Neuroscience 261 (2014) 11–22

INFLUENCE OF THE BRAIN SEXUAL DIFFERENTIATION PROCESS ON DESPAIR AND ANTIDEPRESSANT-LIKE EFFECT OF FLUOXETINE IN THE RAT FORCED SWIM TEST

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Abstract—Sex differences exist in the depressive disorder prevalence and response to treatment. Several studies suggest that females respond better than males to the action of selective serotonin reuptake inhibitors (SSRIs), suggesting that gonadal hormones modulate mood and the response to these drugs. Sexual steroid hormones exert organizational actions (perennial and on early development) and activational effects (transient and on differentiated tissues). The aim of this study was to analyze sex differences in the forced swim test (FST) in animals without treatment and after fluoxetine (FLX, 0, 2.5, 5.0 and 10.0 mg/kg). Initially, we compared male and female adult rats under control conditions or after altering their sexual differentiation process (at day 5 postnatally, PN, 60 μg of testosterone propionate to females and male castration to induce or preclude masculinization, respectively). To further analyze if the sex differences depend on organizational or activational steroid hormone action we tested the same animals before and after adult gonadectomy. To prevent variations depending upon the estrous cycle, control and masculinized females were tested in estrus. Control females showed lower immobility and required lower doses of FLX (5 mg/kg), to show an antidepressant-like effect, than males (10 mg/kg), even after adult gonadectomy. In control males adult orchidectomy prevented FLX's action. Neonatally masculinized females exhibited analogous levels of immobility than control ones; before ovariectomy they responded to FLX similar to controls, but after the surgery they did not respond to fluoxetine. Neonatally orchidectomized males exhibited similar immobility values and response to FLX than control females. The findings suggest that the sex difference in despair depends on the hormones organizational effects and, in males, the response to FLX relies on organizational and activational actions.

Key words: sex differences, neonatally masculinized females, neonatally castrated males, forced swim test, fluoxetine.

INTRODUCTION

Important sex differences in depression have been consistently reported because its prevalence, incidence and morbidity risk is twice higher in young adult women as compared to men and this difference may increase fivefold in middle-aged patients (American Psychiatric Association, 2000; Kessler et al., 2003). The symptoms of depression are also sexually dimorphic with women showing more appetite and sleep disturbances, fatigue, somatic anxiety and hypochondria than men. In addition, women show depression clearly related with changes in gonadal hormone levels during the menstrual cycle, postpartum and menopause (Young et al., 1990; Silverstein, 1999).

Although controversial (see Lewis-Hall et al., 1997), various clinical studies have suggested that antidepressant drugs produce differential therapeutic actions in both sexes. Thus, Davidson and Pelton (1985) showed a poor response to tricyclic antidepressants in women when compared to men treated also with tricyclic antidepressants or with women treated with monoamine oxidase inhibitors (MAOI). Conversely, other studies (Thase et al., 1996; Kornstein et al., 2000) showed that women responded and tolerated better selective serotonin reuptake inhibitors (SSRIs) than men; while men tolerated better imipramine (a tricyclic antidepressant). In line, other authors (Martenyi et al., 2001; Berlanga and Flores-Ramos, 2006) found that women showed a better response to SSRIs (fluoxetine or citalopram) than to selective noradrenaline reuptake inhibitors (SNRI), while men responded similarly to both drugs.

In agreement with clinical reports, recent data have shown sex differences in the action of the tricyclic noradrenergic antidepressant, desmethylimipramine (DMI), with males requiring lower doses than females to display antidepressant-like behaviors in the forced swim test (FST) (Estrada-Camarena et al., 2011a).

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Abbreviations: ANOVA, analysis of variance; FLX, fluoxetine; FST, forced swim test; LSD, Least Significance Difference; NS, non significant; PN, postnatally; RM, repeated measures; SSRIs, selective serotonin reuptake inhibitors; TP, testosterone-propionate.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.12.035

