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Sex and age differences in the antidepressant-like effect of fluoxetine in the forced swim test

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ABSTRACT

This study compared in males and females of three representative ages: young adults (3–5 months old), middle-aged (12–15 months old) and senescent (23–25 months old) the antidepressant-like effect of fluoxetine (FLX, 5.0 and 10 mg/kg) in the forced swim test (FST). Intact (non gonadectomized) rats were evaluated. Young adult females were chosen in proestrus/estrus or in metestrus/diestrus, while middle-aged and senescent females were selected in metestrus/diestrus. Locomotion and motor coordination were also recorded. Under basal conditions (without FLX), young adult and middle-aged females showed less immobility than males. This sex difference disappeared at senescence because males diminished their levels of immobility. Thus, senescent males showed lower immobility than middle-aged and young males. FLX (5 and 10 mg/kg) produced similar actions in young females irrespective of their estrous cycle phase, therefore, these subgroups were pooled in a single one. Young adult and middle aged females clearly responded to 5 and 10 mg/kg of FLX with a reduction in immobility, while young adult and middle-aged males only did to 10 mg/kg. In senescent females 10 mg/kg FLX reduced immobility. Remarkably, in senescent males this FLX dose did not produce an antidepressant-like effect. FLX marginally affected locomotion; however, at its highest dose (10 mg/kg), and only in senescent males, interfered with motor coordination tested in the rotarod. These data show that sex and aging influence behavioral despair without treatment and after FLX.

1. Introduction

Depression is an affective disorder characterized by chronic low mood and loss of interest in most activities as core symptoms (American Psychiatric Association, 2000). Sex and age importantly affect depression (Afifi, 2007; Blazer and Hybels, 2005). Thus, this mental illness is commonly found in the elderly, with a prevalence ranging from 22 to 46% in people over 65 years old (Lebowitz et al., 1997) and in this population becomes a heterogeneous disorder with complex genetic background (Pitychoutis et al., 2013). In addition, clinical studies have constantly observed gender differences among patients with depression, with young women outnumbering men at a rate of 2:1, a prevalence that may exacerbate to 5:1 in perimenopause (Borrow and Cameron, 2014; Cohen et al., 2003; Soares et al., 2001). Another contributing factor for the differences in depression prevalence across a woman's lifetime is changes in levels of gonadal hormones (Freeman et al., 2006; Freeman et al., 2004). Not merely the prevalence of depression varies with age and sex but also the disease symptom profile (American Medical Association Council on Scientific Affairs, 2006; Goodwin and Gotlib, 2004; Nolen-Hoeksema et al., 1999; Silverstein, 2002).

Incredibly, the efficacy/effectiveness of antidepressants in old patients has not been systematically assessed. The first line pharmacotherapy for this population is the selective serotonin reuptake inhibitors (SSRIs) (American Psychiatric Association, 2010; Coupland et al., 2011; Rajji et al., 2008) as fluoxetine (FLX), citalopram, sertraline and paroxetine (Blazer, 2003; Nelson et al., 2008). Although SSRIs are reported to be effective in clinical trials with older patients (Blazer, 2003; Gareri et al., 2000; Salzman et al., 2002), the careful literature review reveals highly variable results (Kasper et al., 2005; Nelson et al., 2008) (see Discussion). Moreover, it has been suggested that the decline in antidepressants' actions begins at middle age (Tedeschini et al., 2011), but no studies have been carried out exploring putative sex differences. The SSRIs are relatively secure compounds that have some side effects such as nausea, diarrhea and sexual dysfunction (Gareri et al., 2000; Owens et al., 1997) that unfortunately are increased in aged patients (Coupland et al., 2011).

Although controversial (Estrada-Camarena et al., 2011a; Hildebrandt et al., 2003; Lewis-Hall et al., 1997), various studies have suggested that women respond and tolerate better SSRIs than men; while men respond better to imipramine (a tricyclic antidepressant) (Kornstein et al., 2000;

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Thase et al., 1996). In line, other authors (Berlanga and Flores-Ramos, 2006; Martenyi et al., 2001) found that women show a better response to SSRIs (FLX or citalopram) than to selective noradrenaline reuptake inhibitors (SNRI), while men respond likewise to both inhibitors. In addition to sex differences, menopausal status has also been suggested as a factor influencing antidepressants effects (Kornstein et al., 2000; Schneider et al., 1997), inviting to test these compounds in middle-aged subjects.

The most used animal model to study the antidepressant-like effect of drugs and non-pharmacological treatments is the forced swim test (FST). In it we and others have found higher immobility scores (interpreted as despair in the FST) in male than in female rats, suggesting a decreased motivation of males to escape from the stressing situation (Alonso et al., 1991; Contreras et al., 1995; Gomez et al., 2014). The lower immobility in females is inversely related to the levels of estrogen and progesterone (Barros and Ferigolo, 1998; Estrada-Camarena et al., 2011a), indicating that the FST is sensitive to steroid hormones (Andrade et al., 2010; Contreras et al., 1998; Estrada-Camarena et al., 2010; Galea et al., 2001; Rodriguez-Landa et al., 2009; Walf and Frye, 2005, 2007; Walf et al., 2004). In relation with age we and others agree that middle aged animals are more susceptible to develop depressive-like behaviors in this test (de Chaves et al., 2009; Recamier-Carballo et al., 2012).

