Progress in Neuro-Psychopharmacology & Biological Psychiatry 44 (2013) 81–87

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Sex- and endocrine-stage-differences in middle-aged rats in an animal model of OCD

Sandra Olvera-Hernández [a], Roberto Chavira [b], Alonso Fernández-Guasti [a,*]

[a] Departamento de Farmacobiología, Centro de Investigación y Estudios Avanzados, Mexico
[b] Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán," México D.F., Mexico

ARTICLE INFO

Article history:
Received 22 November 2012
Received in revised form 28 January 2013
Accepted 31 January 2013
Available online 8 February 2013

Keywords:
8-OH-DPAT
Fluoxetine
Middle-age rats
Obsessive-compulsive disorder
Spontaneous alternation

ABSTRACT

Various clinical studies suggest that many features of OCD are influenced by sex, age and fluctuations in hormonal levels. Animal models have confirmed these differences, and suggest they are mediated by the serotonergic system. We compared the perseveration behavior in a T-maze after the administration of the 5-HT_{1A} agonist, 8-OH-DPAT (2.0 mg/kg) and the preventive action of subchronic fluoxetine (10 mg/kg, 3 times) in middle-aged (11–14 months) malesand female rats in two endocrine states: irregular cycles (tested in diestrus) or persistent diestrus. After 8-OH-DPAT, females with persistent diestrus presented higher perseveration scores than males and females with irregular cycles. Fluoxetine produced an anticompulsive-like effect only in females with persistent diestrus. Females in persistent diestrus showed higher estradiol levels than those in irregular cycles or males. In all groups 8-OH-DPAT increased ambulation and fluoxetine did not modify this action. In males, the combined administration of fluoxetine and 8-OH-DPAT impaired motor coordination. Data are discussed on the basis of estradiol levels and sex differences.

1. Introduction

Obsessive compulsive disorder (OCD) is a chronic and often disabling disorder characterized by intrusive, unwanted and recurrent thoughts (obsessions) and repetitive ritualistic behaviors (compulsions) (American Psychiatric Association, 2000). Obsessions and compulsions are time consuming and interfere with occupational and social activities. The prevalence of OCD is from 1 to 3% worldwide (Bijl et al., 1998; Kessler et al., 2005; Stein et al., 1997).

Various studies suggest that in women the occurrence of reproductive life events such as menarche, menstruation, pregnancy, parturition and menopause influences the increased risk for the onset and the particular symptoms of psychiatric disorders (Avis et al., 1994; Callegari et al., 2007; Soares and Zitek, 2008). Many features of OCD are influenced by the sex, age and endocrine state. For example, boys generally have an earlier disease onset (Burke et al., 1990; Hanna, 1995; Rasmussen and Eisen, 1990) and men have more sexual- and symmetry/exactness-obsessions and checking-, bizarre-compulsions than women. In contrast, women are more prone to have cleaning obsessions and contamination compulsions (Bogetto et al., 1999; Labad et al., 2008; Lensi et al., 1996). Furthermore, symptoms of OCD are initiated or exacerbated at menarche and postpartum (Labad et al., 2005; Uguz et al., 2010; Williams and Koran, 1997). Animal models for the study of OCD have confirmed differences according to sex, age, estrous cycle phase and the reproductive stage (Agrati et al., 2005; Fernández-Guasti et al., 2003, 2006; Flaisher-Grinberg et al., 2009). These data have been interpreted on the basis of the modulatory role of sex hormones on the serotonergic system, one of the neurotransmitter systems more clearly implicated in the pathogenesis of OCD (Hollander et al., 1992; Pigott et al., 1991; Zohar et al., 1987, 1992).

