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A study of time- and sex-dependent effects of vortioxetine on rat sexual behavior: Possible roles of direct receptor modulation

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ABSTRACT

Treatment-related sexual dysfunction is a common side effect of antidepressants and contributes to patient non-compliance or treatment cessation. However, the multimodal antidepressant, vortioxetine, demonstrates low sexual side effects in depressed patients. To investigate the mechanisms involved, sexual behavior was assessed in male and female rats after acute, and repeated (7 and 14 days) treatment with vortioxetine, flesinoxan (a 5-HT_{1A} receptor agonist), CP-94253 (a 5-HT_{1B} receptor agonist), or ondansetron (a 5-HT_3 receptor antagonist). These selective ligands were chosen to simulate vortioxetine's direct modulation of these receptors. Paroxetine was also included in the male study. Acute and repeated treatment with vortioxetine at doses corresponding to clinical levels (based on serotonin transporter occupancy) had minimal effects on sexual behavior in male and female rats. High dose vortioxetine plus flesinoxan (to mimic predicted clinical levels of 5-HT_{1A} receptor occupancy by vortioxetine) facilitated male rat sexual behavior (acutely) while inhibiting female rat proceptive behavior (both acutely and after 14 days treatment). The selective serotonin reuptake inhibitor, paroxetine, inhibited male sexual behavior after repeated administration (7 and 14 days). Flesinoxan alone facilitated male sexual behavior acutely while inhibiting female rat proceptive behavior after repeated administration (7 and 14 days). CP-94253 inhibited sexual behavior in both male and female rats after repeated administration. Ondansetron had no effect on sexual behavior. These findings underline the complex serotonergic regulation of sexual behavior and indicate that the low sexual side effects of vortioxetine found in clinical studies are likely associated with its direct modulation of serotonin receptors.

1. Introduction

Major depressive disorder (MDD) is one of the most common mental disorders and carries a heavy burden of disability in adults (Global burden of disease study 2013 collaborators, 2015). The selective serotonin (5-HT) reuptake inhibitor (SSRI) and serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressants first launched in the 1980s and 1990s are still the current first-line treatments for MDD. However, there is a pressing need for improved treatment options. Only about 50% of MDD patients achieve clinical remission following initial antidepressant treatment, regardless of the drug chosen (Rush et al., 2006). After four consecutive antidepressant treatment attempts, the overall cumulative remission rate is only about 67% (Rush et al., 2006). In addition, the therapeutic response is often delayed, usually requiring several weeks of treatment. Furthermore, drug-related adverse effects are common (Anderson et al., 2012). There is a high prevalence of persistent sexual dysfunction related to antidepressants in both female and male patients, especially those drugs inhibiting the serotonin transporter (SERT) (Serretti and Chiesa, 2009). This adverse effect is a common reason for treatment cessation or noncompliance in patients (Ashton et al., 2005). Therefore, more efficacious MDD treatments with fewer sexual side effects would be extremely valuable.

Several clinical studies examined the treatment-emergent

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sexual dysfunction associated with the multimodal antidepressant, vortioxetine. There was a relatively low incidence of self-reported sexual dysfunction in MDD patients receiving vortioxetine treatment (Baldwin et al., 2016; Sanchez et al., 2015). A study by Jacobsen and colleagues focused on adult MDD patients who experienced treatment-emergent sexual dysfunction despite reduction in depressive mood during SSRI treatment. This study demonstrated that switching to vortioxetine resulted in a greater improvement in sexual function than switching to another SSRI, citalopram (Jacobsen et al., 2015). Furthermore, a recent pooled analysis concluded that there was no increased risk for patients to develop treatment-emergent sexual dysfunction when treated with vortioxetine at any dose, compared to patients that received placebo. In contrast, the risk was increased in patients treated with 60 mg/day duloxetine (Jacobsen et al., 2016). It is plausible that the distinct mechanisms of action of these different classes of antidepressants may underlie their differential effects on sexual function.

The aim of the present study was to use a preclinical model to investigate which of vortioxetine's mechanisms may be related to the low incidence of sexual dysfunction observed in clinical studies. Animal models are critical to obtain objective and quantitative measures of sexual function and to establish the putative roles of different 5-HT receptor subtypes. Vortioxetine is an antidepressant with a multimodal mechanism of action (i.e., an antidepressant exerting its pharmacological actions through two or more different target classes (Zohar et al., 2014)). In addition to being a SERT inhibitor, vortioxetine is a 5-HT_{1A} receptor agonist, a 5-HT_{1B} receptor partial agonist, and a 5-HT_{1D}, 5-HT_3 and 5-HT_7 receptor antagonist (Sanchez et al., 2015). Clinical and preclinical research indicates that several of these 5-HT receptor subtypes are involved in regulating sexual behavior, and the effects of these receptor systems are sex-dependent (Olivier et al., 2011). Although the acute effects of 5-HT_{1A} and 5-HT_{1B} receptor modulation have been reported, there is a paucity of data regarding their chronic effects. As antidepressants typically require several weeks to exert effects in patients, it is important to assess sexual behavior after chronic modulation of the aforementioned receptors. To this end, we examined sexual behaviors in male and female rats after acute, subchronic (7 days) and chronic (14 days) vortioxetine treatment. In addition, the putative roles of 5-HT_{1A}, 5-HT_{1B} and 5-HT_3 receptors on sexual behavior were assessed by examining the effects of selective ligands (flesinoxan, CP-94253, and ondansetron, respectively) following the same treatment schedule.

