured basal conductance levels before and after the acute application of 33 μM fluoxetine for 4 min. This concentration corresponds to the theoretical maximal concentration of fluoxetine when a dose of 10 mg/kg is used in vivo and is ~100 times higher than plasma levels of fluoxetine measured after 10 mg/kg ip or with osmotic minipumps (Czachura and Rasmussen 2000). We found that acute exposure to fluoxetine increased, rather than decreased, the normalized basal conductance level or of 5-HT receptor-mediated GIRK currents. This suggests that fluoxetine treatment affects 5-HT$_{1A}$ receptor function of the signaling pathway that is shared with the GABA$_B$ receptor to the GIRK pathway.

**Social defeat does not affect 5-HT$_{1A}$ receptor responses**

To test whether 5-HT$_{1A}$ receptor function is affected in DRN neurons of animals that exhibit symptoms of depression, we recorded 5-HT$_{1A}$-induced GIRK currents in DRN 5-HT neurons from rats that were subjected to a social stress paradigm (Von Frijtag et al. 2000, 2002). These rats were compared with control rats that had not received social defeat encounters nor isolation. It has previously been reported that rats subjected to this social stress paradigm show anhedonia, a symptom of depression, reflected by a suppression of anticipatory behavior in expectation of a reward. In the present experiments, socially stressed rats were also observed to show a suppressed anticipatory behavior as an indication for anhedonia (Table 1). When 5-HT$_{1A}$-induced GIRK currents were recorded in DRN 5-HT neurons, no differences were found between control and socially stressed animals (Fig. 5).

**TABLE 1. Relief from anhedonia symptoms upon chronic fluoxetine treatment in socially stressed animals**

<table>
<thead>
<tr>
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<th>Baseline (μA ± SE)</th>
<th>Post-Training (μA ± SE)</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Social defeat</td>
<td>16.9 ± 1.09</td>
<td>16.0 ± 0.82</td>
<td>0.4 (n = 9)</td>
</tr>
<tr>
<td>Social defeat + fluoxetine</td>
<td>17.0 ± 1.01</td>
<td>21.1 ± 0.92</td>
<td>0.02 (n = 10)</td>
</tr>
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Mean number of transition between behavioral elements (±SE), displayed in the interval (10 min) between the announcement (light+sound) and the arrival of the reward (sucrose 5%, 5 min), reflecting baseline activity at trial 0 and anticipatory activity (post-training) in expectation of the reward at trial 32.
socially stressed animals. In both groups, GIRK currents were activated by 5-HT in a dose-dependent manner and to a similar extent (Fig. 3, A, B, and E). This suggests that the social stress paradigm does not change 5-HT1A receptor function in DRN 5-HT neurons. Similarly, the maximal GIRK conductance induced by the GABA_B receptor agonist BAC was also not affected on social defeat stress (Fig. 3, C, D, and F). Thus the induction of depression-like symptoms in rats by social defeat stress does not affect 5-HT1A receptors or one of the downstream components.

Rescue of anticipatory behavior after social stress by fluoxetine correlates with reduced 5-HT1A and GABA_B receptor functionality

This insensitivity for reward (anhedonia), reflected by the impaired anticipatory behavior in socially stressed rats was previously shown to be reversed by chronic treatment of the antidepressant imipramine (Von Frijtag et al. 2002). We also observed relief from anhedonia-like symptoms in socially stressed rats after treatment with fluoxetine for 21 days. In fluoxetine-treated animals, anticipatory behavior toward sucrose rewards was restored after 3 wk of treatment, whereas in vehicle-treated animals anticipate behavior remained impaired (Table 1). No effect of social stress and/or fluoxetine treatment on the intake of 5% sucrose consumption was observed (data not shown). To test whether the behavioral effect of chronic fluoxetine treatment correlated with a cellular effect of fluoxetine in the DRN, we examined GIRK currents induced by 5-HT and BAC in DRN 5-HT neurons of animals subjected to the same social stress paradigm with or without subsequent chronic fluoxetine treatment (Fig. 4, A, B, and E). In 5-HT neurons of socially stressed fluoxetine-treated animals, 100 µM 5-HT did not produce larger responses than 5 µM 5-HT (Fig. 4, B and E), similar to the effect seen of chronic fluoxetine treatment in control animals (Fig. 2B and E). Moreover, the responsiveness to either 5 or 100 µM 5-HT was significantly reduced when compared with socially stressed animals not treated with fluoxetine (Fig. 4E). Thus the 5-HT responsiveness of DNR neurons in terms of 5-HT-induced GIRK activation appeared to be suppressed by fluoxetine-treatment in socially stressed animals, similar to the apparent effect of chronic fluoxetine in control animals. However, the apparent relative effect of fluoxetine on social stress appeared to be larger than in control animals (compare Figs. 4E and 2E; see also following text).