In the world, the proportion of 65+ year-old people is gradually increasing (Bongaarts, 2009; Cohen, 2003) but in this population the incidence/follow-up OCD cases and its response to pharmacological treatment are poorly known. OCD is primarily manifested during childhood and adolescence, with a higher number of cases in males, and continues during adult life with equal sex distribution (Bijl et al., 1998; Heyman et al., 2003; Ruscio et al., 2010). Interestingly some studies suggest that the prevalence of OCD in the elderly is higher among women than among men (Kirmizioglu et al., 2009; Nestadt et al., 1998; Regier et al., 1988; Tolin et al., 2005), indicating that there is a relatively high rate of new cases in elderly women. Vulink et al., in, 2006 reported that in women OCD symptoms are exacerbated during menopause. Although Calamari et al. (1994) described that the elderly respond to serotoninergic and tricyclic antidepressants for the treatment of OCD, other reports indicate that during aging the efficacy of antidepressants for treating mood disorders is reduced (Herrera-Pérez et al., 2010;

Abbreviations: OCD, obsessive-compulsive disorder; SAB, spontaneous alternation behavior; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; SSRI, selective serotonin reuptake inhibitor; 5-HT, 5-hydroxytryptamine (serotonin); FSH, follicle-stimulating hormone; LH, luteinizing hormone.

* Corresponding author at: Centro de Investigación y Estudios Avanzados, Departamento de Farmacobiología, Calzada de los Tenorios 235, Colonia Granjas Coapa, México 14330 D.F., Mexico. Tel.: +52 55 54 83 28 70; fax: +52 55 54 83 28 63.
E-mail address: jfernand@cinvestav.mx (A. Fernández-Guasti).

0278-5846/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pnpbp.2013.01.021

Nelson et al., 1995; Reynolds et al., 1996). Furthermore, even if antidepressants treatment does not alter motor coordination in young adult experimental animals (Bomholt et al., 2005; Brocco et al., 2002), clinical studies have shown progressive increase in the risk of fractures with increasing doses of SSRIs (Vestergaard et al., 2006). In the elderly treatment with SSRIs and tricyclics is a key factor associated with falls and fractures that are associated with high morbidity and mortality in this population (Bolton et al., 2008; Darowski et al., 2009; Hanlon et al., 2009; Liu et al., 1998; Ruthazer and Lipsitz, 1993). Pharmacological treatment of OCD requires high doses of antidepressants, compared to those used for the treatment of mood disorders (Neel et al., 2002), that have been correlated with an increase in the incidence of motor alterations (Tollefson et al., 1994).

During midlife there are several endocrine events that exacerbate the rate of loss of the ovarian follicular reserve leading to a decrease fertility and fecundity. In female rodents the incidence of regular estrous cyclicity decreases progressively during aging. After 10 months of age, females display prolonged irregular cycles (Matt et al., 1986), a period named periestropause or recurrent pseudopregnancy and characterized by high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in comparison with young female rats in diestrus (Bestetti et al., 1991). This stage is analogous to perimenopause or menopause transition in women where menstrual cycles irregularities are related with increased levels in FSH, LH and estradiol (Prior, 1998; Rannevik et al., 1995). The state of complete loss of reproductive capacity in rodents is called estropause and characterized by persistent vaginal diestrus (Lu et al., 1979; vom Saal and Finch, 1988). Lu et al., 1979; Bestetti et al., 1991 reported similar low levels of estrogens between young females in diestrus and aged rats in persistent diestrus; however, other authors found high estradiol levels in middle aged diestrus rats as compared with young females in the same estrous-cycle phase (Gore et al., 2000; Wise and Ratner, 1980). These differences seems to depend upon the rat's strain, age and hormone assay sensitivity (Bestetti et al., 1991; Gore et al., 2000; Lu et al., 1979; Wise and Ratner, 1980). Similarly, in male rats, as in men, there is a gradual reduction in testosterone levels during aging (Baulieu, 2002; Herrera-Pérez et al., 2008).

An animal model used for studying OCD is the disruption of spontaneous alternation behavior (SAB). SAB refers to the natural tendency of rats to sequentially explore two equally rewarded arms in a T maze. In this paradigm, SAB is impeded after the administration of the 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) without affecting memory performance in the Morris water maze (Yadin et al., 1991). This model of OCD has similarities with specific characteristics of OCD in humans, such as perservative behaviors (compulsions) and indecision (Seibell et al., 2003). One of the main source of validity for this model refers to the pharmacological evidence revealing that the subchronic and chronic treatment with clomipramine and fluoxetine, drugs that are effective in the treatment of OCD, prevent 8-OH-DPAT-induced perseveration (Fernández-Guasti et al., 2003, 2006; Ulloa et al., 2004; Umathe et al., 2009; Yadin et al., 1991), while benzodiazepines (Whitaker-Azmitia et al., 1990) or desipramine (Fernández-Guasti et al., 2003) lacked an action. Using this animal model we found that young adult females vary in their response to 8-OH-DPAT and to fluoxetine depending on their estrous cycle phase (Fernández-Guasti et al., 2006) and that the anticompulsive-like effects of fluoxetine were higher in young adult females than in males (Agrati, 2004).