In the present study, doses of vortioxetine were chosen based on clinical practice. The low vortioxetine dose (corresponding to 5 mg per day clinical dose) aimed to achieve 50% SERT occupancy. The high vortioxetine dose (corresponding to 20 mg per day clinical dose) aimed to achieve 90% SERT occupancy (Sanchez et al., 2015). Notably, it has been reported that the affinity of vortioxetine for the rat 5-HT_{1A} receptor is markedly lower than its affinity for the human 5-HT_{1A} receptor (Sanchez et al., 2015). Since 5-HT_{1A} receptors are involved in the modulation of some aspects of sexual function (Giuliano and Clement, 2005; Landen et al., 1999; Snoeren et al., 2014a, b), a high dose vortioxetine plus flesinoxan (a 5-HT_{1A} receptor agonist) group was included in this study to better mimic the effects of highest dose of vortioxetine in humans. Doses of flesinoxan, CP-94253, and ondansetron were chosen based on results from pilot studies, to match the levels of receptor occupancy at these targets by vortioxetine at clinically relevant doses (du Jardin et al., 2014; Leiser et al., 2014).

Reference compounds were included in this study. Paroxetine, a standard SSRI, was included as a positive control for sexual dysfunction in male rats. However, although paroxetine has been shown to inhibit male sexual behavior after chronic administration, it does not affect sexual behavior in female Wistar rats. In contrast, acute 8-OH-DPAT inhibits female sexual behavior (Snoeren et al., 2011). Therefore, acute administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT was included in the female study, to ensure deficits in sexual dysfunction could be detected. Sexual behaviors were assessed in male rats focusing on mounts, intromissions and ejaculations (Bijlsma et al., 2014; Chan et al., 2009). In female rats, sexual behaviors were assessed in a paced mating paradigm (Snoeren et al., 2011), focusing on proceptive behaviors rather than receptive behaviors. The proceptive behaviors (characterized by 'ear wiggling', darting and hopping) are more related to sexual motivation, while the often measured lordosis is more of a direct assessment for copulatory function (Martinez and Paredes, 2001).

2. Material and methods

2.1. Animals

Male and female Wistar Crl/Wu rats (Charles River, Germany) were group-housed (4/cage) with a reversed light cycle (lights off from 6:00 a.m. to 6:00 p.m.), upon arrival at approximately 10 weeks of age. Animals had *ad libitum* access to rodent chow and tap water. The study was reviewed and approved by Utrecht University's animal welfare committee.

2.2. Study design

Rats were randomly allocated to the following treatment groups (n = 12–16 per group, Table 1): vehicle (Purina 5001 chow and saline s.c.), low dose vortioxetine (1 mg/kg oral gavage once followed by p.o. administration via food pellet [176 mg vortioxetine per kg Purina 5001 chow, equivalent to approximately 1 mg/kg/day]), high dose vortioxetine (10 mg/kg oral gavage followed by p.o. administration via food pellet [600 mg vortioxetine per kg Purina 5001 chow, equivalent to approximately 10 mg/kg/day]), high dose vortioxetine (same treatment regimen as above) + flesinoxan (2.5 mg/kg b.i.d, s.c.), flesinoxan (2.5 mg/kg b.i.d., s.c.), CP-94253 (5 mg/kg daily, s.c.), and ondansetron (1 mg/kg b.i.d., s.c.). The initial doses of vortioxetine were given by p.o. gavage 60min before the test, as rats would not eat enough drug containing food pellets immediately on first exposure (due to neophobia). Paroxetine (10 mg/kg once a day p.o. gavage in males) and 8-OH-DPAT (0.1 mg/kg s.c. in females on the days of behavioral test, 30min before test) were included as they are known to inhibit sexual behaviors in male and female rats, respectively. All drugs were synthesized by H. Lundbeck A/S (Valby, Denmark), except CP-94253 (Tocris, Minneapolis, MN), ondansetron and paroxetine (Sigma-Aldrich, St. Louis, MO). *Ex vivo* autoradiography was used to measure SERT occupancy to confirm target engagement of vortioxetine and paroxetine. Sexual behaviors were assessed after the first drug treatment (acute), 7 days (subchronic) and 14 days (chronic) of treatment for all groups, except the female 8-OH-DPAT group. This group only received 8-OH-DPAT on the days of behavioral test, 30 min prior to the test sessions.

2.3. Rodent sexual behavior test

The sexual behavior in male and female rats was assessed using the testing paradigm established previously (Chan et al., 2009; Snoeren et al., 2011).

2.3.1. Training and selection of males

Male rats were first trained weekly for 5 consecutive weeks, by exposing them to an estrous female for 30 min in an observation cage (30 × 40 × 60 cm) with a Plexiglas front. Females were brought into estrus with estradiol (Sigma-Aldrich) (50 μg s.c. in the