Interestingly, the FST has also revealed sex differences in untreated rats. Thus, some studies show higher immobility scores (interpreted as despair in the FST) in male rats than in females, suggesting a decreased motivation of males to escape from the stressing situation (Alonso et al., 1991; Contreras et al., 1995). The lower immobility in females (interpreted as a decreased depressant-like behavior) is inversely related to the levels of estrogen and progesterone (Barros and Ferigolo, 1998; Estrada-Camarena et al., 2011b), indicating that the FST is sensitive to hormonal oscillations during the estrous cycle (Contreras et al., 1998). In addition, recent studies have analyzed the effect of gonadectomy on the antidepressant-like effect of some drugs in the FST. Thus, we have shown that adult ovariectomy did not affect fluoxetine's action (Estrada-Camarena et al., 2003, 2011b), while in males adult orchidectomy *per se* did not alter the immobility values, but completely impaired the antidepressant-like action of fluoxetine (FLX) (Martínez-Mota and Fernández-Guasti, 2004), which is recovered after estradiol treatment (Martínez-Mota et al., 2008). These findings indicate that the FLX's antidepressant-like effect in females does not depend on the presence of ovarian hormones, while in males depends on the testicular production of estrogens.

According to the life-period where steroids are produced and to the sort of effect they exert, their actions have been divided into organizational or activational. Organizational effects occur during critical developmental periods and are permanent, for example, the sexual dimorphic brain differentiation (Breedlove et al., 1999; LaCroix-Fralish et al., 2005). Activational actions, in contrast, are transient and take place on already differentiated tissues previously organized, for example, the stimulation of sexual behavior. A bulk of information indicates that the rat brain sexual differentiation process is completed postnatally. Thus, the neonatal administration of androgens to female rats results in adult masculinized animals with polifollicular ovaries (which tonically secrete estradiol) that display masculine sexual behavior. Conversely, the neonatally castrated male, when adult, shows female sexual behavior and a cyclic secretion of gonadotrophins and ovarian steroids after a graft (Young, 1961; Fernández-Guasti and Picazo, 1999).

To date it is unclear whether the sex differences in depressive-like behaviors and in the action of antidepressants in the FST are due to organizational or activational effects of steroid hormones (Dalla et al., 2010). That is, in females the lower levels of immobility and their higher susceptibility to SSRIs – as compared to that shown by males – may be the result of the activational effect of ovarian hormones or may be based on constitutively sex differentiated brains organized by steroid hormones during early development. To analyze these possibilities we tested whether control (non-neonatally manipulated) or neonatally castrated males or neonatally masculinized females differed in their levels of immobility in the FST when adults without pharmacological treatment and after different doses of fluoxetine. As aforementioned, adult neonatally masculinized females as well as control rats of both sexes are exposed to gonadal steroids which impact a normal or an altered sexually differentiated brain, influencing immobility and interacting with FLX, therefore combining organizational and activational actions of gonadal steroids. Thereby, in a second experiment we tested all animals in the FST after adult gonadectomy (with the exception of the neonatally castrated males) with and without FLX treatment. These groups, naturally, lack the activational effect of gonadal steroids and their results – compared with those of the previous experiment – permit to dissect pure organizational from mixed organizational and activational actions of gonadal steroids. Because neonatally masculinized females show persistent vaginal estrus and, although controversial (Alonso et al., 1991 vs. Barros and Ferigolo, 1998; Contreras et al., 1998), immobility may vary depending upon the estrous cycle phase, all groups of non-ovariectomized females were also tested in estrus.

EXPERIMENTAL PROCEDURES

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 with food and water freely available and at a constant temperature of 24 ± 1 °C. All the experiments were approved by the local committee of ethics on animal experimentation and were in accordance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). We certify that all experiments 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 used and their suffering. The day of birth was designated as day 1 and the sex of the newborn established. The litter was culled to eight, usually four males and four females.

Neonatally masculinized females

On the postnatal (PN) fifth day females were assigned to three groups: Testosterone-propionate (TP)-, oil-treated females and neonatally-untreated non disturbed females; the two former groups were crioanaesthetized to match this procedure with the group of males (*vide infra*). The TP-group received a sc injection of 60 μg/rat of TP (Sigma Chemicals, Saint Louis, MA, USA) dissolved in 0.1 ml of corn oil, the oil-treated group received the same volume of the vehicle. This TP dose and day of treatment have been previously shown to be effective for masculinizing (González-Mariscal et al., 1982; Fernández-Guasti and Picazo, 1999). One month after birth, females were checked for the vaginal opening. Once their vagina opened a daily vaginal