To test whether fluoxetine treatment reduced 5-HT1A receptor functionality downstream of the 5-HT1A receptor, we also examined BAC-induced GIRK currents. Similar to the effect of fluoxetine treatment in healthy rats, on social stress conditioning, the BAC-induced GIRK currents were significantly reduced in 5-HT neurons after fluoxetine treatment (Fig. 4, C, D, and F). This suggests that in social defeated animals a reduced functionality of the 5-HT1A receptor coupling to GIRK induced by fluoxetine treatment is expressed downstream of the 5-HT1A receptor and most likely at a level of the signaling pathway that is shared with the GABA_B receptor to the GIRK-pathway. Again the relative effect of fluoxetine on social defeat appeared larger than in control animals (compare Figs. 4F and 2F).

Fluoxetine acts on 5-HT1A receptor functionality independent of social conditions

Fluoxetine appears to have mood-elevating effects predominantly in depressive patients. In healthy human subjects, no significant effects of fluoxetine on mood and other psychological variables were found (Gelfin et al. 1998). As indicated in the preceding text, in our experiments, fluoxetine treatment appeared to affect 5-HT and GABA_B GIRK activation in DRN 5-HT neurons of both control and socially stressed rats but possibly to a different extent. We directly addressed the hypothesis that fluoxetine treatment has a stronger impact on 5-HT1A receptor responses in DRN 5-HT neurons of animals on social stress compared with control conditioning. To this end, we tested 5-HT-induced GIRK activation in fluoxetine-treated, socially stressed rats and in fluoxetine-treated, control rats. Recordings of DRN 5-HT neurons of these animals showed that, whereas µM 5-HT-induced GIRK responses were significantly different from basal levels, the 5-HT-induced GIRK conductance at 5 and 100 µM were not different in either group (Fig. 5, A and B). Thus no significant differences were found in the relative effect of fluoxetine between the control and socially stressed groups (Fig. 5E). Similarly, BAC-induced GIRK currents were similarly suppressed in both groups (Fig. 5F), indicating that also in these experiments fluoxetine acted downstream of the 5-HT1A receptor. The BAC-induced GIRK conductance change was also not different from 100 µM 5-HT responses, either in control and in socially stressed animals (Fig. 5, E and F). These data show that fluoxetine acts on DRN 5-HT neurons independent of whether animals show symptoms of depression.

Subsequent multivariate ANOVA revealed no interaction between social defeat and fluoxetine treatment. Simple contrast (K matrix) testing, for all data pooled in this study, showed no significant effect of social stress (compared with controls, P = 0.563 and P = 0.154). Basal conductances were not different between social stress and control animals. In contrast, chronic treatment with fluoxetine produced robust overall effects (P = 0.006 and P = 0.001, respectively, for 5 and 100 µM 5-HT, with no effects on basal responses). Additional multivariate analysis of possible interaction between social stress and chronic SSRI treatment revealed no significance. This confirms that DRN 5-HT neurons in socially stressed animals are not more sensitive to fluoxetine treatment than 5-HT neurons of control animals.

Discussion

In this study, we addressed the question of whether 5-HT1A receptor function in dorsal raphé nucleus 5-HT neurons is affected in animals that show symptoms of depression and on fluoxetine treatment. Rats exposed to social stress exhibited behavioral symptoms of depression that could be relieved by fluoxetine treatment. In socially stressed animals, neither 5-HT1A nor GABA_B receptor-induced GIRK currents in DRN 5-HT neurons were different in amplitude from GIRK currents in healthy animals. However, when these animals were treated with fluoxetine for 21 days, both 5-HT1A and GABA_B receptor-mediated GIRK responses were markedly reduced. This implies that fluoxetine acts on downstream components shared by both receptor types. We found that fluoxetine treatment also...
affects both 5-HT1A and GABA_B receptor functionality in 5-HT neurons of healthy animals. When directly tested, the effects of fluoxetine treatment on 5-HT1A and GABA_B-induced GIRK currents were not different in healthy and socially stressed animals, indicating that the action of fluoxetine is independent of the animal’s “depressed” state.

