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CITALOPRAM-MEDIATED ANXIOLYSIS AND DIFFERING NEUROBIOLOGICAL RESPONSES IN BOTH SEXES OF A GENETIC MODEL OF DEPRESSION

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Abstract—Disorders such as depression and anxiety exhibit strong sex differences in their prevalence and incidence, with women also differing from men in their response to antidepressants. Furthermore, receptors for corticotrophin releasing hormone (CRHR1) and arginine vasopressin receptor subtype 1b (AVPR1b) are known to contribute to the regulation of mood and anxiety. In the present study, we compared the anxiety profile and CRHR1 and AVPR1b expression levels in control Sprague–Dawley (SD) rats and rats of the SD-derived Flinders Sensitive Line (FSL), a genetic model of depression. Additionally, given the apparent sex differences in the therapeutic efficacy of antidepressants and because antidepressants are commonly used to treat comorbid anxiety and depressive symptoms, we assessed whether the anxiolytic effects of an antidepressant occur in a sex-dependent manner. Male and female FSL rats were treated with citalopram 10 mg/kg once daily for 14 days and were then tested in the open field and the elevated plus maze paradigms. Upon completion of the behavioural analysis, AVPR1b and CRHR1 expression levels were monitored in the hypothalamus and the prefrontal cortex (PFC) using Western blotting. According to our results, male FSL rats were more anxious than control SD rats, a difference abolished by citalopram treatment. Baseline anxiety levels were similar in female FSL and SD rats, and citalopram further reduced anxiety in female FSL rats. Importantly, whereas citalopram altered AVPR1b expression in the hypothalamus of male FSL rats, its actions on this parameter were restricted to the PFC in female FSL rats. In both sexes of FSL rats, citalopram did not alter CRHR1 expression in either the hypothalamus or PFC. Our results demonstrate that antidepressant treatment reduces anxiety levels in FSL rats of both sexes: the magnitude of treatment effect was related to the starting baseline level of anxiety and the antidepressant elicited sexually differentiated neurobiological responses in specific brain regions.

Key words: antidepressant, sex differences, anxiety, depression, females, receptors.

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Abbreviations: ANCOVA, analysis of covariance; AVP, arginine vasopressin; AVPR1b, arginine vasopressin receptor subtype 1b; CRH, corticotropin releasing hormone; CRHR1, corticotropin releasing hormone receptor subtype 1; EPM, elevated plus maze; FSL, Flinders sensitive line; HPA, hypothalamic pituitary adrenal; PFC, prefrontal cortex; SD, Sprague–Dawley; SSRI, selective serotonin reuptake inhibitor.

Stress and dysregulation of the hypothalamic pituitary adrenal (HPA) axis are implicated in the pathophysiology of mood and anxiety disorders (Tsigos and Chrousos, 2002), which show greater comorbidity and prevalence in women (Kessler et al., 1994; Alonso et al., 2004). Two hypothalamic drivers of the HPA axis, corticotropin releasing hormone (CRH) and arginine vasopressin (AVP), also influence mood and emotional behaviour through their actions at extra-hypothalamic sites (Holsboer and Ising, 2008). The actions of CRH are mediated by two receptor subtypes (Lowry and Moore, 2006). The CRH subtype 1 receptor (CRHR1) modulates context-dependent affective responses and is expressed in the hypothalamus and extra-hypothalamic areas, including the frontal cortex (Sanchez et al., 1999). On the other hand, cerebral vasopressin pathways show distinct anatomical and functional patterns (Ermisch et al., 1993); most central actions of this peptide are mediated by subtype 1b vasopressin receptors (arginine vasopressin receptor subtype 1b [AVPR1b]) which are also expressed in the hypothalamus and frontal cortex (Hernando et al., 2001). Increasingly, AVPR1b are being implicated in behaviours relevant to mood and anxiety disorders (Surget and Belzung, 2008). Antagonism of either CRHR1 or AVPR1b results in anxiolytic and antidepressant effects, suggesting these receptors as alternatives to the current monoaminergic drug targets (Surget and Belzung, 2008; Binder and Nemeroff, 2010).

Sex differences in the activity of the HPA axis are well known and the sexually differentiated response to stress is increasingly thought to be an important underlying factor in mood and anxiety disorders (Young, 1998; Goel and Bale, 2009; Young and Korszun, 2010). Women are nearly twice as likely to suffer from depression (Kessler et al., 1994; Alonso et al., 2004). Moreover, as compared with men, depressed women make a greater number of suicide attempts, show more somatisation, anger and hostility, and display increased appetite and weight gain. Importantly, although melancholic depression occurs equally in both sexes, the anxious and atypical forms of depression are more commonly found in women (Frank et al., 1988; Marcus et al., 2005, 2008), although the construct of anxious depression has received some criticism (Nelson, 2008). It is now well-established that estrogens influence depres-

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sive symptoms, including irritability, insomnia, appetite, and general physical well-being (Young et al., 2007; Kornstein et al., 2010). Together, these observations suggest the potential importance of considering the role of sex and the ovarian steroid milieu in measuring the efficacy of antidepressant therapy. Indeed, several studies have reported that women respond better to selective serotonin re-uptake inhibitors (SSRI) (Kornstein et al., 2000; Joyce et al., 2003; Baca et al., 2004; Khan et al., 2005); a similar conclusion was reached in a recent large multicentre trial of citalopram (Marcus et al., 2008). Nevertheless, some authors have argued that sex differences in response to antidepressant treatment may be clinically irrelevant (Quitkin et al., 2002; Hildebrandt et al., 2003; Parker et al., 2003; Wohlfarth et al., 2004; Thiels et al., 2005).