The main hypothesis of this study is that in middle-aged rats there are sex and endocrine-stage differences in the perseverative actions of 8-OH-DPAT and in the anti-perseverant and motor side actions of fluoxetine. In addition, due to the variations in estradiol levels reported for aged females in persistent diestrus and the lack of information on the levels of this steroid in middle-aged females with irregular cycles, selected in diestrus, we measured the serum levels of estradiol in these groups and in aged males.

2. Material and methods

2.1. Animals

Female and male Wistar rats, 11 to 14 months of age were used in this study. All animals were kept in groups of five in cages measuring $45 \times 28 \times 26$ cm. They were housed in a room with controlled conditions of temperature (22 ± 2 °C) under an inverted 12 hour light-dark cycle (lights off at 10:00 h); with *ad libitum* access to water and food throughout the experiment, except for the 24 h food deprivation period required before testing. All experimental procedures were performed in accordance with the Mexican Official Norm for the use and care of laboratory animals "NOM-062-ZOO-1999" and NIH publication 85-23, 1985 and were approved by the local Ethics Committee (Cinvestav-IPN). All efforts were made to minimize the number of animals used and their suffering. Animals were externally inspected and rats with overt signs of respiratory distress, infection or tumors were excluded.

The estrous cycle stage was determined by daily vaginal smears that were taken 1 h before lights off. The estrous cycle phases (proestrus, estrus, metestrus and diestrus) were characterized by the predominance of specific vaginal cells (Freedman, 2006). Females were classified in irregular cycles when the same phase was observed during 4–5 days or when there was a loss of the normal sequence of proestrus, estrus, metestrus and diestrus at least during 15 days before the test, and in persistent diestrus when the female showed the diestrus phase at least during 10 consecutive days. All behavioral tests for females were done in diestrus to avoid putative differences due to estrus-cycle phase (Fernández-Guasti et al., 2006). Males were similarly manipulated for at least 15 days before the test. Independent groups of animals were used for the experiments. The number of animals employed in the present study ranged from 10 to 12 per group.

2.2. Drugs

The selective 5-HT_{1A} agonist, 8-OH-DPAT (Sigma Chemicals, St. Louis, USA) at the dose of 2.0 mg/kg, was dissolved in 2.0 ml/kg saline solution and injected s. c. 15 min before the test. The selective serotonin reuptake inhibitor (SSRI), fluoxetine (kindly donated by Bioquimed, Mexico City, Mexico) at a dose of 10 mg/kg was dissolved in distilled water and s.c. administered sub-chronically: 24, 4 and 1 h before 8-OH-DPAT. These doses and schedules were chosen because they produce differential effects in young adult females, depending on the phase of the estrous cycle (Agrati et al., 2005; Fernández-Guasti et al., 2006).

2.3. SAB in the T maze

The testing apparatus for alternation consisted of a T-maze with two goal arms characterized by distinctive visual cues (Yadin et al., 1991). All arms measured $50 \times 10 \times 10$ cm and were separated from the main body of the maze with guillotine doors. Small plastic cups filled with chocolate milk were placed at the end of each goal arm. The maze was covered with clear Plexiglas lids and cleaned after each animal was tested to eliminate possible odor cues.

The animals were first exposed to chocolate milk in their home cages in order to acquaint them with the novel taste, thus reducing neophobia. Afterwards, the rats were exposed to three training sessions before the experiment. In the first training session, the animals were habituated to the maze for 20 min during which they were allowed to explore the entire area. In the second training session, the rats were confined for 5 min to each goal arm. In the third training session, the animals were placed in the main arm, the guillotine doors were lifted and rats were allowed to choose between the two goal arms. An arm choice was considered when an animal placed all four paws in one of the goal arms and stayed in it for at least 90 s. Thereafter, the rats were removed and placed in a holding cage for 10 s. This procedure was repeated for a total of nine runs. In all