5-HT1A receptor function in the DRN and depression

Previous studies on depressed patients suggest either a positive or an inverse correlation between chronic depression and 5-HT1A receptor levels in the brain. In postmortem brains of patients suffering from major depression, increased (Stockmeier et al. 1998) and decreased (Arango et al. 2001) levels of 5-HT1A receptors were reported. In patients with bipolar or late-life depression, reduced levels of 5-HT1A receptors were found (Drevets et al. 1999, 2000; Meltzer et al. 2004). In socially stressed rats that show behavioral symptoms of depression, we found no changes in levels or functionality of 5-HT1A receptors at the level of DRN neurons. Our results suggest that in DRN neither receptor levels nor downstream components, such as G proteins or GIRK channel activity, are affected after severe social stress. This suggests that the behavioral symptoms of depression observed in these animals, such as a loss of reward sensitivity, an impaired social memory, and social interaction behavior that resemble anhedonia and motivational deficits in humans (Von Frijtag et al. 2000–2002), are brought about by other mechanisms than an alteration in 5-HT1A-receptor signaling in DRN 5-HT neurons. These results appear in line with findings in monkeys that show symptoms of behavioral depression. In these animals, 5-HT1A receptor binding at the level of the raphé nucleus (Shively et al. 2006) were not affected.

Chronic treatment with the SSRI fluoxetine did affect 5-HT1A receptor functionality in DRN 5-HT neurons, both in healthy and socially stressed animals. Fluoxetine treatment also restored anticipatory responses in the latter group (Table 1), just as the antidepressant imipramine reverses the symptoms of anhedonia after social stress (Von Frijtag et al. 2001). Previous studies have shown that fluoxetine treatment affects 5-HT1A receptor-stimulated G protein binding in the DRN in healthy rats (Elena Castro et al. 2003; Hensler 2002; Pejchal et al. 2002; Shen et al. 2002). In transgenic mice lacking 5-HT transporter protein, similar adaptive changes in 5-HT1A receptor signaling were found in DRN (Fabre et al. 2000; Gobbi et al. 2001; Holmes et al. 2003; Li et al. 2000; Mannoury la Cour et al. 2001, 2004). Recently, it was confirmed that fluoxetine treatment can alleviate behavioral deficits evoked by chronic social stress in rats (Rygula et al. 2006). How reduced 5-HT1A receptor functionality in the DRN contributes to the suppression of depressive symptoms in socially stressed animals is not clear. However, previous studies show a clear relation between 5-HT1A receptor-mediated autoinhibition at the DRN level and 5-HT levels in the projection areas. 5-HT levels in DRN innervated regions and the activity of serotonergic neurons are both reduced after acute application of SSRIs (Artigas et al. 1996a,b; Czachura and Rasmussen 2000). This is attributed to the inhibiting action of 5-HT1A receptors that are activated by the increased concentration of 5-HT in the DRN after blocking 5-HT reuptake. Long-term treatment with SSRIs produces a robust increase in extracellular 5-HT levels in the projection areas and restores firing activity of serotonergic cells, which is consistent with the desensitization of 5-HT1A receptors in the DRN (Bel and Artigas 1993; Czachura and Rasmussen 2000; Ferrer and Artigas 1994). The similar time course of 5-HT levels in frontal cortex and the antidepressant response by SSRIs suggests that these are related. This idea is supported by the fact that coapplication of SSRIs with a 5-HT1A antagonist like pindolol prevents the initial reduction of 5-HT in forebrain projection areas and accelerates antidepressant action (Artigas et al. 1996b). Our findings therefore suggests that the restoration of hedonic behavior in socially stressed animals after treatment with fluoxetine might possibly be due to augmented serotonergic output of DRN as a result of reduced 5-HT1A receptor functionality in DRN.