Experimental studies from our group and others have consistently demonstrated increased female vulnerability to the detrimental effects of stress on mood- and anxiety-related behaviours (Patchev and Almeida, 1998; Dalla et al., 2010; Pitychoutis and Papadopoulou-Daifoti, 2010; Pitychoutis et al., 2010). Furthermore, our previous studies showed that antidepressants elicit similar, albeit differing in magnitude, behavioural responses in male and female rats of the Flinders Sensitive Line (FSL); serotonergic and glutamatergic responses also differ between the two sexes following antidepressant treatment (Kokras et al., 2009a,b). FSL rats, derived by selective breeding of Sprague–Dawley (SD) rats, have several key characteristics which support the face, construct and predictive validity of this model of depression (Overstreet, 2002). FSL rats exhibit decreased weight and disturbed appetite, have elevated rapid eye movement sleep, present anhedonia in chronic mild stress, and are less active in novel environments; at the same time they are inherently more immobile in the forced swim test. Furthermore, in brain limbic areas, FSL rats show significant regional abnormalities in levels of biogenic amines and their receptors, reduced serotonin synthesis, impaired serotonin-induced dopamine release, and impairments in their immune system (Overstreet et al., 2005). Importantly, chronic and not acute treatment with antidepressants restores those observed behavioural and neurobiological abnormalities (Yadid et al., 2000).

Despite the large body of evidence supporting the validity of the FSL model of depression, it still remains unclear whether FSL rats can model comorbid anxious depression. Previous research on male FSL showed increased levels of anxiety in the social interaction test (Overstreet, 2002; Janowsky et al., 2004; Lavi-Avnon et al., 2005) but not in the elevated plus maze (EPM) (Schiller et al., 1991; Overstreet et al., 1995). Although male FSL rats have been well studied, relatively fewer studies have focused on female FSL animals. While our previous studies confirmed the validity of female FSL rats as a model of depression (Kokras et al., 2009a,b), there is no information with respect to their anxiety profile and expression levels of CRHR1 and AVPR1b.

Based on the aforementioned sex-differences and the documented validity of male and female FSL rats as a model of depression, a first aim of the present study was to explore the potential suitability of the FSL rat as a model of comorbid anxious depression. Further, given that male FSL rats were previously reported to be sensitive to the behavioural actions of CRHR1 and AVPR1b antagonists (Janowsky et al., 2004; Overstreet et al., 2004, 2005) we examined the expression of brain CRHR1 and AVPR1b in male and female FSL rats under baseline conditions and after repeated citalopram treatment.

EXPERIMENTAL PROCEDURES

Animals

Twenty-four adult male and female FSL rats, weighing 275±17 g and 200±15 g, respectively, and aged 10–11 weeks at the beginning of the experiment, were used. In addition, 24 male and female, similarly aged, SD rats, weighing 325±26 g and 245±20 g, respectively, were used as controls as previously described (Overstreet et al., 2005). Animals were group-housed, according to sex, under controlled 12:12-light/dark cycles (lights on at 7:00 AM) and temperature (22±2 °C), with free access to food and tap water. All animal experiments were carried out in accordance with the EEC directive 86/609. Efforts were made in order to minimize the number of animals used and to reduce their suffering.

Oestrous cycle

In the case of females, a semi-random process controlled for disparities regarding the phases of the oestrous cycle. Specifically, female rats were selected from a larger pool of experimental animals on the basis of a normal 4–5 day cycle and assigned to groups on the basis of an equal distribution of oestrous cycle phases; the latter was monitored by vaginal smears until the day of sacrifice, as described elsewhere (Becker et al., 2005). Oestrous cycle phases on the day of behavioural testing and sacrifice are reported in Table 1.

Treatments

FSL and control SD rats were gently handled, daily by the same researcher. Male and female animals were given intra-peritoneal injections of either saline (FSL n=12; SD n=12) or 10 mg/kg of citalopram (Lundbeck S.A., Denmark; FSL n=12; SD n=12) during the morning, over 14 days. This dose of citalopram was previously shown to be effective and has been routinely used in male and female rats (Burghardt et al., 2004; Overstreet et al., 2004; Hasegawa et al., 2005).

Open field

Spontaneous activity under novelty stress was measured for 5 min, approximately 22–26 h after the last injection of saline or citalopram (day 15). As previously described (Pitychoutis et al., 2009b), all rats were acclimatized to the test room for 1 h and

Table 1. Oestrous cycle

	Vehicle		Citalopram	
	FSL	SD	FSL	SD
Proestrous	1	2	2	2
Estrous	2	1	1	1
Diestrous I	1	2	1	2
Diestrous II	2	1	2	1

Distribution of the phases of the oestrous cycle in female FSL and Sprague–Dawley (SD) rats treated with vehicle and citalopram.