Surprisingly, the experimental analysis of putative changes in antidepressant-like actions considering sex and age differences is scant [see the excellent review by (Kokras et al., 2015)]. In males, Bourin and colleagues found that some antidepressants were less effective in 40-week-old mice compared with 4-week-old subjects (Bourin et al., 1998; David et al., 2001a). In another study, middle-aged male rats (12–15 months old) required a longer treatment than young adults (3–5 months old) to show the antidepressant-like effect of citalopram (10 mg/kg/day) (Herrera-Perez et al., 2010). These data suggested that middle-aged males were less responsive to the antidepressant-like effects. A recent report from our group systematically analyzed this proposition and found that young adult males were sensitive to the antidepressant-like effect of FLX, at 10 mg/kg in the FST, while senescent males (23–25 months old) were completely insensitive to the antidepressant-like effects of this SSRI at 5, 10 and even 20 mg/kg (Olivares-Nazario et al., 2015). In females FLX (10 mg/kg) produced an analogous response in young adult and middle-aged ovariectomized subjects in the FST, although the young ones seem slightly more sensitive, because they showed a reduced immobility at 2.5 mg/kg that was absent in middle-aged females (Recamier-Carballo et al., 2012). These data, taken together, support the hypothesis of a lower sensitivity to antidepressants with age, particularly in males. It is also worth mentioning that working with females has the extra consideration of the estrous cycle phase.

To reduce the number of subjects, researchers have abused of ovariectomized females to explore sex differences, using intact (non orchidectomized) males. Such comparison has the flaw that one group lacks ovarian steroids while in the other there is a testicular androgen and estrogen production, which importantly modify the antidepressants action (Gomez et al., 2014; Martinez-Mota et al., 2008; Martinez-Mota and Fernandez-Guasti, 2004). Another important issue regards the different age concepts. Although it is impossible to analogously establish age correlations between rats and humans, most studies using aged rats include animals of around one year. The lifespan of laboratory rodents is about 24–34 months (Nadon, 2006), and it is quite difficult and expensive to maintain senescent animals. However, this information is essential in depression studies evaluating sex differences. On these bases the purpose of this study was to compare in males and females of three representative ages: young adults (3–5 months old), middle-aged (12–15 months old) and senescent (23–25 months old) the antidepressant-like effect of FLX in the FST. Intact (non gonadectomized) rats were used. The groups of females were selected according to their vaginal smear cytology: young adult rats were chosen in proestrus/estrus and in metestrus/diestrus, while middle-aged and senescent females were selected in metestrus/diestrus.

2. Materials and methods

2.1. Animals

Wistar male and female rats born in the local vivarium were used in this study. Animals were housed under an inverted 12-h light/12-h dark cycle (lights off at 10:00 h) in a room with constant temperature of 24 ± 1 °C. Rats had *ad libitum* access to water and commercial food during the complete study and were maintained in accordance with the Official Norm of Technical Specification for the Production, Care and Use of Laboratory Animals (NOM-062-ZOO-1999). All protocols were approved by the local committee of ethics on animal experimentation. We certify that all procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23 revised 1996) and that all efforts were made to minimize the number of animals and their suffering.

2.2. Forced swim test

Swimming sessions were conducted by placing rats in individual glass cylinders (46 cm height; 20 cm diameter) containing water at 23–25 °C, 30 cm deep, so the rats could not support themselves by touching the bottom. For males, an initial 15-min session (termed pre-test) was followed 24 h later by a 5-min session (termed test). In our experience (unpublished data) the FST alters the estrous cycle in around 30% of the subjects. In view of that, in the present experiments with females the pre-test was made one week before the test (Vega-Rivera et al., 2013). The impact of the pretest on expression of despair in the test is maintained up to three weeks (Detke et al., 1997). Following each swim session, the rats were removed from the cylinders, dried with paper towels and placed into heated cages for 30 min, and then returned to their home cages. Test sessions were run between 1200 and 1500 h and videotaped for later scoring. A single observer, who was unaware of the treatments, did all the behavioral scoring. A time sampling technique was employed to score three different behaviors (Detke et al., 1995). During the test session, the scorer rated at the end of each 5-s period the following behaviors: (1) immobility—floating without struggling, and doing only those movements necessary to keep the head above the water; (2) swimming—showing active swimming motions, more than those necessary to merely keep the head above water, i.e., moving around in the cylinder or diving; and (3) climbing—presenting active movements with the forepaws, usually directed against the walls (Estrada-Camarena et al., 2003; Martinez-Mota et al., 2008).

2.3. Experimental design

Rats of different ages were randomly assigned to receive vehicle (saline solution 0.9%), 5.0 or 10 mg/kg of fluoxetine hydrochloride (Bioquimed, Mexico City, Mexico, dissolved in saline solution). These treatments were applied following a sub-acute schedule, i.e., three s.c. injections administered between pre-test and test sessions (24 h, 5 h, and 1 h before the test). The number of subjects per group is shown in Table 1. FLX was injected in a volume of 2 mL/kg and doses (expressed in terms of salt) were those given in each individual injection. FLX doses and latencies were selected from previous reports (Estrada-Camarena et al., 2003, 2004; Gomez et al., 2014; Martinez-Mota et al., 2008; Martinez-Mota and Fernandez-Guasti, 2004). Only young adult females with regular 4–5 days estrous cycles were selected. They were tested in proestrus/estrus or metestrus/diestrus, while middle aged and senescent rats were chosen in metestrus/diestrus for the 5-min session of the FST. The few aged female rats with constant estrous were discarded. Due to the shortage of senescent animals in this study we include only