Action of fluoxetine at the cellular level

The observed reduction of 5-HT-induced GIRK currents after chronic SSRI treatment may result from desensitization of 5-HT1A receptors or may be due to desensitization of a component downstream of the receptor. The fluoxetine-induced reduction in 5-HT1A receptor functionality is paralleled by a reduction in GABA_B receptor-mediated GIRK activation after treatment with fluoxetine, both in healthy as well as in socially stressed animals. Although a parallel effect of fluoxetine on both receptors cannot be excluded it is more likely that fluoxetine treatment affects 5-HT1A receptor signaling in DRN 5-HT neurons downstream of the receptors because both 5-HT1A and GABA_B receptors couple to the same type of GIRK channel through G_i proteins in these neurons (Innis and Aghajanian 1987; Innis et al. 1988; Williams et al. 1988). Radioligand binding assays suggest that chronic fluoxetine treatment reduces the coupling of 5-HT1A receptors and G proteins without changing 5-HT1A-receptor levels (Elena Castro et al. 2003; Hensler 2002; Pejchal et al. 2002; Shen et al. 2002). In transgenic mice in which 5-HT reuptake is prevented by knocking out the 5-HT transporter (5-HT null mice), functionality of 5-HT1A as well as GABA_B receptors is strongly reduced in DRN 5-HT cells (Mannoury la Cour et al. 2004), supporting the idea that both receptor types partly share a common signal transduction pathway in 5-HT neurons.

Thus far, the best supported candidate mechanism by which fluoxetine treatment leads to inhibition of GIRK channel activity is through uncoupling of G_i proteins from their receptors (Elena Castro et al. 2003; Hensler 2002; Pejchal et al. 2002; Shen et al. 2002). Alternative mechanisms could be through PKC-dependent phosphorylation of GIRK channels (Mao et al. 2004), depletion of PIP2, an endogenous co-factor necessary for GIRK channel activity (Cho et al. 2005; Lei et al. 2003) or through a glucocorticoid-mediated modulation of GIRK responsiveness (Fairchild et al. 2003). Recently the background potassium channel TREK-1 was proposed as a target for antidepressant action in DRN neurons (Heurteaux et al. 2006). In this model, fluoxetine acts as antidepressant through direct inhibition of TREK-1 channels. However, in our experiments, we could not detect a direct inhibition of background whole cell potassium currents by fluoxetine in DRN 5-HT neurons that would support this model.
GABA<sub>B</sub> receptors and the action of SSRIs

There is an emerging body of data purporting a role for GABA<sub>B</sub> receptors as potential targets for antidepressant actions (Mombereau et al. 2004a, 2005; Petty 1995; Slattery et al. 2005). Although previous studies reported no change in GABA<sub>B</sub> receptor levels (Cross et al. 1988) or functionality (Monteleone et al. 1990) in depressed patients, more recent studies showed antidepressant effects of GABA<sub>B</sub> antagonists. Transgenic mice lacking either GABA<sub>B1</sub> or GABA<sub>B2</sub> receptor subunits show enhanced resistance against symptoms of depression in the forced swim test (Mombereau et al. 2004b, 2005; Slattery et al. 2005). We show that chronic SSRI treatment suppresses GABA<sub>B</sub> receptor functionality of DRN neurons, confirming a possible role for GABA<sub>B</sub> receptors as target for antidepressants. Because local GABAergic interneurons in the DRN are activated by 5-HT through 5-HT<sub>2A/C</sub> receptors (Liu et al. 2000), GABA signaling through GABA<sub>B</sub> receptors is likely to provide an additional negative feedback loop of 5-HT neuron activity. Therefore reduction of GABA<sub>B</sub> receptor functionality may have a similar disinhibiting effect of 5-HT neuron activity as decreased 5-HT<sub>1A</sub> receptor functionality. This model may further suggest that co-application of GABA<sub>B</sub> antagonists might speed up the antidepressant action of SSRIs by blocking GABA<sub>B</sub> receptor activity.

Significance from a clinical perspective

The prevalent view on the cellular mechanisms underlying the origin of depression in humans and the cellular mechanisms of SSRI treatment is that they both reside in the serotonergic system. Our results suggest that these mechanisms could diverge. On the one hand, severe social stress can induce behavioral symptoms of depression, such as anhedonia, without affecting 5-HT<sub>1A</sub> receptor functionality in 5-HT neurons. On the other hand, fluoxetine treatment can alleviate these symptoms of depression by reducing 5-HT<sub>1A</sub> receptor functionality in DRN 5-HT neurons. Thus although the possibility remains that stress hormones affects components in the serotonergic cell system other than GIRK currents (Abrams et al. 2005; Lowry et al. 2000), it becomes more and more likely that nonaminergic pathways located in target areas of the DRN may also be involved (Berton and Nestler 2006). Also in the latter model, antidepressants are thought to act primarily on the serotonergic system (Duman 2002; Duman et al. 1999). Cause and therapy of depression may therefore diverge. Using animal models, such as social stress in rodents, to identify the neuronal mechanisms underlying both cause and therapy of mood disorders is indispensable to eventually get from palliative to curative treatments of these disorders in the future.

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References


